EVALUATION AND UTILIZATION OF PLANT PIGMENTS AS NATURAL FOOD COLOURANTS

By

NETRAVATI

(2019-22-020)

DEPARTMENT OF POSTHARVEST MANAGEMENT

COLLEGE OF AGRICULTURE

VELLANIKKARA, THRISSUR – 680 656

KERALA, INDIA

2023

EVALUATION AND UTILIZATION OF PLANT PIGMENTS AS NATURAL FOOD COLOURANTS

By

NETRAVATI

(2019-22-020)

THESIS

Submitted in partial fulfillment of the requirement for the degree of

Doctor of Philosophy in Horticulture

Faculty of Agriculture

Kerala Agricultural University, Thrissur

DEPARTMENT OF POSTHARVEST MANAGEMENT

COLLEGE OF AGRICULTURE

VELLANIKKARA, THRISSUR – 680 656

KERALA, INDIA

2023

DECLARATION

I, hereby declare that this thesis entitled "**EVALUATION AND UTILIZATION OF PLANT PIGMENTS AS NATURAL FOOD COLOURANTS**" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellanikkara
Date: 18.03.2023

Netravati
(2019-22-020)

CERTIFICATE

Certified that this thesis entitled "**EVALUATION AND UTILIZATION OF PLANT PIGMENTS AS NATURAL FOOD COLOURANTS**" is a bonafide record of research work done independently by **Ms. Netravati (2019-22-020)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Vellanikkara
Date: 18.03.2023

Dr. Saji Gomez
(Major Advisor, Advisory Committee)
Professor (PHM) and Head
Department of Postharvest Management
College of Agriculture, Vellanikkara
Kerala Agricultural University
Thrissur, Kerala

CERTIFICATE

We, the undersigned members of the advisory committee of **Ms. Netravati (2019-22-020)**, a candidate for the degree of **Doctor of Philosophy in Horticulture**, with major in **Postharvest Management**, agree that the thesis entitled "**EVALUATION AND UTILIZATION OF PLANT PIGMENTS AS NATURAL FOOD COLOURANTS**" may be submitted by **Ms. Netravati**, in partial fulfillment of the requirement for the degree.

Dr. Saji Gomez
(Major Advisor, Advisory Committee)
Professor (PHM) and Head
Department of Postharvest Management
College of Agriculture, Vellanikkara
Kerala Agricultural University
Thrissur, Kerala

Dr. Berin Pathrose
(Member, Advisory Committee)
Associate Professor
Department of Entomology
College of Agriculture Vellanikkara,
Kerala Agricultural University
Thrissur, Kerala

Dr. Anupama T V
(Member, Advisory Committee)
Assistant Professor
Department of Postharvest Management
College of Agriculture, Vellanikkara
Kerala Agricultural University
Thrissur, Kerala

Dr. Suma A
(Member, Advisory Committee)
Scientist
ICAR–National Bureau of Plant Genetic Resources
Regional Station
Thrissur, Kerala

Dr. Anu Mary Markose
(Member, Advisory Committee)
Assistant Professor
Department of Postharvest Management
College of Agriculture, Vellanikkara
Kerala Agricultural University
Thrissur, Kerala

Dr. Shynu M
(Member, Advisory Committee)
Professor
Department of Veterinary Biochemistry
College of Veterinary and Animal Sciences, Mannuthy
Kerala Veterinary and Animal Sciences University, Thrissur, Kerala

EXTERNAL EXAMINER
(VASUDEVA, K.R)

ACKNOWLEDGEMENT

No scientific endeavor is the outcome of a single person's efforts. Therefore, I reflect on the path I traveled during this quest and, with the utmost respect, take this opportunity to thank those who motivated me to fulfill the goal.

I feel incredibly privileged to thank my esteemed major advisor, ***Dr. Saji Gomez***, Professor and Head, Department of Postharvest Management, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur, for introducing me to this exciting research topic and for his invaluable advice, consistent encouragement, critical suggestions, and belief in me throughout this investigation. For my doctoral work, I could not have asked for a greater mentor and advisor. His excitement, holistic perspective on study, commitment, editorial, and technical guidance were crucial to the successful completion of my thesis. I will always be inspired by his unwavering strength and determination, and I hope to carry on cooperating with his admirable ideas.

I would like to thank ***Dr. Anupama T. V.,*** Assistant Professor, Department of Postharvest Management, College of Agriculture, Vellanikkara, and member of my Advisory Committee for the support and suggestions. I would also like to thank ***Mrs. Anu Mary Markose***, Assistant Professor, Department of Postharvest Management, College of Agriculture, Vellanikkara, and member of my Advisory Committee, for her advice and willingness to help me during the preparation of processed products.

I am deeply indebted to ***Dr. Berin Pathrose***, Associate Professor, Department of Entomology, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur, and member of my Advisory Committee, for his valuable help in research, experimentation, and suggestions while writing my thesis and articles. Without his instant help, it would have been extremely difficult.

I am thankful for the help and support provided by ***Dr. Suma A.*** Scientist, ICAR-NBPGR, Regional Station, Vellanikkara and member of my Advisory Committee, in getting the raw materials required for the experiment. I am happy to

express all my feelings and heartfelt gratitude to ***Dr. Shynu M.*** Professor, Department of Veterinary Biochemistry, College of Veterinary and Animal Sciences, Mannuthy, and member of my Advisory Committee, for extending the help with the biochemical analysis procedures and suggestions while thesis writing.

I was impressed by the kindness and caring gesture shown by ***Mrs. Meagle Joseph P***., Associate Professor (Retd.), Department of Postharvest Management, College of Agriculture, Vellanikkara, for the students. I'm grateful to her for setting up a comfortable stay for me in Thrissur, Kerala. I want to express my sincere gratitude to her at this time. I thankfully acknowledge the help and support offered by ***Dr. Mini Raj N.*** Professor (Retd.), Department of Plantation crops and Spices, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur.

I am grateful to ***Dr. K. M. Indiresh***, Vice Chancellor, University of Horticultural Sciences, Bagalkote, Karnataka, for providing me an opportunity to pursue a doctoral degree programme. My diction is poor to convey how grateful I am to ***Dr. S. L. Jagadeesh*** for his unending support and encouragement. I take this opportunity to thank him from the bottom of my heart. My acknowledgement will never be complete without the special mention of ***Dr. A. Nataraja***, Professor (Horticulture), Department of Horticulture, College of Agriculture, Hassan, UAS, GKVK, Bengaluru, for his moral support and motivation. I also take this opportunity to thank ***Dr. Vikram H. C***., Assistant Professor, Department of Plantation crops and Spices, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur, for his support and suggestions.

Many thanks to ***Radhakrishnan uncle***, gac fruit farmer, Irinjalakuda, for his incredible help in timely providing me with gac fruits. Also, I am extremely grateful for his constant care and concern for me. I am also thankful to ***Mr. Nithin Gowda***, PhD Scholar, IIHR, Bengaluru, Karnataka, for providing me basella berries.

Special thanks are expressed to ***Ms. Bintu Kuruvila***, Analyst, Quality Control Lab, Department of Postharvest Management, College of Agriculture, Vellanikkara, for her valuable help and best support extended to me during biochemical analysis

work. I also acknowledge my thanks to ***Lathika chechi***, ***Seena***, ***Manju chechi***, ***Shiji chechi***, ***Karishma***, ***Rigzen***, ***Maneesha***, ***Shrija chechi,*** and ***Prasanna chechi*** of the Department of Postharvest Management for their cooperation and invaluable help in times of need rendered during the course of the investigation.

I avail myself of this opportunity to express my heartfelt gratitude to my friends ***Thatayone Malikongwa***, ***Rajeesha***, ***Nivya*****,** ***Geethu,*** and juniors ***Anjali***, ***Janmitha***, ***Sharon,*** ***Amrutha Lakshmi***, ***Sandhya***, ***Ismath,*** and ***Anupam*** for the affection and moral support extended to me all the time.

I am grateful to my beloved husband, ***Dr. Jagadish Hosamani,*** for always believing in me, encouraging me to follow my dreams, celebrating each of my accomplishments, being ready to take care of me, and helping in whatever way he could during this challenging period. My sincere and humble thanks are due to my mother, ***Smt. Chandrabhaga***, father, ***Shri. Pandit Noola***, sisters, ***Smt. Jaishree*** and ***Smt. Baby Nanda,*** father-in-law, ***Shri. Yellappa Hosamani***, mother-in-law, ***Smt. Kasturavva***, sister-in-law, ***Smt. Vijayalakshmi,*** and brother-in-law, ***Shri. Shashidhar Hosamani,*** who were with me to comfort, morally support, and boost my zeal in pursuing this task. I heartily thank you all for being with me through the ups and downs of life, for withstanding my frustration at times, and for helping me get through this period in the most positive way possible.

Last but not least, I would like to thank my best friend ***Nilambika***, who in all ways has been by my side since the days of graduation, post-graduation, and throughout this PhD, encouraging me every single minute of it. She has been with me ever since as a friend, a companion, and a motivator.

Netravati

Dedicated to My

Beloved Husband

TABLE OF CONTENTS

LIST OF PLATES

LIST OF TABLES

LIST OF APPENDICES

Appendix No.	Title
I	Score card for sensory evaluation of pigmented guava squash
II	Score card for sensory evaluation of pigmented guava jelly
III	Score card for sensory evaluation of pigmented ash gourd candy

LIST OF ABBREVIATIONS

°C	:	Degree Celsius
µg	:	microgram
mg	:	milli gram
OD	:	Optical Density
h	:	hour
min	:	minute
s	:	seconds
Fig.	:	figure
mM	:	milli Molar
et al.	:	co-workers
%	:	per cent
Eq.	:	equivalent
ml	:	milli litre
viz.	:	namely
CD	:	critical difference

Introduction

1. INTRODUCTION

A well-known culinary proverb says, "You eat with your eyes first". This aphorism refers to the reality that unappealing foods are frequently avoided. Discoloured, unusually shaped, or otherwise unusual foods are looked at with distrust. Visual appearance, on the other hand, can change not only the acceptability of foods but also how their taste, odour, and flavour are perceived (Delwiche, 2004). Colour is one of the most important and fundamental characteristics of food because it can influence other sensory qualities and help establish a positive initial impression (Ngamwonglumlert *et al*., 2017; Verma *et al*., 2018). Food tastes better when it is presented in appealing colours. The colour of food items influences customer acceptance of those items more than any other element. Colour is frequently linked to quality and is used as a trustworthy indicator of a product's worth and safety. Sadly, the majority of food processing procedures, especially those that include heat, cause colour degradation or even colour loss. As a result, a variety of food colourants, such as pigments and dyes, have been utilized to improve or intensify food colour. Furthermore, colourants can be employed to ensure consistency in colour, enhance food presentation, or impart colour to otherwise colourless foods (Delgado-Vargas and Paredes-López, 2002; Mortensen, 2006; Ngamwonglumlert *et al*., 2017).

The food industry, cosmetics, pharmaceuticals, livestock feed, and other industries use food colour additives that are synthetic, natural (derived from plant, animal, or mineral sources), or nature identical (similar to those found in nature but developed by humans, such as synthetic carotenoids) (Oplatowska-Stachowiak and Elliott, 2015). The pigments extracted from the plants are being used extensively as colourants in foods, beverages and cosmetics since the consumer is showing interest towards plant-based sources which are undoubtedly safer and provide health benefits. Despite the century-long dominance of synthetic colours, nature continues to offer a wide range of visually appealing hues. The global demand for natural pigments is around 10,000 tonnes, or around 1 per cent of the global demand for synthetic colours (Singh *et al*., 2020).

In 2019, the market for natural food colouring additives was worth USD 4864.90 million. By the end of 2026, the market will be worth USD 5481.50 million, with a CAGR of 1.7% from 2021 to 2026. The market for natural food colours in India was valued USD 74.09 million in 2019. It is expected to grow by 3.90% CAGR between 2020 and 2027 to reach USD 92.96 million (Anon., 2021).

Plant pigments are divided into four groups based on how they naturally occur: chlorophylls, carotenoids, anthocyanins, and betalains (Ngamwonglumlert *et al*., 2017). Chlorophyll and carotenoids, which are found in the protoplasts of plants, are among those that are fat-soluble pigments. Anthocyanins and betalains, which are water-soluble pigments, are found in the cell sap (Boo *et al*., 2012).

Due to the increased awareness regarding the safety and established health benefits of naturally occurring pigments from fruits, vegetables, and edible flowers, consumers are tending towards the purchase and consumption of products that are enriched with the pigments from plant sources. And hence, an important factor of food processing is identifying the characteristics of plant pigments, their interactions, and their stability in different types of food during storage. This also presents an undiscovered opportunity to use the health advantages of these pigments in the production of novel naturally coloured food products.

Anthocyanins are widely available water-soluble flavonoids that can be found in a variety of flowers, fruits, and leaves (such as butterfly pea, red onion, grape, and purple maize). Anthocyanins vary in colour depending on pH, ranging from orange (pelargonidin) to red (cyanidin) to blue (beta-carotene) (delphinidin) (Delgado-Vargas and Paredes-López, 2002; Stintzing and Carle, 2004).

The butterfly pea, or *Clitoria ternatea* L., belongs to the Fabaceae family and has edible flowers that range in colour from dark blue to light blue to white. It is commonly grown in temperate and tropical areas of the world, including Central and South America, the Caribbean, Southeast Asia, and Asia. Flavonoids, particularly anthocyanins, which are produced from the fundamental classes of delphinidin, are the main pigment molecules in blue butterfly pea flowers. The coloured flowers are frequently employed as a natural source of food colouring in a wide range of dishes.

Tea brewed from the dried flowers of blue butterfly pea is a common herbal brew in Southeast Asia (Adisakwattana *et al*., 2020).

Mangosteen (*Garcinia mangostana* L.), a tropical fruit belonging to the Clusiaceae family, is generally regarded as the Queen of fruits. The fruits are smooth and spherical, 3.4 to 7.5 cm in diameter, bear 4 to 8 triangular, flat stigma remnants in a rosette at the tip, and are capped by a huge calyx at the stem end. The mature and ripe fruit is dark-purple to reddish–purple in colour. The rind (pericarp) is 6 to 10 mm thick and spongy, with a crimson exterior and purplish-white interior cross section. There are four to eight triangular segments of snow-white, juicy, soft, and delicious aril inside the pericarp that adhere to the seeds. The development in total anthocyanins is closely associated with the development of colour in the mangosteen pericarp. The two main cyanidin components of anthocyanins that are found to rise with fruit colour development are cyanidin-3-sophoroside and cyanidin-3-glucoside (Palapol *et al*., 2009).

Betalains are N-heterocyclic, water-soluble pigments (Delgado-Vargas and Paredes-López, 2002), and based on their structural differences, betalains can be divided into two groups: betaxanthins (a yellow pigment) and betacyanins (a red-purple pigment) (Chandrasekhar *et al*., 2015).

Basella (*Basella alba* var. *rubra* L.), also known as Malabar spinach, Indian spinach, vine spinach, and Ceylon spinach, is a member of the Basellaceae family and is a widespread ethnic green leafy perennial vegetable in tropical areas of the world (Deshmukh and Gaikwad, 2014; Natesh *et al*., 2021). It produces berries with rich red-violet pulp and a dark blue skin that contain betalains (Reshmi *et al*., 2012).

Amaranth (*Amaranthus tricolour* L.) is a plant that is extensively spread in warm and tropical areas of the world (Li *et al*., 2015; Rastogi and Shukla, 2013). Large amounts of betalains, such as betaxanthins and betacyanins, as well as flavonoids, alkaloids, and other substances are found in red amaranthus plants (Li *et al*., 2015). These substances have anti-oxidative, anti-cancer, anti-viral, anti-parasitic, and radical-scavenging properties and may be used to treat some oxidative stress-related disorders (Aguilera *et al*., 2016). Amaranthus plants are used instead of beet

because they can be grown in a wider range of environmental conditions and are therefore a more reliable source for the extraction of natural betalains (Cai *et al*., 2005).

Carotenoids are lipid-soluble pigments that give many fruits, vegetables, and flowers their vivid red, orange, and yellow hues (Mortensen, 2006). According to their functions, they can be classified as primary (-carotene, lutein, zeaxanthin, and antheraxanthin) and secondary (-carotene, lycopene, astaxanthin, and canthaxanthin) carotenoids (Delgado-Vargas *et al*., 2000).

Marigold (*Tagetes erecta*) is an annual plant claimed to be native to Mexico (Barzana *et al*., 2002). On a significant industrial scale, its flowers are commercially grown, collected, and processed as a source of high-value carotenoids. Lutein, a dihydroxylated compound, accounts for 85 per cent of the total carotenoids present in marigold flowers, making them one of the most significant sources of carotenoids for use in the food industry. Marigold flowers are the most concentrated and common source of carotenoids (Barzana *et al*., 2002; Philip and Berry, 1975).

Gac fruit (*Momordica cochinchinensis* Spreng) of Cucurbitaceae family is indigenous to Southeast Asia and consumed there for dietary as well as medicinal uses. According to reports, it is a good source of bioactive substances such as carotenoids, phenolic compounds, and flavonoids. (Chuyen *et al*., 2017). Due to its vibrant red colour from its high carotenoid concentration, the ripe fruit's seed membrane (seed pulp or aril) is frequently used as a rice colourant (Vuong *et al*., 2006).

Since the pigments are tightly bound to the cell membranes, the solid-liquid leaching process used to remove the pigments presents a mass-transfer problem (Sivakumar *et al*., 2011). The recovery of the desired pigments has been found to be significantly impacted by the extraction process. Innovative methods are required to improve the major pigment extraction mechanisms, including cell wall rupture, pigment release, and pigment transport into the external medium. The predicted pigment's quality and quantity can be improved by using the right extraction method. The extraction solvents used are also very important. There hasn't yet been a single

extraction method that can be applied to extract bioactive substances, particularly pigments, from a range of sources. So, there is a growing need for innovative extraction techniques that are quick, efficient, provide larger extraction yields, and most importantly, are safe for the environment (Manzoor *et al*., 2019; Roobab *et al*., 2018; Zia *et al*., 2020).

Because of consumer concerns about the safety of artificial food dyes and the potential health advantages of natural pigments, using natural colours is currently a promotional tool. However, it is difficult to replace synthetic colourants with natural ones since natural colourants are typically less stable, more expensive, require more material to attain the same level of colour strength, and have a smaller colour spectrum than synthetic colourants (Rodriguez-Amaya, 2017). With this background, the present study is formulated to evaluate the efficacy of different eco-friendly solvents in the extraction of anthocyanins, betalains, and carotenoids from plant sources and subsequently, application of the extracted pigment in different categories of food products to study their stability with the following objectives:

1. Standardization of the process for extraction of plant pigments from selected fruits (mangosteen), vegetables (red amaranthus, basella, gac fruit), and flowers (butterfly pea, marigold).
2. To assess extracted pigment application in food products.

Review of literature

2. REVIEW OF LITERATURE

Due to the increased accessibility of producer-to-consumer communication, the food sector has grown very quickly, and consumers' understanding of food has substantially increased. The issue of the use of colour additives in food is one of the subjects sparking a lot of debate. The producers focus on using natural pigments to satisfy consumer expectations because the additives obtained synthetically are finding it difficult to gain market acceptance, but replacing the artificial colours with natural ones comes with an array of difficulties with respect to pigment extraction, low stability, obtaining identical colour strength, *etc*. With this background, the present study was formulated to determine which eco-friendly solvents are most effective at extracting anthocyanins, betalains, and carotenoids from plant sources, and then to apply the extracted pigment to various categories of food products in order to study their stability. The reviews pertaining to the set objectives are presented hereunder.

2.1 Introduction to plant pigments

'You eat with your eyes first', according to a well-known proverb in food refers to the fact that unpleasant foods are commonly avoided. Foods that are oddly coloured, shaped, or otherwise distinctive are viewed with suspicion. Contrarily, visual elements can alter the perception of taste, odour, and flavour as well as the acceptability of foods (Delwiche, 2004). One of the most significant and fundamental aspects of food is colour, which can help create a favourable first impression and influence other sensory qualities (Ngamwonglumlert *et al*., 2017; Verma *et al*., 2018). Food tastes better when it is presented in appealing colours. The colour of a piece of food influences its consumer appeal more than any other component, possibly. Colour is usually used as a trustworthy indicator of product safety and value because it is frequently linked with quality. Unfortunately, the majority of food processing procedures lead to colour change and degradation, especially when heat is involved. As a result, many different food colourants (also known as pigments and synthetic colourants) have been utilized to improve or intensify food colour. Additionally, colourants can be employed to ensure colour uniformity, enhance food presentation,

or impart colour to otherwise uncoloured (Delgado-Vargas and Paredes-Lopez, 2002; Mortensen, 2006; Ngamwonglumlert *et al*., 2017).

2.1.1 Types of pigments

Chlorophylls, carotenoids, anthocyanins, and betalains are the different types of plant pigments based on natural occurrence. Chlorophyll and carotenoids found in the protoplasts of plants are fat soluble, whereas anthocyanins and betalains are water soluble, which are dissolved in the cell sap (Boo *et al*., 2012).

Considering that the pigments are firmly attached to the cell membranes, the solid-liquid leaching process used to remove the pigments presents a mass-transfer problem (Sivakumar *et al*., 2011). On the recovery of the desired pigments, it has been discovered that the extraction process has a significant influence. Innovative methods are needed to improve the key mechanisms of pigment extraction, including cell wall rupture, pigment release, and pigment transport into the external medium. By employing the proper extraction technique, it is possible to increase the recovery and quality of extracted substances. The extraction solvents that are utilised are also crucial.

To date, there is no one single extraction method developed that can be utilized to extract bioactive substances, particularly pigments, from different sources. Because of that, there is a rising need for novel and inventive extraction techniques that are quick, efficient, provide larger extraction recovery, and especially, are eco-friendly (Manzoor *et al*., 2019; Zia *et al*., 2020).

Due to recent publicity in the media, consumers are now aware that many naturally occurring pigments from fruits, vegetables, and edible flowers are safe, cleanly marked and also packed with health benefits, which they may impart when used as ingredients in processed foods (De Mejia *et al*., 2020). Therefore, an essential part of food processing is to know the characteristics of plant pigments, their interactions, and stability in diverse types of food. This also presents an unexploited opportunity to use the health advantages of these pigments in the development of new naturally coloured food products.

2.2 Anthocyanins

Ludwig Marquart, a German botanist, gave anthocyanins their original name in 1835, combining the Greek terms anthos (means flower) and kyanos (means blue), which had previously been used to refer to them as "coloured cell sap" (Boldt *et al*., 2014). Anthocyanins are now understood to be a class of naturally occurring, water-soluble, bioactive phenolic chemicals that are soucrce for many of the orange, red, violet, and blue colours found in nature (Pazmiño-Durán *et al.,* 2001).

Anthocyanidin (an aglycon base), sugar(s) (glycone), and organic acid(s), in the case of acylated anthocyanins, make up the basic structural components of anthocyanins. Cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin are the six anthocyanidins that have been found to be extensively distributed in plants. Almost 700 anthocyanins from diverse plants have been identified so far. (Clifford, 2000; Wu *et al*., 2006). Anthocyanins are found in many flowers, fruits, and leaves (*e.g.,* butterfly pea, red onion, grape and purple corn) with pH dependent hues ranging from orange (pelargonidin) to red (cyanidin) to blue (delphinidin) (Delgado-Vargas and Paredes-Lopez, 2002; Stintzing and Carle, 2004).

Anthocyanins are renowned for their potent antioxidant characteristics, which allow them to either directly, scavenge free radicals by providing them with the hydrogen atom or chelating free metal ions can indirectly stop them. It also offers additional health benefits such as hypoglycemic, antibacterial, anti-proliferative, *etc* (Gamage *et al*., 2021; Li *et al*., 2019).

The degradation of anthocyanins during food processing appears to be largely influenced by temperature. The use of anthocyanins in food systems is limited by their low stability under varied food processing, formulation, and storage conditions (Giusti and Wrolstad, 2003).

2.2.1 Butterfly pea flower

The plant, *Clitoria ternatea* L., belongs to the kingdom Plantae, the phylum Tracheophyta, the class Magnoliopsida, and the family Fabaceae (Jamil *et al*., 2018). The plant is a perennial climber that grows to a height of 2 to 3 metres. Its popular

names include butterfly pea and blue pea flower (Mukherjee *et al*., 2008). Other names for it include dangchan (Thai), cunha (Brazilian), kajroti (India) and bunga telang (Malaysian) (Kosai *et al*., 2015; Subramanian and Prathyusha, 2011). It is extensively found in Madagascar, South and Central America, the Caribbean, India, the Philippines, and other tropical Asian countries (Sivaranjan and Balachandran, 1994).

In Ayurvedic medicine, butterfly pea is regarded as a smart drug (Chauhan *et al*., 2017). It thrives under full sunshine or partial shade, and seed germination takes 1-2 weeks while flowering takes place over the course of four weeks (Jamil *et al*., 2018). There are numerous *C. ternatea* lines with 4-5 cm long flowers in a variety of colours, including light blue, dark blue, white, and mauve.

Nutritional examination of *C. ternatea* flowers showed that their moisture content was 92.4 % and that their percentages of protein, fibre, carbohydrate, and fat were 0.32, 2.1, 2.2, and 2.5 %, respectively. A high concentration of calcium (3.09 mg/g), magnesium (2.23 mg/g), potassium (1.25 mg/g), zinc (0.59 mg/g), sodium (0.14 mg/g), and iron (0.14 mg/g) were also reported in the flower (Neda *et al*., 2013).

2.2.2 Mangosteen fruit

Mangosteen (*Garcinia mangostana* L.) is belongs to one of the plants of the Guttiferae family. It is one of the best-tasting tropical fruits and is primarily grown in Southeast Asian nations including Thailand, Sri Lanka, India, Myanmar, Malaysia, and the Philippines. Due to the distinctive sweet-sour flavour of the edible pulp, which is primarily enjoyed fresh as a dessert, the fruit is also referred to as "the queen of fruits"; its dark red peel is thrown and viewed as a waste (Suttirak and Manurakchinakorn, 2012).

The peel constitutes 25–29 % edible flesh and more than 60–65 % of skin waste with 14 % dye waste (Cheok *et al*., 2018). Anthocyanins and antioxidant phenolic substances such phenolic acids, tannins, and xanthones are present in this fruit's peel (Zadernowski *et al*., 2009). The major anthocyanin in mangosteen was cyanidin-3-sophoroside (Du and Francis, 1977). Moreover, it has proven anti-

inflammatory, anti-tumor, anti-diabetic, and antibacterial activities (Gopalakrishnan *et al*., 1980).

2.2.3 Extraction of anthocyanin pigment

Extraction is a vital first step in extracting active compounds from plant sources (Jeyaraj *et al*., 2020). The highest yield with the highest concentration of the target compounds is the aim of selecting an efficient extraction procedure. A appropriate extraction technique must be used in order to obtain the greatest quantity of anthocyanin without causing any deterioration because anthocyanin are sensitive to heat, light, acids, and alkalis (Chandrasekhar *et al*., 2012; Jeyaraj *et al*., 2020).

When using traditional solvent extraction techniques, the extraction yield and total anthocyanin content (TAC) of an anthocyanin extract from blue pea flowers may be impacted by the kind of solvent, substrate: solvent ratio, extraction temperature, extraction time, and soaking time (Rocha *et al*., 2020). Depending on how the anthocyanin extract will be used, a solvent should be selected. Therefore, it is advisable to refrain from using potentially harmful chemical solvents while extracting anthocyanin from blue pea flowers for food applications (Chemat *et al*., 2019).

Hydro-alcoholic extraction has been employed in several studies to extract anthocyanin from blue pea blossom, including 37 per cent ethanol (Jaafar *et al*., 2020), 50 per cent ethanol (Pham *et al*., 2019), and 50 per cent methanol (Shen *et al*., 2019). However, the FDA (2018) has classified ethanol as a class III solvent that should be restricted by good manufacturing standards (GMP) and other quality-based regulations and methanol as a class II solvent with inherent toxicity. Distilled water is the ideal solvent for extracting anthocyanin for food applications because it is non-toxic, non-flammable, and economical (Chemat *et al*., 2019).

According to Nisha and Narayanan (2020), ethanol acidified with one per cent citric acid as test solvents has given higher yield with the mean and standard values of 89.37±0.24 mg/100 g cyanidin equivalent in mangosteen fruit. Ludin *et al*. (2018) reported that ethanol extract had the highest anthocyanin extraction efficiency, whereas ethyl ether extract has the least amount of effectiveness when they tested the

extraction effectiveness of anthocyanins using different solvents (high to low polarity). According to the study, ethanol is more effective in extracting polar chemicals like anthocyanins than solvents with lower polarity.

Plant materials are typically powdered, dried, or ground up before extraction in order to enhance the surface area for mixing with solvent. The majority of investigations on *C. ternatea* flowers used air or oven-dried, fresh flowers or ground/ powdered, dried flowers (Kamkaen and Wilkinson, 2009; Lakshan *et al*., 2019; Mehmood *et al*., 2019; Prado *et al*., 2019; Rabeta and An Nabil, 2013; Srichaikul, 2018). Some experiments (Chong and Gwee, 2015) used fresh flowers that were cleaned, cut into smaller pieces, and kept in a freezer at -25 °C for a month before being extracted. Other studies used freeze-dried flowers that were then crushed (Shen *et al*., 2016).

Response surface approach was used by Chaudhary and Mukhopadhyay (2012) to optimize the solvent for the extraction of anthocyanin from *Syzygium cumini* L. Skeels. The findings revealed that 20% ethanol in combination with 1% acetic acid could produce the maximum anthocyanin content (763.80 mg/100 ml), highest chroma (4.41), and largest hue angle (336.22) in the red colour spectrum.

According to Oancea *et al*. (2013), a discontinuous extraction method at temperature of 50 °C, extraction period of 2 h, with solvent containing 50 per cent ethanol (v/v), and shielding from light may be used to improve anthocyanin recovery from high bush blueberries (*Vaccinium corymbosum* L.). The samples under investigation showed a 20 per cent increase in anthocyanin levels after being kept at -18 °C for a prolonged period of time. According to Mai and Tan (2013), the optimal conditions for anthocyanin extraction from mangosteen rind was obtained when ethanol was used as solvent with 1.5 per cent HCl, 1:10 ratio of rind and solvent at 60 °C temperature for 40 min.

The use of non-conventional extraction techniques, such as pressurized liquid extraction (PLE), microwave assisted extraction (MAE), enzyme assisted extraction (EAE), and ultrasound assisted extraction (UAE), is more recent, highly effective, and environmentally friendly (Azmir *et al*., 2013; Wen *et al*., 2018).

In MAE, the release of solutes from the sample matrix into the solvent is facilitated by the conversion of electromagnetic energy to heat *via* the ionic conduction and dipole rotation mechanisms (Alupului *et al*., 2012; Jain, 2009). Sinha *et al*. (2012) compared MAE to conventional extraction using water and heating to determine which method was more effective at extracting the anthocyanin colour from *C. ternatea.* With a higher dye yield, MAE (3985 mg/L) proved more effective than traditional extraction (2022 mg/L). It was significantly quicker, taking only two minutes as opposed to the traditional extraction method's three hours.

2.2.4 Phytochemical properties of the anthocyanin pigment

A comparison of the anthocyanin pigment powder from purple sweet potato and butterfly pea with the best phytochemical properties was done in the study. The butterfly pea presented the best characteristics after a 10 % addition of maltodextrin, with a total anthocyanin concentration of 53.02 mg/L, colour intensity L^* (brightness) of 51.72, a^* (redness) of 23.50, b^* (yellow) of 8.42, water content of 2.87 per cent, hygroscopicity of 8.33 per cent, 97.33 per cent of solubility, 187 seconds of soluble time, pH value (Hariadi *et al*., 2018).

The bioactive components from *C. ternatea* flower were examined, identified, and isolated in a number of researches. Blue ternatin anthocyanins that are acylated on the basis of delphinidin are anthocyanins. The six main anthocyanins found in flowers are ternatins A_1, A_2, B_1, B_2, D_1, and D_2 (Mukherjee *et al*., 2008; Terahara *et al*., 1998). Fourteen flavonols, including kaempferol, quercetin, and myricetin glycosides, have been found in the petals (Kazuma *et al*., 2003; Mukherjee *et al*., 2008).

In several studies, the antioxidant activity of *C. ternatea* flowers was examined using a variety of antioxidant assays, including 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) radical scavenging, ferric reducing antioxidant power (FRAP), hydroxyl radical scavenging activity (HRSA), hydrogen peroxide scavenging, oxygen radical absorbance capacity (ORAC), superoxide radical scavenging activity (SRSA). Ascorbic acid (vitamin C) was shown to be more potent than 100 per cent methanol extract of *C. ternatea* flower extract in the DPPH assay

(Nithianantham *et al*., 2013), however the water extract was found to be less potent (Chayaratanasin *et al*., 2015).

Antioxidant activity of the extracts employing various solvents was tested and compared (DPPH assay), and at 15 min of extraction time, the water extract was found to be more effective than the 100 per cent ethanol extract (Kamkaen and Wilkinson, 2009). The optimal extraction period for the water extract, 100 per cent methanol extract, and 50 per cent methanol extract was found to be six hours, wherein the water extract and 50 per cent methanol were shown to be similarly powerful and to have a higher activity than 100 per cent methanol extract (Prado *et al*., 2019).

With the aid of MAE, antioxidants can be successfully and economically extracted from mangosteen peel using ethanol as solvent as the microwave power has a key role in the antioxidant conversion. The antioxidant conversions at 35 min and 300, 450, and 600 W (Watt) were 15.45, 17.00, and 18.33 per cent, respectively. The total phenolic content ranged between 156 and 202 mg GAE/g (Megawati *et al*., 2020).

DPPH radical scavenging activity was used to evaluate the antioxidant activities of mangosteen peel extracts (ethanol 7 %), which have been prepared in oral solution dosage forms. The active ingredients in mangosteen peel, called xanthones, are not soluble in water; as a result, co-solvents like polyethylene glycol (PEG) 400-glycerol (20-20, 20-40, 40-20, and 40-40) were used to increase their solubility. The maximum DPPH radical scavenging activity was found in an oral solution with a co-solvent ratio of PEG 400: glycerol (40:40), with an IC_{50} of 24.81 g/ml (Sumarny *et al*., 2014). Due to the inclusion of co-solvents in the formula but without any activity as radical scavengers, the IC_{50} achieved was in fact lower than that of its extract.

2.2.5 Application of anthocyanins in food products

Anthocyanins, a group of water-soluble, bioactive flavonoids are present throughout all plant species and give plant tissues their pink, red, purple, and blue hues (Grotewold, 2006). Anthocyanins are a safe, incredibly nutritive, and versatile food colouring additive. Anthocyanins, however, are extremely unstable in nature

and are quickly destroyed by external factors such as temperature, pH, light, oxygen, enzymes, metal ions *etc* (Escher *et al*., 2020).

During the processing of food, extreme pH and temperature conditions are commonly encountered, and discoloration and anthocyanin degradation usually happen while the food is being stored and processed (Zang *et al*., 2021). The preservation of anthocyanins is significantly hampered by the aforementioned stability related problem (Fu *et al*., 2021).

Albuquerque *et al*. (2020) improved a heat- and ultrasound-assisted ANC-rich (anthocyanin) extract from jaboticaba [*Myrciaria jaboticaba* (Vell.) Berg] epicarp, for use as a natural colourant in French macarons production. The delphinidin-3-O-glucoside and cyanidin-3-O-glucoside (C_3G) levels indicated that heat-assisted extraction was the most successful extraction method. Up to six days of storage (13 min at 130 °C, conventional oven), the prepared macarons retained their lightness (L^*), redness (a^*), and yellowness (b^*), and little changes in glucose, fructose, and sucrose were observed throughout the same evaluation period.

Jiménez-López *et al*. (2019) assessed the feasibility of adding a C_3G extract from *Arbutus unedo* L. fruits to wafers. An optimised heat-assisted extraction was used to produce an antioxidant C_3G-rich extract with antioxidant potential, with a *Salmonella enteritidis* minimum inhibitory concentration (MIC) of 150 g/mL and a *Salmonella typhimurium* minimum bactericidal concentration (MBC) of 200 g/mL (DPPH half-maximal effective concentration, EC50: 295 g/mL). The extract was most stable when the temperature was below 20 °C. In comparison to untreated wafers, the manufactured product had a golden hue, contained more sucrose, had greater quantities of fatty acids (palmitic, stearic, and linoleic acids), and had better antioxidant activity.

Rubus ulmifolius Schott has been investigated as a novel source of food colourant used in baked products (Da Silva *et al*., 2019). When a heat-assisted extraction yielded an ANC-rich extract, the principal identified ANC (cyanidin-O-di-hexoside, C_3G, Pr_3G, cyanidin-3-O-xyloside, and cyanidin-3-O-dioxayl-glucoside) were used as responses for a response surface analysis. Thirty three mg of ANC were

present per gram of the reddish-burgundy extract. When the addition was used, the donuts' lightness and yellowness (*b**) decreased (by 24.34 to 25.97 % and 44.67 to 48 %, respectively), while their redness increased (+109 to 338.67 %) in comparison to control. The designed donuts also had lower levels of carbohydrates and calorie value, greater levels of free sugars, and no variations were discovered in the amount of free fatty acids.

Coloured tubers like purple-fleshed sweet potato (*Ipomea batatas* L.) coloured flours were used in biscuit recipes (Aziz *et al*., 2018). The flour contained substantial levels of total phenolic content (TPC) (80.89 mg GAE/100 g) and TAC (38.90 mg C_3G equivalents/100 g), despite the absence of colourants in the raw materials. Potato-supplemented biscuits outperformed control biscuits in terms of TPC and TAC, despite the fact that baking (160 °C, 20 min and electric oven) resulted in a loss of 67.24 per cent TPC and 27.79 per cent TAC. High FRAP and DPPH levels were generated by the improved nutritional composition.

Black rice flour was used in place of some of the wheat flour in the muffins made by Croitoru *et al*. (2018). The 50 and 100 per cent black rice formulations exhibited significant TPC, total flavonoids (TF), and TAC concentrations compared to muffins manufactured simply with wheat, which were reflected in their antioxidant capacity. Overall acceptance of the innovative muffins was unaffected, showing the potential of colourful additives to improve the nutritional composition without detracting from the sensory qualities.

Anthocyanins were extracted from *Phaseolus vulgaris* L. black bean seed coat using water at 40 °C for 4 h, then centrifuged, filtered, and kept at -20 °C. For pH adjustment, citric acid was utilised. Extracts were added to 250 ml of a commercial sports drink with glacier cherry taste at concentrations of 0.1 mg/ml or 0.26 mg/ml. The concentration of β-cyclodextrin was then increased to 2 g/100 ml. In comparison to commercial sports drinks, beverages with anthocyanin extract added had a longer half-life, were co-pigmented with β-cyclodextrin, were equivalent in lightness, had a lower *a**, and higher *b** (Aguilera *et al*., 2016).

Lakshan *et al*. (2019) developed a beverage with beneficial qualities using blue pea flower extract. For this experiment, healthy, unblemished blue pea flowers were dried in an oven at 50 °C for 24 h. The dried flowers were ground in a mechanical grinder for five minutes, sieved through a one-millimeter sieve, and stored at room temperature in sealed, airtight low-density polyethylene bags (300 gauge). According to the findings, three gram of powdered blue pea flower/L of distilled water at 59.6 °C for 37 min was the ideal extraction procedure for blue pea flower utilizing response surface methodology (RSM). Total phenolic content (mg GAE/L of sample), TF content (mg QE/L of sample), ferric reducing antioxidant power (mg TE/L of sample), DPPH radical scavenging activity (mg TE/L) and $ABTS^{+}$ (mg TE/L) radical scavenging activity of blue pea flower extract (BFE) were 80.17, 42.75, 15.39, 35.92 and 192.14, respectively.

Mangosteen peel extract was used to extract anthocyanin, which was then used to create a drink with useful properties. Mangosteen peel extract was added to the mixture in amounts of 10, 20, 30 and 40 per cent. Antioxidant activity, total microbiological throughout storage, an organoleptic test, and the stability test using anthocyanin as a colouring agent were characterized. On the second day of storage, the antioxidant activity was 78 per cent, and the log total microorganism was 2.62. Anthocyanin was comparatively constant at the storage temperatures of 5 and 25 °C utilized (Suttirak and Manurakchinakorn, 2012).

According to Abdel-Moemin *et al*. (2016), cupcakes with roselle (*Hibiscus sabdariffa* L.) calyces (20 g) extract per 100 g of cupcake had an improved chemical composition and received good overall like scores. Dry calyces were used to create a fine powder (0.55 mm) that was then combined with water and cooked for one hour (80 °C) to create this functional extract. The formulated cupcakes had less total carbohydrates (11.28 %), lipids (16.48 %), and higher dietary fibre (126.18 %) and ash (179.68 %) than the control cupcakes (without roselle). Moreover, the addition of roselle maintained 77 per cent of the anthocyanin content from the dry calyces (435 mg/100 g cyanidin-3-glucoside) in cupcakes, potentially providing up to 32 times the daily allowance for Americans (12.5–215 mg). Although the surface Maillard reactions that developed during the baking process (175 ºC for 20 min) gave the

finished cupcakes a crust and crumb pink colour, no differences were found for the sensory evaluation of the colour, appearance, texture, taste, volume, and aroma compared to control cupcakes, but they were less well-liked.

Since butterfly pea flower extract has high antioxidant activity and contains bioactive compounds, the addition of 10 per cent butterfly pea flower extracts showed an increase in antioxidant activity in fermented beverages made from all types of plant-based milk (almond, soy, and combination of almond and soy milk) (Lakshmi *et al*., 2014).

2.3 Betalains

The seeds, fruits, flowers, leaves, stems, and roots of the Amaranthaceae, Cactaceae, and Chenopodiaceae families contain betalains, which are secondary nitrogenous metabolites. These plants benefit due to their antioxidant, anti-cancer, anti-lipidemic, and antibacterial capabilities (Gengatharan *et al*., 2016; Otálora *et al.*, 2019). They also impart a distinctive red-yellow pigmentation.

The presence of hydroxyl groups (-OH) on the structures of betalains results in charge polarisation and the creation of hydrogen bonds, which gives them their high hydrophilicity (Fathordoobady *et al*., 2016).

Red-violet (betacyanins) and yellow (betaxanthins) betalain pigments are water-soluble pigments and in the majority of plant families of the order Caryophyllales, these pigments replace anthocyanins (Cai *et al*., 2005). The only plants in the order Caryophyllales that contain betalains are those in the family Amaranthaceae, which contains several significant genera such as Amaranthus, Celosia, Gomphrena, and Iresine (Bhattacharyya and Johri, 1998).

2.3.1 Basella

Basella alba var. *rubra* also known as Malabar spinach, is an underutilized plant with great food and medicinal potential, belongs to the family Basellaceae, which are most frequently grown in Asia, have branched, ascending stems with alternately mucilaginous and succulent leaves. They put forth tiny, dark violet-red

stone fruits throughout the summer that are rice source of betalains along with carbohydrates, proteins, lipids, niacin, ascorbic acid, tocopherols (Sutor-Świeży *et al*., 2022).

2.3.2 Red amaranthus

Amaranth (*Amaranthus tricolor* L.) plants are widely distributed in warm and tropical regions worldwide (Rastogi and Shukla, 2013). In addition to flavonoids, alkaloids, and other compounds with anti-oxidative, anti-cancer, anti-viral, anti-parasitic, and radical scavenging properties, red amaranthus plants also contain significant amounts of betalains, including betaxanthins and betacyanins (Liu *et al*., 2019). These compounds may be useful for treating specific oxidative stress-related disorders (Aguilera *et al*., 2016). Amaranthus plants are used instead of beet because they can be grown in a wider range of environmental conditions and are therefore a more reliable source for the extraction of natural betalains (Cai *et al*., 2005).

2.3.3 Extraction of betalain pigment

The methods established for betalains' extraction from various natural sources include the use of water, methanol- and ethanol-water combinations in various ratios and ethyl acetate, since betalains are hydrophilic (Calva-Estrada *et al*., 2022). It should be noted that the final extraction product's applicability must be considered when choosing the best solvent for extracting any phytochemical because, if it is to be used as a food additive, certain alternatives (such as methanol and ethyl acetate) are not recommended due to their potential toxicity. In view of this problem, it is recommended to use solvents like water, ethanol, and mixes of these solvents.

Due to the nucleophilic attack of ethanol on the aldimine bond (N = CH) of betalains resulting from their breakdown *via* decarboxylation, the extraction of betacyanins with water has demonstrated improved efficiency compared to aqueous ethanol solutions (Das *et al*., 2019; Sanchez-Gonzalez *et al*., 2013).

Selecting the right liquid/solid ratio, pH, temperature, and time, in addition to a suitable solvent, has been proven to considerably increase the effectiveness of betalains extraction (Kumar *et al*., 2017; Zin *et al*., 2020a). The most efficient method

for extracting betalains from *Bougainvillea glabra* floral bracts is an aqueous methanol solution (Kumar *et al*., 2017). Employing an extraction temperature of 20 °C, Zin *et al*. (2020b) found that an increased betacyanin and betaxanthin extraction yields from red beetroot (*Beta vulgaris* L.).

The types and quantities of chemicals extracted from the material depend on the ratio of water to ethanol; for example, extracts with higher ethanol concentrations have higher phenolic and betaxanthin contents, while extracts with lower and higher ethanol concentrations have higher betacyanin concentrations (Thiyajai and Koyama, 2022). Neagu and Barbu (2014) found that the pH affects the extraction process favourably when it is carried out at 20 °C as against the 70 °C temperature, when the pH has no effect. With the pH of medium being lowered to 5, red amaranth (*Amaranthus cruentus*) yields for betalains extraction increased (Das *et al*., 2019).

Plant parts containing betalain are often crushed or macerated. Water can be used to extract pigments, although methanol or ethanol solutions (20-50 %) are typically required to complete the extraction process (Delgado-Vargas *et al*., 2000). However, according to Castellar *et al*. (2006), water was able to extract more pigment from Opuntia fruits than ethanol:water. According to Barrera *et al*. (1998), ethanol-HCl had a higher betalain extraction rate than water (v/v ratio, 99:1). However the pigments' stability was improved by the aqueous extraction. A slight acidity of the extraction media improves betacyanin stability and prevents polyphenoloxidases from oxidising it (Strack *et al*., 2003).

Total betalain content of *Basella rubra* L. fruits was evaluated by Pawar *et al.* (2018) and it was reported that the fruits contained high amount (48.15 mg) of betacyanins and sparing amounts (2.53 mg) of betaxanthins and the total betalains accounted for 50.69 mg/100g of fresh fruit pulp.

2.3.4 Phytochemical properties of betalain pigment

The two structural components of betalains, a phenolic and a cyclic amine are efficient electron donors and give them their antioxidant properties (Kanner *et al*., 2001). The antioxidant and antiradical characteristics of betanin have all been

evaluated and explained using its electron donor capacity, bond dissociation energy, and ionisation potential (Gliszczyska-Swiglo *et al*., 2006). Betalains, from a structural standpoint, are immonium derivatives of betalamic acid that contain an aromatic amino component that can stabilise radicals. This stability is closely related to betalain's capacity for electron donation (Slimen *et al*., 2017).

The levels of betalain in the flowers/bracts of *Amaranthus* spp., ranged from 0.95 to 6.02 mg/100 g (Li *et al*., 2015). Inhibition of the radicals ABTS and DPPH by *Bougainvillea* spp., was shown to be 72.68 and 61.24 per cent and 116 mg of betaxanthines/100 g, respectively (Orozco-Villafuerte *et al*., 2019). The red dragon fruit peel's reducing power (measured by FRAP) and antioxidant activity (measured as an inhibition percentage by the ABTS assay) were each 3.04 per cent and 200.83 mol Fe2 +/g, respectively, however there was only a weak relationship between the two (Ramli *et al*., 2014).

The level of betalains in Amaranthus species and the antioxidant activity as determined by the FRAP method (0.63-62.21 mmol AAE/g) and ORAC (30.67-451.37 mmol TE/g) had a significant positive correlation (Li *et al*., 2015). The results showed that the antioxidant activities of *Amaranthus hypochondriacus* leaves were much higher than those of the species' seeds, flowers, stems, and fruits (Li *et al*., 2015).

There were moderate to strong correlations between betaxanthin (BX) and antioxidant activities (FRAP assay: r = 0.885 and 0.917; DPPH assay: r = -0.673 and -0.729; ORAC assay: r = 0.926 and 0.920 (p 0.01 or 0.05) for dopamine-BX and 3-methoxytyramine-BX, respectively). Phenolic compounds were the major contributor (Thiyajai and Koyama, 2022).

2.3.5 Application of betalains in food products

Betacyanin and its derivatives were extracted from beetroot (*Beta vulgaris* L.) pomace, further encapsulated, and employed in wheat einkorn (*Triticum monococcum*) water biscuits that were enhanced with pseudocereals (amaranth, buckwheat, and quinoa). In contrast to control biscuits, all extract-added biscuits

displayed a dose-dependent rise in betanin, isobetanin, and derivatives of betanin (5.7, 10.4, 14.9, and 10.8 % extract addition), independent of the presence of the pseudo-cereal. The maximum TPC value was found in buckwheat (*Fagopyrum esculentum*) biscuits (2500 mg GAE/kg dry matter, DM). The highest levels of furosine were found in quinoa (*Chenopodium quinoa*) biscuits (275 mg/100 g protein), which may indicate that the encapsulated extract for this ingredient is less effective at preventing heat damage (Luzardo-Ocampo *et al*., 2021).

Betalains from *Basella rubra* can be used as a colouring agent for banana spread, which has a stability of 95 per cent after a year of storage at 5 °C, for an intermediate moisture food (creating a gel-like product), which has a stability of 60 per cent after two months, and for juices, bananas, and lemons, which have a stability of 58 and 76 per cent, respectively, after three months. These revelations resulted from Kumar *et al*. (2020). Each time, the proposed colour inhibited the growth of microorganisms and provided a product that was well-liked by customers.

Betaxanthins from yellow pitaya (*Stenocereus pruinosus*) fruit were used by Rodriguez-Sánchez *et al*. (2017) as a colour for jelly candies. It was discovered that when the product was kept in low temperatures and darkness, the betaxanthin stability was at its highest. In addition, scientists discovered that the gummies' food matrix (which offered a protective effect due to their interactions with proteins) and low water activity made these pigments more stable. Pitaya betaxanthins (betalain-type pigments) extracts in a model yellow beverage, it was found that they produced a range of colours of yellow-orange and greatly dispersed chroma (21.38-87.78) and hue (53.9-87.8) values. However, the beverages with the closest colour resemblance to their commercial equivalents were those coloured with five per cent pitaya juice. The first nine days of storage showed up to 75 per cent of the total betaxanthins being retained by beverages that had been made. The ability of this pigment to replace synthetic yellow colours in commercial beverages was suggested.

Betalains from berries (*Rivina humilis*) were used by Khan *et al*. (2015) as a colouring agent for fruit spread and banana juice. It was found that the stability of the betalains in the fruit spread was less than 40 per cent after six months of storage at 5

°C. The absolute loss of betalamic colour during pasteurisation rendered the intended colourant inert in the beverage. Betalains have been also used as colourants in ice cream, which increased consumer acceptance of the product and provides high colour stability for 180 days when stored at 20 °C (Kumar *et al*., 2015). Betalains from cactus pears were used as natural colours in dairy products (yoghurt and cream) by Coria-Cayupán and Nazareno (2015), who observed a greater than 80 per cent prevention of oxidative damage in yoghurt and a 50 per cent inhibition in cream during the oxidation of the systems without the additional pigments.

In a study by Attia *et al*. (2013) who examined the sensory effects of red beet extract added to jelly and ice sherbets, found that the products' overall acceptability depends on the amount of betalains added and on characteristics similar to those of a synthetic red colourant.

Cai and Corke (1999) evaluated the colour properties and stability of commercial colourants and amaranthus betacyanins in model food systems at various temperatures. The red colour of betacyanins was more intense than that of red radish anthocyanin. Between 14 and 25 °C, the colour stability of both pigments was comparable, but at 37 °C, betacyanin's colour was less stable than red radish anthocyanin's. Moreover, a synthetic colourant was examined, and it was generally more stable than betacyanins.

2.4 Carotenoids

Many of the brilliant red, orange, and yellow hues of fruits, vegetables, and flowers are produced by lipid soluble pigments called carotenoids (Mortensen, 2006). According to their function, they can be classified as primary (β-carotene, lutein, zeaxanthin and antheraxanthin) and secondary (α-carotene, lycopene, astaxanthin and canthaxanthin) carotenoids (Delgado-Vargas *et al*., 2000). Carotenoids are isoprenoids that are abundantly present in foods that have traditionally been a staple of the human diet. Some carotenoids can be transformed into retinoids that exhibit vitamin A activity, which is necessary for humans, unlike the other so-called dietary bioactives. Additionally, they are far more adaptable due to their inclusion in food as

natural colours, antioxidants, and health-improving substances in addition to serving as sources of vitamin A.

2.4.1 Gac fruit

Gac fruit (*Momordica cochinchinensis* Spreng.) is a tropical vine originating from South and South-East Asia and belonging to the Cucurbitaceae family. It is known as the Fruit of Heaven in Vietnam, a moniker that honours the fruit's mythological past. It is referred to by a variety of names in various publications around the world, including Tu Mu Bie in China, Fakkao in Thailand, Kakrol in Bangladesh, Bhat Kerala in India, Makkao in Laos, and Gac as a common term in Vietnam, among others.

Gac fruit has reportedly been shown to be a high source of bioactive substances, such as carotenoids, phenolic, and flavonoid compounds (Chuyen *et al*., 2017). Due to its bright red colour from its high carotenoid concentration, the seed membrane (also known as the seed pulp or aril) of the ripe fruit is frequently used as a rice colourant (Vuong *et al*., 2006).

2.4.2 Marigold

Marigold (*Tagetes erecta*) is an annual plant reported to be native to Mexico (Barzana *et al*., 2002). As a source of high-value colourants from the carotenoids family, it is commercially grown, collected, and processed from its flowers on a large industrial scale. Lutein, a dihydroxylated molecule, accounts for 85 per cent of the total carotenoids present in marigold flowers, making them one of the most significant sources of carotenoids for use in the food industry. Marigold flowers are the most concentrated common sources of carotenoids (Barzana *et al*., 2002; Philip and Berry, 1975).

2.4.3 Extraction of carotenoid pigment

Organic solvent extraction is a common procedure in the food and pharmaceutical industries. The technique works well, although it necessitates using and discarding organic solvents. Remaining traces of any harmful organic solvent

used to extract the carotenoids will render the extract unsafe for consumption by people (Sapkale *et al*., 2010).

To determine the solvents that extract the highest amounts of lycopene and β-carotene from gac aril fruit powders, Saadedin *et al*. (2017) studied three different organic solvent mixtures: ethanol/ethyl acetate (6:4), hexane/ethyl acetate/ethanol (2:1:1), and hexane/acetone/ethanol (2:1:1). The results showed that the best extraction efficiency for lycopene (40640 g/g) was from the mixture of ethanol and ethyl acetate (6:4), while the best extraction efficiency for β-carotene (2912 g/g) resulted from the combination of hexane, acetone, and ethanol (2:1:1).

In order to maximize the recovery of lycopene from tomato pomace, Pandya *et al*. (2017) optimized the solvent extraction process by choosing the appropriate solvent system, temperature-time combination, and feed-to-solvent ratio *i.e*., Acetone: Ethyl acetate (1:1), 40 °C/ 5 hr and 1:30 (w/v). The highest amount of lycopene, which had a refractive index of 1.37604, a colour value of 5.59 *L**, 8.00 *a**, and 6.14 *b**, and a lycopene content of 611.105 mg/100 g, was extracted utilizing an improved solvent extraction procedure.

Chuyen *et al.* (2017) reported the optimal extraction time, temperature and solvent to the solid ratio for carotenoid extraction in gac fruit peel as 150 min, 40.7 °C and 80 mL/g, respectively which yielded 271mg/100 g on dry weight basis. Recent studies have examined the effects of extraction time, temperature, and ultrasonic power on the recovery of total carotenoids from gac peel using a response surface technique approach. An extraction yield of 269 mg/100 g dry weight was achieved utilizing a 76 min extraction period, 50 °C and 250 W (Chuyen *et al*. 2019).

Ethyl lactate (ethyl 2-hydroxypropanoate) is a lactate ester. Lactate esters are utilized as food additives as well as in biochemicals, pharmaceuticals, and cosmetics. Ethanol lactate is a solvent that is favourable to the environment since it satisfies eight of the twelve purported requirements of "green chemistry" (Pereira *et al*., 2011). Through the fermentation of a feedstock comprised of carbohydrates from the corn and soybean industries, ethanol lactate is produced. The European Union (EFSA, 2012) and the U.S. Food and Drug Administration have approved its use in food

products (FDA, 2014). Due to the wide variety of polarity of the various families of polyphenols, which vary from benzoic and cinnamic acids to quercetins and other flavonols, it is miscible with both hydrophilic and hydrophobic molecules and is hence, potentially an useful solvent for plant phenolics (Lores *et al*., 2015).

Ishida and Chapman (2009) reported that ethyl lactate as an excellent solvent for extracting food grade lycopene. The maximum amount of total lycopene extracted from red tomato was obtained at 60 °C, using ethyl lactate with added antioxidant. Kua *et al*. (2014) reported that as an effective solvent, ethyl lactate is capable of dissolving in both aqueous (polar) and hydrocarbon (non-polar) environment. Hence, it has the potential to recover compounds with a wide range of polarity without the presence of co-solvent from crude palm oil. It was demonstrated the use of ethyl lactate and ethanol as green and safe solvents to extract phytonutrients such as carotenes and tocols from crude palm olein (CPO) before they are lost during oil refining process (Kua *et al*., 2018).

Pataro *et al*. (2020) studied the extraction of lycopene from tomato pomace using an electric field. The experiment used two solvents: acetone, a popular compound used in carotenoid extraction, and ethyl lactate, which has a low environmental impact. It was observed that the most important parameter in the process was the extraction time used; the most optimal time was determined to be 240 min. The application of an electric field in the extraction process significantly improved the efficiency of the process. Additionally, in the case of ethyl lactate, higher amounts of all-trans lycopene of about 23 per cent (for acetone, it was 18 %) were observed, whose presence stabilizes and intensifies the colour of the extract. After 40 min of novel assisted extraction, UAE at 20 and 60 °C revealed that water extract had the highest concentrations of total phenolics. Remarkably, phenolic compound production from ethyl lactate extract at 20 °C was higher than that from ethanol and isopropanol extracts (El-Malah *et al*., 2015).

Nie *et al*. (2021) opined that the ethyl lactate had similar efficiency to ethanol/acetone in the extraction of fucoxanthin from *Sargassum fusiforme,* edible brown macro-alga rich in carotenoid content, and therefore could be used as a

substitute for conventional solvents. Szabo *et al*. (2022) reported that the highest values for lycopene (1324.89 µg/g DW) were obtained when ethyl lactate was applied as a solvent, followed by ethyl acetate with slightly smaller differences (1313.54 µg/g DW) in tomato processing by-products.

2.4.4 Phytochemical properties of carotenoid pigment

The edible red aril (seed membrane) of gac fruit contains a very high concentration of carotenoids including lycopene and β-carotene. It was reported that the β-carotene content in gac aril is five times greater than the levels measured in carrots, and the lycopene content eight times greater compared to the levels measured in tomatoes (Aoki *et al*., 2002). The greatest antioxidant activities of ethanolic extract from the aril of ripe gac fruit were 4.87 mg AAE/g fresh weight (FW) and 0.016 mg AAE/g FW compared to peel and pulp extract when examined by DPPH and FRAP (Tinrat *et al*., 2014). Bharathi *et al*. (2014) reported that 500 g of fruit samples collected at 25-day maturity post-pollination contained 45.06 mg ascorbic acid equivalent (AAE)/100 g by using DPPH assay and 5.84 mg ascorbic acid equivalent antioxidant capacity (AEAC)/ 100 g by using FRAP assay.

The FRAP values of differently dried materials of marigold indicated that combination of far-infrared radiation with hot air convection (FIR-HA) drying had the greatest reducing power (972.7 µmol FeSO4/g DW), followed by fresh (821.0 µmol FeSO4/g DW), freeze drying (FD) (811.0 µmol FeSO4/g), and then hot-air (HA) (730.7 µmol FeSO4/g DW). The FIR-HA dried gave high FRAP values that may be due to their higher levels of TPC, TFC, lycopene, β-carotene and lutein. The increase in antioxidant activity of the thermally processed marigold could be explained by the increased amount of lycopene, β-carotene and lutein, a major phytochemical in marigold, and other bound phytochemicals released from the matrix with thermal processing (Siriamornpuna *et al*., 2012).

The antioxidant activity of a gac aril powder, which was prepared with 10 per cent malto-dextrin by spray drying at 120 °C to a moisture content of 4.9 per cent, was shown to be equivalent to 1.4 mmol Trolox per gram in an ABTS assay (Kha *et al*., 2010). Gac aril and products from gac aril have been demonstrated to have very

high antioxidative activity because of their extremely high levels of carotenoids, especially lycopene. Lycopene has been reported as one of the most bioactive carotenoids which contribute to a variety of health benefits by having anticancer, cardio-protective and anti-inflammatory effects (Mordente *et al*., 2011).

2.4.5 Application of carotenoids in food

Gac fruit has potential to be utilized by the pharmaceutical, cosmetic, and food industries. It is processed for components to be used as ingredients or fortification. Recently, commercial gac products such as frozen aril, puree, gac oil, dried gac aril powder, and gac juice have been introduced into the market. Food manufacturers could take the bulk of gac fruit and utilize it in a range of mainstream food such as fruit bars, yogurt, breakfast cereal, or in specific health food such as juices, jams, natural colourants, and health supplements.

Kumkong *et al*. (2020) reported that gelatin gummy jelly is a chewable snack with attractive synthetic colour and flavour. The use of natural carotenoid colourant, found in gac aril or pulp, potentially benefits consumer health. The prototype containing gelatin, sucrose, and glucose syrup at 10, 50, and 40 per cent, respectively, was selected based on its hardness, gumminess, and chewiness values. The addition of whey protein concentrate (WPC) (0.75 %) to the selected prototype increased the values of hardness, springiness, and gumminess but reduced the values of cohesiveness and chewiness. Coloured WPC-mixed gelatine gummy with blends (0.5 g/100 g) of freeze-dried (FD) gac aril and pulp at a ratio of 75:25 appeared yellow-orange and received the highest acceptance score. The quality of coloured WPC-mixed gelatin changed to a dull colour and a softer texture gel during storage.

Yellow bell pepper pigments were coated with cyclodextrin by Lobo *et al*. (2018) to test their stability in isotonic beverages (pH: 2.9; addition of extracts of 0.02, 0.05 and 0.06 %). The key carotenoids identified were lutein, zeaxanthin, β-cryptoxanthin, and β-carotene. Drinks with extract added showed a dose-dependent increase in brightness and redness but a decrease in yellowness. There were no differences in the beverages' lightness over time (21 days), although the yellowness considerably decreased.

In a study by Alim-un-Nisa *et al*. (2018), colour was extracted from *T. erecta*, which gave a lemon yellow colour shade. When applied to food products, the lemon yellow colour boosted the attractiveness of the food. With the aid of a spectrophotometer set at 474 nm, the stability of the extract in both its raw form and as candies was evaluated. At 4 °C, the colour was shown to be stable in both its crude and candy forms. At higher temperatures, such as 25 and 45 °C, the colour indicated deterioration in the mean lutein concentration. The microbiological investigation of the crudely extracted colour from *T. erecta* and the candies that were dyed with it revealed that the colour, both in crude form and in the candies, had antibiotic activity. Since no clinical symptoms were seen in rabbits after receiving the maximal dose of 1000 ml of extracted colour, the lethal dose and toxicity analysis of the marigold colour extraction revealed that it is safe for consumption.

Lycopene was obtained from tomato waste and utilised in cakes and biscuits. Lycopene from tomato waste (fibrous pulp without peel or seeds) has 300.85 mg of lycopene per 100 g and 654.8 mg of total carotenoids. Oil from the recipe for the cake and butter from the ingredients for the cookies were substituted out for 1, 3, and 5 per cent lycopene, and the finished products were assessed. Cakes containing lycopene displayed a dose-dependent increase in volume, higher DPPH inhibition, and increased lightness in the crust and crumb, however only 5 per cent of the formulation displayed a higher volume than control cakes (without lycopene). The cakes' colour and texture in the crust and crumb were significantly different from the control cakes According to sensory evaluation (the cakes appeared to have more yellow and redness). However, there were no variations amongst panellists in terms of general acceptability, taste, or odour. The results for the lycopene-added cookies were the same (Eletr *et al*., 2017).

Domingos *et al*. (2014) incorporated lutein as a dye to yoghurt and assessed both the yogurt's oxidative stability and lutein's stability. Spectrophotometer was used to quantify the amount of lutein in yoghurt that was refrigerated. It was discovered that the lutein concentration of samples maintained in light and darkness remained the same.

Materials and methods

3. MATERIALS AND METHODS

The present investigation, "Evaluation and utilization of plant pigments as natural food colourants" was carried out at the Department of Postharvest Management, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur, Kerala, during 2019-22. The materials and methods adopted in the study are furnished below.

3.1 Materials

The present study focused on the extraction of anthocyanin, betalain and carotenoid pigments. Two crops for each pigment group were taken: butterfly pea flowers and mangosteen fruit rind for anthocyanin pigment; red amaranthus leaves and basella berries for betalain pigment; gac fruit seed aril and marigold flower petals for carotenoid pigment.

Fresh butterfly pea flowers (dark blue-dark purple double type) were harvested from the Medicinal and Aromatic field block, College of Agriculture, Vellanikkara. Fresh mangosteen fruits (local type) were collected from college orchard, Dept. of Fruit Science, College of Agriculture, Vellanikkara. Fresh red amaranthus (Arun variety) was procured from the local market the of Thrissur district. Ripe basella berries (local type) were collected from well maintained field of Mr. Nithin Gowda, Bengaluru district, Karnataka. Gac fruits (local type) were freshly harvested from the field of a progressive farmer Mr. Radhakrishanan, Irinjalakuda, Thrissur district. Marigold flowers (local type) were collected from the local market of the Thrissur district.

3.2 Methods

The study was conducted under three experiments with two sections. The first section of each experiment was standardization of extraction method for anthocyanins, betalains and carotenoids, whereas the second section of each experiment was the evaluation of anthocyanin, betalain, and carotenoid pigment stability in processed products.

3.3 Standardization of extraction method for anthocyanins

3.3.1 Collection and preparation of butterfly pea (*Clitoria ternatea* L.) flowers

Fresh butterfly pea flowers were collected in the early morning at the Medicinal and Aromatic field block, College of Agriculture, Vellanikkara, and were washed with running tap water and drained free of water. Petals were separated from the sepals and dried in a cabinet drier at 40±2 °C until a constant weight was obtained, then the dried petals were ground in a commercial blender and sieved through an 80 mesh size sieve. Later, the petal powder was packed in an aluminium foil laminated pouch and stored in a freezer until pigment extraction.

3.3.2 Collection and preparation of mangosteen (*Garcinia mangostana* L.) fruit rind

Fresh mangosteen fruits were collected with uniform colour was chosen at the 6^{th} stage of maturation based on colour index that reveals purple-black colour on the fruit skin (Palapol *et al*., 2009). The fruits were washed thoroughly with distilled water and shade dried to remove excess moisture content. The exocarp was peeled using a stainless steel peeler, and the peel was dried in a cabinet drier at 40±2 °C until a constant weight was obtained and it was ground in a commercial blender and sieved through an 80 mesh size sieve. Later, the peel powder was packed in an aluminium foil laminated pouch and stored in a freezer until pigment extraction.

3.3.3 Extraction of anthocyanin pigment

The extraction was carried out with the conventional solid-liquid extraction method which included: T_1 - Aqueous (distilled water) extraction, T_2 - Acidified aqueous (1 % citric acid) extraction, T_3 - Solvent extraction (50 % ethanol), T_4 - Acidified solvent extraction (50 % ethanol with 1 % citric acid) and T_5 - Microwave assisted extraction with aqueous (distilled water) and acidified solvent (50 % ethanol with 1 % citric acid) in case of butterfly pea flowers and mangosteen fruit rind, respectively. The sample was stirred in the solvent at a temperature of 45 °C for 45 min. The ratio of the plant sample and solvent was 1:20 (w/v). In the case of microwave assisted extraction, the sample was mixed with the respective solvent for

each crop, and the tube containing the suspension was irradiated in the microwave device at 300 W for 120 s with the temperature maintained between 45 and 50 °C. The extract was filtered through filter paper. The filtrate was collected and evaporated using a rotary vacuum evaporator (Heidolph rotary evaporator, Germany) at 60 °C and 114 mbar (Azima *et al*., 2017). The concentrated filtrates were kept in the glass vials, packed in an aluminium foil laminated pouch and stored under refrigerated (4-7 °C) condition until analysis (Plate 1 and 2).

3.3.4 Layout

The experiment was laid out in a completely randomized design (CRD) with four replications.

3.3.5 Observations recorded

3.3.5.1 Recovery percentage of the pigment concentrate (%)

The recovery percentage of the pigment concentrate was determined using the following formula (Gonfa *et al*., 2020) as stated below.

$$\text{Recovery percentage (\%)} = \frac{\text{Weight of pigment concentrate (g)}}{\text{Weight of powdered sample (g)}} \text{ x } 100$$

3.3.5.2 Moisture content (%)

Moisture content of pigment concentrate was determined using an infra-red moisture analyzer (Hallmark Mechatronics, Model-Sartorius, MA 150C, Germany) which records moisture in percentage.

3.3.5.3 Total monomeric anthocyanin content (TMAC) (mg/L)

The TMAC was estimated by pH differential method as described by Wrolstad *et al*. (2005). Aliquots of the concentrated pigment extract for different treatments were diluted with pH 1.0 and 4.5 buffers using potassium chloride (0.025 M) and sodium acetate (0.4 M), respectively using the determined dilution factor. Then, the dilutions were let to equilibrate for 15 min. The absorbance of each sample solution

was recorded using a spectrophotometer (UV-Visible 1800 spectrophotometer, Shimadzu, Kyoto, Japan) calibrated with distilled water as the blank at the wavelength of 530 and 700 nm. The difference in absorbance between pH values and wavelengths was calculated using the formula as stated below.

$$A = (\mathrm{A}_{530\mathrm{nm}} - \mathrm{A}_{700\mathrm{nm}})_{\text{pH 1.0}} - (\mathrm{A}_{530\mathrm{nm}} - \mathrm{A}_{700\mathrm{nm}})_{\text{pH 4.5}}$$

The TMAC in the sample was calculated as cyanidin-3-glucoside using the following equation:

$$TMAC\ (mg/L) = \frac{\mathrm{A}\ x\ \mathrm{MW}\ x\ \mathrm{DF}\ x\ 1000}{\varepsilon\ x\ l}$$

Wherein, MW is molecular weight (449.2 g/mol for cyanidin-3-glucoside), DF is dilution factor, ε is molar coefficient (26900 L/mol/cm for cyanidin-3-glucoside), l is path length (1 cm).

3.3.5.4 Colour properties

3.3.5.4.1 Instrumental colour values

The visual colour of the samples in terms of *L**, *a**, *b**, *chroma* and *hue angle* instrumental colour values was measured by reflectance measurement with a Minolta CM-3600D (Konica Minolta Sensing, Inc., Osaka, Japan) using JAYPAK 4808 software (Quality Control System, Version 1.2) based on the CIELAB colour space. D_{65} lamp was used as a reference light source. The colorimeter measured lightness (*L**) value and two coordinates *a** and *b**. Lightness (*L**) values 100 and 0 represent absolute white and absolute black, respectively, while positive and negative *a** (+*a** and -*a**) values denoted the direction of redness and greenness, respectively. Values of positive and negative *b** (+*b** and -*b**) were in the direction of the vector for yellowness and blueness, respectively. Chroma (*C**) value was a measure of intensity (or saturation), whereas *hue angle* (*h*°) angle was explained and characterized by the colour wheel, wherein the angle 0°, 30°, 60°, 90°, 120°, 150°, 180° (or -180°), 210° (-150°), 240° (or -120°), 270° (-90°), 300° (-60°) and 330° (-30°) were represented by red, orange, yellow, lime, green, turquoise, cyan, cobalt, blue, violet, magenta and crimson colour, respectively (Azima *et al*., 2017).

1. Butterfly pea flowers

2. Flower petals

3. Drying in cabinet drier

4. Dried flower petals

5. Petal powder

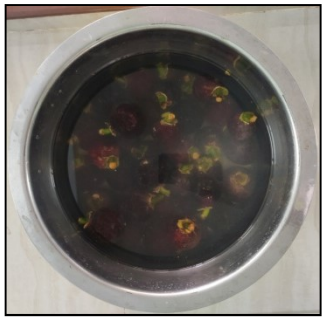
6. Extraction of pigment

7. Vacuum concentration

8. Concentrated pigment

Plate 1: Steps followed for the extraction of anthocyanin pigment from butterfly pea flower petals

1. Mangosteen fruits



2. Washing of fruits

3. Peeling

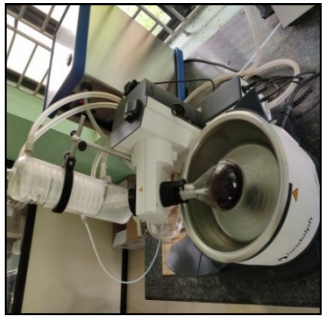
4. Fruit rind

5. Drying in cabinet drier

6. Dried rind

7. Rind powder

8. Extraction of pigment



9. Vacuum concentration

10. Pigment concentrate

Plate 2: Steps followed for the extraction of anthocyanin pigment from mangosteen fruit rind

3.3.5.5 Antioxidant assays

3.3.5.5.1 DPPH (2,2-diphenyl-1-picrylhydrazyl) assay (µl/ml)

The DPPH radical scavenging activity of samples was determined based on method developed by Chaovanalikit *et al*. (2012). The following formula calculated the radical scavenging percentage:

$$\text{Per cent inhibition} = \frac{[\text{Absorbance (control)} - \text{Absorbance (sample)}]}{\text{Absorbance (control)}} * 100$$

The sample concentration providing 50 % reduction in colour (IC_{50}) was calculated from the graph of the per cent inhibition versus the concentration. Gallic acid was used as standard.

3.3.5.5.2 FRAP (Ferric reducing antioxidant power) assay (µl/ml)

The FRAP assay determined reducing power capacity (Benzie and Strain, 1996). The FRAP reagent was prepared by adding 10 parts of 300 mM acetate buffer, pH 3.6, one part of 10 mM 2,4,6-Tripyridyl-S-triazine (TPTZ) prepared in 40 mM Hydrochloric acid (HCl) and one part of 20 mM Ferric chloride ($FeCl_3$). The mixture was diluted to one-third with methanol and pre-warmed at 37 ºC. This reagent (3 ml) was mixed with 0.1 ml diluted test samples. The mixture was shaken and incubated in the dark at 37 ºC for 20 min and the absorbance was read at 593 nm. Ascorbic acid was used as standard. The sample concentration providing 50 % reduction in colour (IC_{50}) was calculated from the graph of the per cent inhibition versus the concentration.

3.3.5.5.3 ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] assay (µl/ml)

The ABTS radical scavenging activity was carried out according to Brand-Williams *et al*. (1995) with some modifications. To prepare ABTS stock solution, potassium persulfate (2.45 mM) and ABTS (7 mM) were mixed in equal ratio (1:1) and left in the dark for at least 16 h. The working solution was prepared by combining the ABTS stock solution with methanol before use to obtain an absorbance of

0.706±0.01 Units. After allowing the sample to react with the ABTS solution for seven minutes, the absorbance was measured at 734 nm using a spectrophotometer. An appropriate solvent blank was used. The ABTS scavenging capacity of the pigment concentrate was compared with ascorbic acid, and the percentage inhibition was calculated by using the following equation:

$$\text{Scavenging activity (\%)} = \frac{[\text{Absorbance (control)} - \text{Absorbance (sample)}]}{\text{Absorbance (control)}} * 100$$

Wherein, Absorbance (control) is the absorbance of ABTS radical in methanol; Absorbance (sample) is the absorbance of ABTS radical solution mixed with sample/standard.

The sample concentration providing 50 per cent reduction in colour (IC_{50}) was calculated from the graph of the per cent inhibition versus the concentration.

3.3.5.6 Total phenolics (mg GAE/100 g)

Estimation of total phenolics was carried out with Folin-Ciocalteau reagent as described by Lakshan *et al*. (2019). The absorbance readings were taken at 765 nm on a spectrophotometer (UV-Visible 1800 spectrophotometer, Shimadzu, Kyoto, Japan). Total phenolics were quantified using a calibration curve prepared with a gallic acid standard. The results were expressed as mg gallic acid equivalent (GAE) per 100 g sample.

3.3.5.7 Total flavonoid content (mg QE/100 g)

Total flavonoid content was determined as per the method suggested by Mehmood *et al*. (2019). The sample was added with 0.3 ml of 5 % sodium nitrite, 0.3 ml of 10 % aluminium chloride, and one ml of 1 M sodium hydroxide solution. The volume was made up to 10 ml with distilled water. Absorbance of the reaction mixture was measured at 415 nm. Quercitin was used as standard, and results were expressed as mg of quercitin (QE) per 100 g of sample.

3.3.5.8 Non-enzymatic browning

Non-enzymatic browning was determined using standard method suggested by Zhu *et al.* (2009). Five ml of the sample was homogenized with 5 ml of 95 % ethyl alcohol and centrifuged at 5000 rpm for 10 min. The supernatant of the centrifuged sample was measured using a spectrophotometer at 420 nm using 95 % ethanol as a blank, and the absorbance (optical density value) obtained was considered the non-enzymatic browning index**.**

3.4 Evaluation of anthocyanin pigment's stability in processed products

The pigment concentrate obtained by aqueous extraction and acidified solvent extraction methods yielded the highest TMAC from butterfly pea flowers and mangosteen fruit rind, respectively. Hence, they were considered for the assessment of pigment's stability in fruit and vegetable products of liquid (squash), semi-solid (jelly), and solid (candy) nature. The quantity of anthocyanin pigment standardized by conducting preliminary studies was 0.3g/200 ml for all the product groups. The specified concentration of pigment was incorporated in the products, packed in appropriate packaging material (glass bottles for squash and, glass jars for jelly and candy), and stored under ambient as well as refrigerated conditions for three months. Observations were recorded at initial and monthly intervals.

3.4.1 Guava squash coloured with anthocyanin pigment

The guava squash was prepared by the standard procedure by following the specifications (25 % pulp; 40 ºB TSS; 1 % acidity) suggested by Food Safety and Standard Authority of India (FSSAI, 2011).

3.4.1.1 Procedure followed for the preparation of guava squash with anthocyanin pigment is as follows:

Firm ripe guava fruits (white pulp variety) were selected, washed, stalk ends were removed and cut into pieces. Fruits pieces were blended to obtain a smooth pulp. The pulp was mixed with strained sugar syrup and the pigment concentrate was

added. Later, the mixture was mixed with sodium benzoate (preservative). Then the whole mixture was homogenized, packed in glass bottles and stored.

3.4.2 Guava jelly coloured with anthocyanin pigment

The guava jelly was prepared by the standard procedure following the specifications (45 % pulp; 65 ºB TSS) suggested by FSSAI (2011).

3.4.2.1 Procedure followed for the preparation of guava jelly is as follows:

Matured guava fruits were selected, washed, stalk ends were removed and cut into pieces. Fruit pieces were boiled with citric acid for 30 min (1 kg fruit, 1 kg water, 2.5 g cirtic acid) and strained to obtain pectin rice extract. Later, the fruit pieces were boiled again with 500 g water for 15 min. The extract obtained was strained and mixed with previous batch of extract and kept undisturbed for clarification for 2 h. Clear extract was taken and added with equal quantity of sugar, and remaining 2.5 g citric acid. The mixture was boiled and scum was removed as and when formed. End point was judged to add the pigment concentrate. The jelly was then filled into clean sterilized glass jars and stored.

3.4.3 Ash gourd candy coloured with anthocyanin pigment

The ash gourd candy was prepared by the standard procedure, following the specifications [percentage of total sugar (w/w) not less than 70] suggested by FSSAI (2011).

3.4.3.1 Procedure followed for the preparation of ash gourd candy is as follows:

Matured ash gourds were selected. The fruits were washed; peeled, fluffy portion was removed, pricked and cut into cubes. The cubes were then soaked in lime solution for 24 h. Later, they were washed and soaked in alum solution for 24 h. The cubes were washed and drained free of water. The cubes were blanched in water containing KMS for 2 min. The sugar syrup (70 °B + 1 % citric acid + 0.25 % sodium benzoate) was prepared, strained, cooled and pigment concentrate was added. Then the cubes were steeped in the pigmented syrup for one week. Later, the cubes were

drained free of syrup and dried in cabinet drier. Then the candy was packed in glass jars and kept for storage.

Treatments

T_1 – No pigment

T_2 – Butterfly pea pigment

T_3 – Mangosteen pigment

3.4.4 Observations

The sample was crushed/ mixed with an equal quantity of water and filtered through the filter paper for recording the observations.

3.4.4.1 TMAC (mg/L)

Total monomeric anthocyanin content was estimated as given in 3.3.5.3

3.4.4.2 Instrumental colour values

The instrumental colour values **(***L**, *a**, *b**, *chroma* and *hue angle)* were evaluated based on the procedure given in 3.3.5.4.1

3.4.4.3 Antioxidant assays

All the antioxidant assays were performed as given in 3.3.5.5

3.4.4.4 Non-enzymatic browning

Non-enzymatic browning was performed as given in 3.3.5.8

3.4.4.5 Sensory evaluation (9-point Hedonic scale)

A panel of 15 semi-trained judges of different age groups were selected to evaluate the naturally coloured guava squash, guava jelly and ash gourd candy (Plate 3). The samples were coded and presented to the judges and no discussion during the sensory evaluation was allowed. Plain water was given to the judges to rinse their mouth between the sample's evaluations. The attributes such as appearance, colour, flavour, body and consistency, after-taste and overall acceptability for guava squash; visual appearance and transparency, colour, flavour, after-taste and overall

acceptability for guava jelly and visual appearance, colour, flavour, texture, after-taste and overall acceptability for ash gourd candy were evaluated on a 9-point hedonic scale rating (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much and 9 = like extremely) at monthly intervals for three months. A score of 5.50 and above was considered acceptable (Stone *et al*., 2020).

3.5 Standardization of extraction method for betalains

3.5.1 Collection and preparation of basella (*Basella alba* var. *rubra* L.) berries

Fresh, fully ripe, black purple coloured basella berries were collected and brought to the laboratory, washed with running tap water and drained free of water and dried in a cabinet drier at 40±2 °C until a constant weight was obtained, then the berries were ground in a commercial blender and sieved through an 80 mesh size sieve. Later, the powdered basella berries were packed in an aluminium foil laminated pouch and stored in a freezer until pigment extraction.

3.5.2 Collection and preparation of amaranthus (*Amaranthus tricolour* L.) leaves

Freshly harvested red amaranthus bunch was brought to the laboratory. The leaves were sorted out to remove the damaged and infected ones and were washed thoroughly with running tap water and drained free of moisture content. Then the leaves were dried in a cabinet drier at 40±2 °C until a constant weight was obtained and it was ground in a commercial blender and sieved through an 80 mesh size sieve. Later, the leaf powder was packed in an aluminium foil laminated pouch and stored in a freezer until pigment extraction.

3.5.3 Extraction of betalain pigment

The extraction was carried out with the conventional solid-liquid extraction method which included: T_1 - Aqueous (distilled water) extraction, T_2 - Acidified aqueous (1 % citric acid) extraction, T_3 - Solvent extraction (50 % ethanol), T_4 - Acidified solvent extraction (50 % ethanol with 1 % citric acid) and T_5 - Microwave assisted extraction with solvent (50 % ethanol) for both basella and amaranthus. The

Plate 3: Sensory evaluation of the products

sample was stirred in the solvent at a temperature of 45 °C for 45 min. The ratio of the plant sample and solvent was 1:20 (w/v). In the case of microwave assisted extraction, the sample was mixed with 50 per cent ethanol, and the tube containing the suspension was irradiated in the microwave device at 300 W for 120 s with the temperature maintained between 45 and 50 °C. The extract was filtered through filter paper. The filtrate was collected and evaporated using a rotary vacuum evaporator (Heidolph rotary evaporator, Germany) at 60 °C and 114 mbar (Azima *et al*., 2017). The concentrated filtrates were kept in the glass vials, packed in an aluminium foil laminated pouch and stored under refrigerated (4-7 °C) condition until analysis (Plate 4 and 5).

3.5.4 Layout

The experiment was laid out in a completely randomized design (CRD) with four replications.

3.5.5 Observations recorded

3.5.5.1 Recovery percentage of pigment concentrate (%)

The recovery percentage was determined as per the procedure given in 3.3.5.1

3.5.5.2 Moisture content (%)

The moisture content percentage was determined as per the procedure given in 3.3.5.2

3.5.5.3 Total betalain content (mg/g)

The total betalain content in the pigment concentrate was estimated by a spectrophotometric method using a UV-visible spectrophotometer (UV 1800, Shimadzu, Kyoto, Japan) using the method suggested by (Kumar and Giridhar, 2017) with slight modifications. A known amount of sample was added with a known volume of distilled water and the absorbance was measured in a UV-visible spectrophotometer. Betacyanins and betaxanthins were measured in terms of betanin and vulgaxanthin I. Basis on the measurement of extinction at 600 nm, a correction

factor resulting from light absorbance by impurities present in the sample was included while calculating total betalains. The sum of betacyanins and betaxanthins was used to calculate the total pigment content (Kumar and Giridhar, 2017).

For quantitative analysis, the betalain content was calculated according to following equation:

$$\text{Betalain content (mg/g)} = \frac{\text{A x DF x MW x V}}{\varepsilon \text{ x l x } W_d}$$

Wherein,

A = absorption value at the absorption maxima of 535 nm and 477 nm for betacyanins and betaxanthins, respectively

DF = dilution factor

MW = Molecular weight (550 g/mol and 339 g/mol for betacyanins and betaxanthins, respectively)

V = solution volume (ml)

ε = Molar extinction coefficient (60000 L/(mol cm) and 48000 L/(mol cm) in water for betacyanins and betaxanthins, respectively)

l = path length (1 cm) of the cuvette, and

W_d = sample weight (g)

3.5.5.4 Instrumental colour values

The instrumental colour values **(***L**, *a**, *b**, *chroma* and *hue angle)* were evaluated based on the procedure given in 3.3.5.4.1

3.5.5.5 Antioxidant assays

All the antioxidant assays were performed as given in 3.3.5.5

3.5.5.6 Total phenolics (mg GAE/100 g)

The total phenolics content was determined as per the procedure given in 3.3.5.6

1. Fresh basella berries

2. Dried basella berries

3. Basella berry powder

4. Pigment extract

5. Vacuum concentration

6. Pigment concentrate

Plate 4: Steps followed for the extraction of betalain pigment from basella berries

1. Fresh red amaranthus

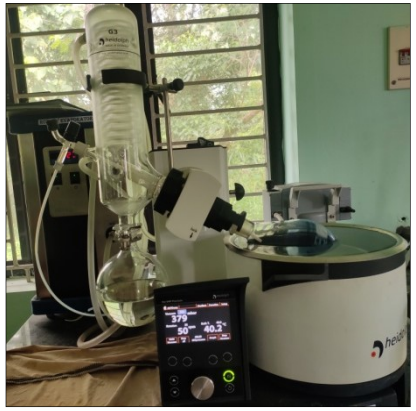
2. Washing

3. Draining and drying

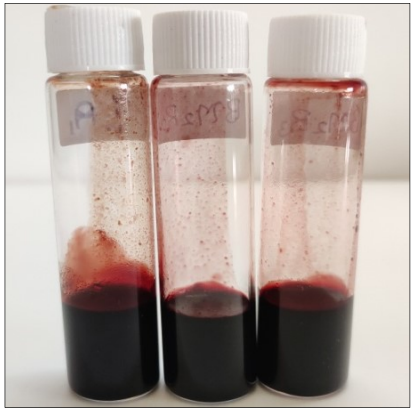
4. Dried red amaranthus

5. Leaf powder

6. Pigment extract



7. Vacuum concentration



8. Concentrated pigment

Plate 5: Steps followed for the extraction of betalain pigment from red amaranthus leaves

3.5.5.7 Total flavonoid content (mg QE/100 g)

The total flavonoid content was determined as per the procedure given in 3.3.5.7

3.5.5.8 Non-enzymatic browning

Non-enzymatic browning was performed as given in 3.3.5.8

3.6 Evaluation of betalain pigment's stability in processed products

The pigment concentrate obtained by MAE with solvent (50 % ethanol) method yielded the highest total betalain content from basella berries and red amaranthus. Hence they were considered for the assessment of pigment stability in guava squash, guava jelly, and ash gourd candy processed products of liquid, semi-solid and solid nature respectively. The quantity of betalain pigment standardized by conducting preliminary studies is 0.3 g/200 ml for all the product groups. The specified concentration of pigment was incorporated in the products, packed in appropriate packaging material (glass bottles for squash and, glass jars for jelly and candy) and stored under ambient as well as refrigerated condition for three months. Observations were recorded at initial and monthly intervals.

3.6.1 Guava squash coloured with betalain pigment

The guava squash was prepared by the standard procedure, following the specifications (25 % pulp; 40 ºB TSS; 1 % acidity) suggested by the FSSAI (2011).

3.6.1.1. Procedure followed for the preparation of guava squash with betalain pigment is as mentioned in 3.4.1.1

3.6.2 Guava jelly

The guava jelly was prepared by the standard procedure, following the specifications (45 % pulp; 65 ºB TSS) suggested by FSSAI (2011).

3.6.2.1 Procedure followed for the preparation of guava jelly with betalain pigment is as mentioned in 3.4.2.1.

3.6.3 Ash gourd candy

The ash gourd candy was prepared by the standard procedure by following the specifications suggested by FSSAI (2011).

3.6.3.1 Procedure followed for the preparation of ash gourd candy with betalain pigment is as mentioned in 3.4.3.1.

Treatments

T_1 – No pigment

T_2 – Basella pigment

T_3 – Amaranthus pigment

3.6.4 Observations recorded

The sample was crushed/ mixed with an equal quantity of water and filtered through the filter paper for recording the observations.

3.6.4.1 Total betalain content (mg/g)

The total betalain content was estimated as in 3.5.5.3

3.6.4.2 Instrumental colour values

The instrumental colour values **(***L**, *a**, *b**, *chroma* and *hue angle)* were evaluated based on the procedure given in 3.3.5.4.1

3.6.4.3 Antioxidant assays

All the antioxidant assays were performed as given in 3.3.5.5

3.6.4.4 Non-enzymatic browning

Non-enzymatic browning was performed as given in 3.3.5.8

3.6.5 Sensory evaluation (9-point Hedonic scale)

The sensory evaluation was performed as per the procedure given in 3.4.4.5

3.7 Standardization of extraction method for carotenoids

3.7.1 Collection and preparation of gac fruit (*Momordica cochinchinensis* Spreng) seed aril

Fully ripe gac fruits were collected and brought to the laboratory. The fruits were cut open and the seeds covered with bright red aril were carefully separated from the fruit meat (yellow part). The seeds with aril were partially dried as a whole in a cabinet drier at 40±2 °C. This was done because after partial drying, the aril will easily separate from the seed. Later the seed aril was subjected to drying until a constant weight was obtained and then the aril was ground in a commercial blender and sieved through an 80 mesh size sieve. Later, the aril powder was packed in an aluminium foil laminated pouch and stored in a freezer until pigment extraction.

3.7.2 Collection and preparation of marigold (*Tagetes erecta*) flowers

Fresh marigold flowers were collected and brought to the laboratory. The flowers were sorted to remove the damaged ones. Petals were separated from the flower head, washed thoroughly, drained water by placing the petals on a fine cloth. Then they were dried in a cabinet drier at 40±2 °C until a constant weight was obtained, and it was ground in a commercial blender and sieved through an 80 mesh size sieve. Later, the petal powder was packed in an aluminium foil laminated pouch and stored in a freezer until pigment extraction.

3.7.3 Extraction of carotenoid pigment

The extraction was carried out with the conventional solid-liquid extraction method using different solvents, which include: T_1 - Ethanol, T_2 – Ethyl lactate, T_3 – Ethanol: Ethyl lactate (1:1 ratio), T_4 – Microwave assisted extraction with ethanol and T_5 - Microwave assisted extraction with Ethanol: Ethyl lactate (1:1 ratio) for both gac fruit and marigold. The sample was stirred in the solvent at a temperature of 45 °C for 45 min. The ratio of the plant sample and solvent was 1:20 (w/v). In the case of MAE,

the sample was mixed with the respective solvents, and the tube containing the suspension was irradiated in the microwave device at 300 W for 120 s with the temperature maintained between 45 and 50 °C. The extract was filtered through filter paper. The filtrate was collected and evaporated using a rotary vacuum evaporator (Heidolph rotary evaporator, Germany) at 60 °C and 50 mbar. The concentrated filtrates were kept in the glass vials, packed in an aluminium foil laminated pouch and stored under refrigerated (4-7 °C) condition until analysis (Plate 6 and 7).

3.7.4 Layout

The experiment was laid out in a completely randomized design (CRD) with four replications.

3.7.5 Observations recorded

3.7.5.1 Recovery percentage of the pigment concentrate (%)

The recovery percentage was determined as per the procedure given in 3.3.5.1.

3.7.5.2 Moisture content (%)

The moisture content percentage was determined as per the procedure given in 3.3.5.2

3.7.5.3 β-carotene content (µg/g)

The β-carotene content of the pigment concentrate from gac fruit and marigold flowers was determined by using the method mentioned by Vieira *et al*. (2020). A known quantity of sample was put in a test tube. The mixed solvents of acetone and hexane in the ratio of 4:6 (volume per volume) were added and mixed well with the sample. Then the mixture was centrifuged at 15000 rpm for one min. Absorbance (A) values at 453 and 505 nm wavelength were recorded to determine the β-carotene content in each sample and calculated by using the following equation.

$$C_{\beta-caroten} = 4.624 \times A_{453} - 3.091 \times A_{505}$$

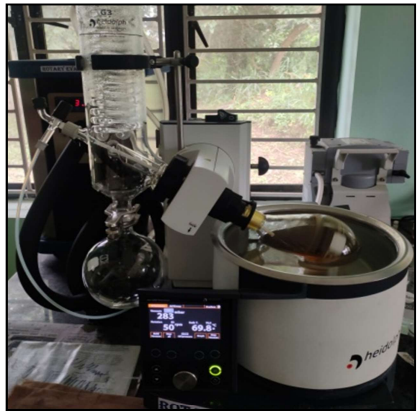
			
1. Fresh Gac fruits	**2. Preparation**	**3. Gac fruit seed with aril**	**4. Dried gac fruit seed aril**
			
5. Seed aril powder	**6. Pigment extract**	**7. Vacuum concentration**	**8. Concentrated pigment**

Plate 6: Steps followed for the extraction of carotenoid pigment from gac fruit seed aril

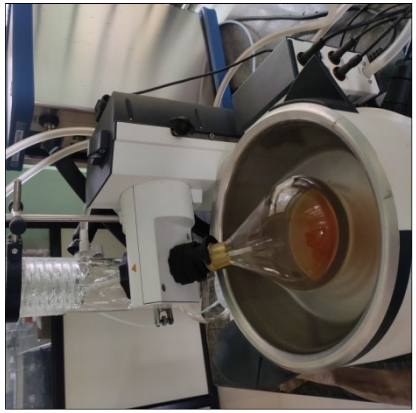
1. Fresh marigold flowers

2. Preparation

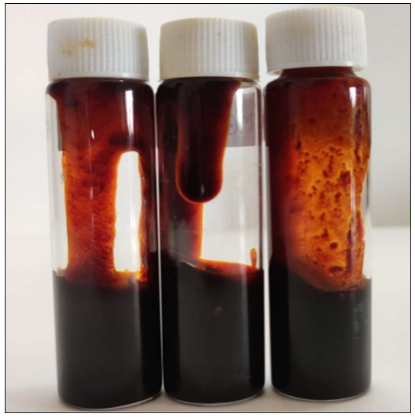
3. Dried marigold petals

4. Petal powder

5. Pigment extract



6. Vacuum concentration



7. Concentrated pigment

Plate 7: Steps followed for the extraction of carotenoid pigment from marigold flower petals

Wherein,

$C_{\beta\text{-carotene}}$ is the concentration of β-carotene expressed in µg/g.

A_{450} and A_{503} represent the absorbance at 450 nm and 503 nm, respectively

3.7.5.4 Lycopene content (µg/g)

The lycopene content of the pigment concentrate from gac fruit and marigold flowers was determined by using the method mentioned by Vieira *et al*. (2020). A known quantity of sample was put in a test tube. The mixed solvents of acetone and hexane in the ratio of 4:6 (volume per volume) were added and mixed well with the sample. Then the mixture was centrifuged (Kamalambigeswari and Rebecca, 2016) at 15000 rpm for one min. Absorbance (A) values at 453 and 505 nm wavelength were recorded for the determination of the lycopene content in each sample and were calculated by using the following equation.

$$C_{Lycopene} = 3.956 \text{ x } A_{453} - 0.806 \text{ x } A_{505}$$

Wherein, $C_{Lycopene}$ is the concentration of lycopene expressed in µg/g.

A_{453} and A_{505} represent the absorbance at 453 nm and 505 nm, respectively

3.7.5.5 Lutein content (µg/g)

Absorbance was measured using UV-Vis spectrophotometer at the wavelength of 445 nm (Kamalambigeswari and Rebecca, 2016). The concentration of lutein was calculated using the following equation:

$$\text{Concentration of lutein } (\mu g/g) = \frac{\text{A x V(ml) x dilution factor}}{E^{1\%}\text{cm x W (g)}}$$

Wherein,

A = Absorbance

V = Volume (ml)

$E^{1\%}$ 1cm = Extinction coefficient of solvents

W = weight of the sample (g)

3.7.5.6 Instrumental colour values

The instrumental colour values **(***L**, *a**, *b**, *chroma* and *hue angle)* were evaluated based on the procedure given in 3.3.5.4.1

3.7.5.7 Antioxidant assays

All the antioxidant assays were performed as given in 3.3.5.5

3.7.5.8 Total phenolics (mg GAE/100 g)

The total phenolics content was determined as per the procedure given in 3.3.5.6

3.7.5.9 Total carotenoid content (µg/g)

A known quantity of sample was dissolved in two ml of n-hexane, and mixed thoroughly for two min. Then, the absorbance was measured using a spectrophotometer ((UV-Visible 1800 spectrophotometer, Shimadzu, Kyoto, Japan)) at 445 nm wavelength. Finally, the absorbance of each sample was compared with a standard curve (R^2 = 0.99) to calculate the concentration of carotenoids in µg/g of pigment extract (Natnoi and Pirak, 2019).

3.7.5.10 Non-enzymatic browning

Non-enzymatic browning was performed as given in 3.3.5.8

3.8 Evaluation of carotenoid pigment's stability in processed products

The pigment concentrate obtained by MAE with ethyl lactate as solvent yielded the highest β-carotene and lutein content in gac fruit and marigold. Hence, they were considered for the assessment of pigment stability in guava squash, guava jelly, and ash gourd candy processed products of liquid, semi-solid and solid nature, respectively. The quantity of carotenoid pigment used was 0.3 g/200 ml for all the product groups. The specified concentration of pigment was incorporated in the products, packed in appropriate packaging material (glass bottles for squash and glass

jars for jelly and candy) and stored under ambient as well as refrigerated condition for three months. Observations were recorded at initial and monthly intervals.

3.8.1 Guava squash coloured with carotenoid pigment

The guava squash was prepared by the standard procedure, following the specifications (25 % pulp; 40 ºB TSS; 1 % acidity) suggested by FSSAI (2011).

3.8.1.1. Procedure followed for the preparation of guava squash with carotenoid pigment is as mentioned in 3.4.1.1

3.8.2 Guava jelly

The guava jelly was prepared by the standard procedure by following the specifications (45 % pulp; 65 ºB TSS) suggested by FSSAI (2011).

3.8.2.1 Procedure followed for the preparation of guava jelly with carotenoid pigment is as mentioned in 3.4.2.1

3.8.3 Ash gourd candy

The ash gourd candy was prepared by the standard procedure by following the specifications suggested by FSSAI (2011).

3.8.3.1 Procedure followed for the preparation of ash gourd candy with carotenoid pigment is as mentioned in 3.4.3.1

Treatments

T_1 – No pigment

T_2 – Gac fruit pigment

T_3 – Marigold pigment

3.8.4 Observations recorded

The sample was crushed/ mixed with an equal quantity of acetone and filtered through the filter paper for recording the observations.

3.8.4.1 β-carotene content (mg/g)

The β-carotene content (mg/g) was determined as per the procedure given in 3.7.5.3

3.8.4.2 Lycopene content (µg/g)

The lycopene content was determined as per the procedure given in 3.7.5.4

3.8.4.3 Lutein content (µg/g)

The lutein content was determined as per the procedure given in 3.7.5.5

3.8.5.4 Instrumental colour values

The instrumental colour values **(***L**, *a**, *b**, *chroma* and *hue angle)* were evaluated based on the procedure given in 3.3.5.4.1

3.8.5.5 Antioxidant assays

All the antioxidant assays were performed as given in 3.3.5.5

3.8.5.6 Total carotenoid content (µg/g)

The total carotenoid content was determined as per the procedure given in 3.7.5.7

3.8.5.7 Non-enzymatic browning

Non-enzymatic browning was performed as given in 3.3.5.8

3.8.5.8 Sensory evaluation (9-point Hedonic scale)

The sensory evaluation was performed as per the procedure given in 3.4.4.5

3.9 Statistical analysis

The experiment was carried out and results were expressed as mean values with standard deviation (±SD) (Panse and Sukhatme, 1985). One-way analysis of variance (ANOVA) was carried out to determine significant group differences ($p \leq 0.05$) between means. Duncan Multiple Range Test (DMRT) was used to compare

mean values. Kendall's coefficient of concordance (W) indicates the degree of association of ordinal assessments made by multiple appraisers when assessing the same samples. Kendall's coefficient values can range from 0 to 1. Higher the value, stronger is the association (Legendre, 2005).

Results

4. RESULTS

The results obtained in the present investigation entitled "Evaluation and utilization of plant pigments as natural food colorants" are presented below.

4.1 Standardization of extraction method for anthocyanin from butterfly pea flowers and mangosteen fruit rind

4.1.1 Butterfly pea flowers

4.1.1.1 Recovery percentage of pigment concentrate (%)

The observations on per cent recovery of anthocyanin pigment concentrate of butterfly pea flowers as affected by different extraction methods are recorded in the Table 1. Considerable variation in per cent recovery of anthocyanin pigment was recorded which had a range between 63.30±1.82 to 74.03±0.84 per cent. Significantly higher per cent recovery of anthocyanin pigment was recorded in acidified aqueous extraction method (T_2) (74.03±0.84 %) which was found to be at parity with microwave assisted extraction (MAE) with aqueous medium (T_5) (73.17±1.76 %). Lowest per cent recovery was observed in solvent extraction method (T_3) (63.30±1.82 %) which is on par with acidified solvent extraction (T_4) (65.27±1.21 %) method.

4.1.1.2 Moisture content (%)

The results on the moisture content percentage in the pigment concentrate of butterfly pea flower are presented in the Table 1. Non-significant variation was recorded in the moisture content as influenced by different extraction methods. The values ranged from 15.58±0.02 to 15.62±0.02 per cent.

4.1.1.3 Total monomeric anthocyanin content (TMAC) (mg/L)

The results on the TMAC present in the pigment concentrate of butterfly pea flower are presented in the Table 1. Significant variation was recorded in the TMAC as influenced by different extraction methods that had a range from 3772.28±116.74 to 7925.29±36.07 mg/L. Statistically higher TMAC was registered in aqueous extraction method (T_1) (7925.29±36.07 mg/L) followed by MAE with aqueous

Table 1: Effect of different extraction methods on recovery percentage, moisture content and TMAC of anthocyanin pigment concentrate from butterfly pea flower petals

Treatments		Recovery percentage	Moisture content (%)	TMAC (mg/L)
T_1	Aqueous (distilled water) extraction	72.03±1.24[a]	15.58±0.03	7925.29±36.07[a]
T_2	Acidified aqueous extraction (1 % citric acid)	74.03±0.84[a]	15.58±0.02	4373.44±110.77[d]
T_3	Solvent extraction (50 % ethanol)	63.30±1.82[b]	15.62±0.02	3772.28±116.74[e]
T_4	Acidified solvent extraction (50 % ethanol with 1 % citric acid)	65.27±1.21[b]	15.60±0.01	5771.13±54.19[c]
T_5	Microwave assisted extraction with aqueous medium	73.17±1.76[a]	15.60±0.01	6146.86±22.98[b]
S.Em±		1.42	0.02	78.31
CD (0.05)		4.54	NS	249.53

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

medium (6146.86±22.98 mg/L) and the lowest was reported in solvent extraction method (3772.28±116.74 mg/L).

4.1.1.4 Colour properties

4.1.1.4.1 Instrumental colour values (*L**, *a**, *b**, *chroma* and *hue angle*)

The observations pertaining to *L*, a**, *b**, *chroma* and *hue angle* values are given in the Table 2. All the treatments showed significant variations as influenced by different extraction methods.

The results on instrumental *L** value showed significant variations as influenced by different extraction methods which ranged from 18.09±3.95 to 49.24±1.13. However, significantly higher *L** value was recorded in acidified aqueous extraction method (49.24±1.13), followed by MAE with aqueous medium (27.74±0.73) and the lowest value was registered in solvent extraction method (18.09±3.95).

Instrumental *a** value varied significantly among different treatments which ranged from 36.78±0.92 to 46.90±0.77. However, significantly higher value was reported in the MAE with aqueous medium (46.90±0.77) which was on par with acidified solvent extraction method (46.42±1.37) and significantly lower value was recorded in acidified aqueous extraction method (36.78±0.92).

The data with respect to instrumental *b** values varied significantly among the treatments as influenced by different extraction methods where the values ranged from 63.25±0.13 to -38.02±0.41. Significantly higher *b** value was recorded in acidified aqueous extraction method (-38.02±0.41) which was found to be at parity with acidified solvent extraction method (-39.72±1.04), whereas the lowest *b** value was recorded in MAE with aqueous medium (-63.25±0.13).

Chroma values of different treatments as influenced by different extraction methods had a range from 52.90±0.92 to 78.74±0.38. Significantly higher value was reported in MAE with aqueous medium (78.74±0.38) followed by aqueous extraction

method (69.30±0.13). Significantly lower value was reported in acidified aqueous extraction method (52.90±0.92).

Hue angle values of different treatments were in the range of 306.56±0.50 to 319.44±1.56. However, statistically higher *hue angle* value (319.44±1.56) was recorded in acidified solvent extraction method which was followed by acidified aqueous extraction method (314.38±0.09), whereas lowest value was registered in MAE with aqueous medium (306.56±0.50).

4.1.1.5 Antioxidant properties

4.1.1.5.1 DPPH assay (µl/ml)

According to the data analysed, antioxidant activity of butterfly pea flower showed least significant difference among the treatments as influenced by different extraction methods (Table 3). Antioxidant activity of butterfly pea flower pigment as depicted by DPPH method had a range from 3.49±0.59 to 9.31±1.72 µl/ml. However, the highest antioxidant activity was recorded in MAE with aqueous medium (3.49±0.59 µl/ml), while lowest antioxidant activity (9.31±1.72 µl/ml) was noticed in aqueous extraction method which was statistically on par with acidified solvent extraction method (5.80±1.02 µl/ml).

4.1.1.5.2 FRAP assay (µl/ml)

The data with respect to antioxidant activity of butterfly pea flower pigment obtained through FRAP method showed least statistical variation among different treatments as displayed in the Table 3. Free radical scavenging activity of the antioxidants present in the butterfly pea flower pigment was in the range of 3.99±1.10 to 10.00±1.70 µl/ml. However, highest antioxidant activity was recorded in MAE with aqueous medium (3.99±1.10 µl/ml), whereas the significantly lowest antioxidant activity was registered in aqueous extraction method (10.00±1.70 µl/ml) which was followed by acidified solvent extraction method (6.20±0.93 µl/ml).

Table 2: Effect of different extraction methods on instrumental colour values of anthocyanin pigment concentrate from butterfly pea flower petals

Treatments		*L** value	*a** value	*b** value	*Chroma*	*Hue angle*
T_1	Aqueous (distilled water) extraction	25.34±1.30^{b}	42.40±0.91^{c}	-54.79±0.88^{b}	69.30±0.13^{b}	307.75±1.04^{cd}
T_2	Acidified aqueous extraction (1 % citric acid)	49.24±1.13^{a}	36.78±0.92^{d}	-38.02±0.41^{a}	52.90±0.92^{d}	314.38±0.09^{b}
T_3	Solvent extraction (50 % ethanol)	18.09±3.95^{c}	43.56±1.06^{bc}	-49.88±4.43^{b}	66.30±4.05^{bc}	311.35±1.75^{bc}
T_4	Acidified solvent extraction (50 % ethanol with 1 % citric acid)	27.32±2.28^{b}	46.42±1.37^{ab}	-39.72±1.04^{a}	61.14±0.41^{c}	319.44±1.56^{a}
T_5	Microwave assisted extraction with aqueous medium	27.74±0.73^{b}	46.90±0.77^{a}	-63.25±0.13^{c}	78.74±0.38^{a}	306.56±0.50^{d}
S.Em±		7.04	1.03	2.08	1.87	1.17
CD (0.05)		2.20	3.27	6.64	5.98	3.73

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

4.1.1.5.3 ABTS assay (µl/ml)

The values for ABTS antioxidant activity of butterfly pea flower pigment as affected by different extraction methods are illustrated in Table 3. Here, different extraction methods showed statistical differences. According to the data analysed, the ABTS antioxidant activity of different methods had a range from 2.42±0.01 to 8.55±0.05 µl/ml. Among the different treatments, highest antioxidant activity (2.42±0.01 µl/ml) was reported in MAE with aqueous medium, while significantly lowest antioxidant activity (8.55±0.05 µl/ml) was recorded in aqueous extraction method, followed by acidified aqueous extraction method (4.86±0.10 µl/ml).

4.1.1.6 Total phenolics (mg GAE/100 g)

The results on the total phenolics of butterfly pea flower pigment are demonstrated in the Table 3. Total phenolics of five treatments as influenced by different extraction methods showed significant variations and it ranged from 7.87±0.09 to 29.78±1.79 mg GAE/100 g. However, highest total phenolics was reported in MAE with aqueous medium (29.78±1.79 mg GAE/100 g) followed by acidified aqueous extraction method (11.30±1.45 mg GAE/100 g), while the lowest total phenolics was noticed in acidified aqueous extraction method (7.87±0.09 mg GAE/100 g).

4.1.1.7 Total flavonoid content (mg QE/100 g)

The observations pertaining to total flavonoid content of butterfly pea flower as influenced by different extraction methods showed significant variations among different treatments. The results with respect to total flavonoid content are displayed in the Table 3. The total flavonoid content of different treatments was in the range of 6.50±0.82 to 20.13±0.40 mg QE/100 g. However, the highest value for total flavonoid content was reported in MAE with aqueous medium (20.13±0.40 mg QE/100g), followed by acidified aqueous extraction method (11.23±2.40 mg QE/100g), while the lowest value (6.50±0.82 mg QE/100 g) was recorded in acidified aqueous extraction method.

Table 3: Effect of different extraction methods on antioxidant properties of anthocyanin pigment concentrate from butterfly pea flower petals

Treatments	DPPH (µl/ml)	FRAP (µl/ml)	ABTS (µl/ml)	Phenols (mg GAE/100g)	Flavonoids (mg QE/100g)	Non-enzymatic browning
T_1	9.31±1.72^{a}	10.00±1.70^{a}	8.55±0.05^{a}	9.83±0.42^{bc}	9.17±0.52^{bc}	ND
T_2	4.44±1.26^{b}	4.97±1.39^{b}	3.04±0.05^{c}	7.87±0.09^{c}	6.50±0.82^{c}	ND
T_3	4.17±1.10^{b}	4.02±0.97^{b}	3.31±0.23^{c}	10.70±0.27^{bc}	9.17±0.81^{bc}	ND
T_4	5.80±1.02^{ab}	6.20±0.93^{ab}	4.86±0.10^{b}	11.30±1.45^{b}	11.23±2.40^{b}	ND
T_5	3.49±0.59^{b}	3.99±1.10^{b}	2.42±0.01^{d}	29.78±1.79^{a}	20.13±0.40^{a}	ND
SE(m)	1.20	5.83	0.12	1.05	1.23	
CD (5%)	3.82	1.253	0.37	3.36	3.91	

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)
GAE: Gallic Acid Equivalent; **QE:** Quercetin Equivalent; **ND:** not detected

Treatments details:

T_1 Aqueous (distilled water) extraction
T_2 Acidified aqueous extraction (1 % citric acid)
T_3 Solvent extraction (50 % ethanol)
T_4 Acidified solvent extraction (50 % ethanol with 1 % citric acid)
T_5 Microwave assisted extraction with aqueous medium

4.1.1.8 Non-enzymatic browning

The observations with respect to non-enzymatic browning of the anthocyanin pigment concentrates from butterfly pea flower as affected by the different extraction methods were not detected in the samples (Table 3).

4.1.2 Mangosteen fruit rind

4.1.2.1 Recovery percentage of pigment concentrate (%)

The per cent recovery of anthocyanin pigment concentrates from mangosteen fruit rind as affected by the different extraction methods are presented in Table 4. Per cent recovery of anthocyanin pigment showed considerable differences among the methods and ranged from 30.23±1.37 to 55.03±2.98 per cent. Statistically maximum per cent recovery of anthocyanin pigment concentrate was observed in MAE with acidified solvent (55.03±2.98 %) followed by acidified solvent extraction method (40.43±1.66 %) and acidified aqueous extraction method (38.73±0.27 %), whereas the lowest value in comparison to all other methods was registered in aqueous extraction method (30.23±1.37 %).

4.1.2.2 Moisture content (%)

The results on the moisture content percentage in the pigment concentrate of butterfly pea flower are presented in the Table 4. Non-significant variation was recorded in the moisture content as influenced by different extraction methods that had a range from 15.55±0.02 to 15.58±0.03 per cent.

4.1.2.3 Total monomeric anthocyanin content (mg/L)

Significant variations pertaining to TMAC of mangosteen fruit rind as influenced by different extraction methods were observed (Table 4). However, the TMAC of mangosteen fruit rind varied from 8712.95±382.80 to 17652±139.47 mg/L. Statistically higher TMAC value (17652±139.47 mg/L) was registered in acidified solvent extraction method which had parity with MAE with acidified solvent (16282.20±837.54 mg/L). However, the lowest TMAC value (8712.95±382.80 mg/L) among different methods in this study was recorded in aqueous extraction method.

Table 4: Effect of different extraction methods on recovery percentage, moisture content and TMAC of anthocyanin pigment concentrate from mangosteen fruit rind

Treatments		Recovery percentage	Moisture content (%)	TMAC (mg/L)
T_1	Aqueous (distilled water) extraction	30.23±1.37^{c}	15.56±0.07	8712.95±382.80^{d}
T_2	Acidified aqueous extraction (1 % citric acid)	38.73±0.27^{b}	15.55±0.02	11171.17±239.58^{c}
T_3	Solvent extraction (50 % ethanol)	32.70±1.08^{c}	15.57±0.03	12614.34±309.76^{b}
T_4	Acidified solvent extraction (50 % ethanol with 1 % citric acid)	40.43±1.66^{b}	15.58±0.03	17652.54±139.47^{a}
T_5	Microwave assisted extraction with acidified solvent (50 % ethanol with 1 % citric acid)	55.03±2.98^{a}	15.56±0.02	16282.20±837.54^{a}
S.Em±		1.72	0.04	451.85
CD (0.05)		5.48	NS	1442.20

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

4.1.2.4 Colour properties

4.1.2.4.1 Instrumental colour values (*L**, *a**, *b**, *chroma* and *hue angle*)

The data on instrumental *L**, *a* b**, *Chroma* and *Hue angle* colour values of anthocyanin pigment concentrate from mangosteen fruit rind as influenced by different extraction methods are presented in Table 5. The data with respect to instrumental colour values showed significant differences among the treatments.

The range of instrumental *L** value was from 3.17±0.3861 to 61.65±0.94. Significantly higher *L** value was recorded in aqueous extraction method (61.65±0.94) which was at parity with acidified aqueous extraction method (58.34±2.30). Minimum *L** value was noticed in MAE with acidified solvent (3.17±0.38) which was on par with solvent extraction method (6.14±4.14).

The *a** value as influenced by different extraction methods were in the range of 19.24±1.08 to 59.53±0.85. Significantly highest *a** value was recorded in MAE with acidified solvent (59.53±0.85) and lower value was recorded in aqueous extraction method (19.24±1.08).

The *b** values of different treatments were in the range of 5.09±0.52 to 23.75±2.04. Statistically higher *b** value was recorded in acidified aqueous extraction method (23.75±2.04) which was on par with aqueous extraction method (20.19±0.75) and the lowest value was registered in MAE with acidified solvent (5.09±0.52).

The range of *chroma* value was from 25.33±1.28 to 60.80±1.00. Significantly highest *chroma* value was recorded in MAE with acidified solvent method (48.66±2.70) and lower *chroma* value was noticed in acidified aqueous extraction (25.33±1.28).

Hue angle values of different treatments ranged from 28.06±0.33 to 46.42±2.43. Significantly highest *hue angle* value was recorded in aqueous extraction method (46.42±2.43) which was on par with solvent extraction method (45.47±6.26) and the lowest value was recorded in MAE with acidified solvent (28.06±0.33).

Table 5: Effect of different extraction methods on instrumental colour values of anthocyanin pigment concentrate from mangosteen fruit rind

Treatments		*L** value	*a** value	*b** value	*Chroma*	*Hue angle*
T_1	Aqueous (distilled water) extraction	61.65±0.94^{a}	19.24±1.08^{c}	20.19±0.75^{a}	27.93±0.54^{c}	46.42±2.43^{a}
T_2	Acidified aqueous extraction (1 % citric acid)	58.34±2.30^{a}	23.61±1.82^{c}	23.75±2.04^{a}	25.33±1.28^{c}	39.44±0.80^{ab}
T_3	Solvent extraction (50 % ethanol)	32.29±9.58^{b}	20.55±4.73^{c}	19.78±0.33^{a}	28.86±3.60^{c}	45.47±6.26^{ab}
T_4	Acidified solvent extraction (50 % ethanol with 1 % citric acid)	6.14±4.14^{c}	42.46±1.96^{b}	9.65±6.26^{b}	43.48±1.27^{b}	35.35±2.79^{bc}
T_5	Microwave assisted extraction with acidified solvent (50 % ethanol with 1 % citric acid)	3.17±0.38^{c}	59.53±0.85^{a}	5.09±0.52^{b}	60.80±1.00^{a}	28.06±0.33^{c}
S.Em±		4.80	2.50	2.98	1.87	3.28
CD (0.05)		15.32	7.99	9.51	5.96	10.45

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

4.1.2.5 Antioxidant properties

4.1.2.5.1 DPPH assay (µl/ml)

The observations pertaining to DPPH antioxidant activity are given in Table 6. Antioxidant properties differed significantly with extraction methods. The DPPH radical scavenging activity of anthocyanin pigment concentrate from mangosteen fruit rind prepared by various extraction methods was in the range of 2.29±0.24 to 9.57±1.28 µl/ml. However, significantly higher antioxidant activity was recorded in MAE with acidified solvent (2.29±0.24 µl/ml), whereas lowest antioxidant activity was recorded in aqueous extraction method (9.57±1.28 µl/ml).

4.1.2.5.2 FRAP assay (µl/ml)

According to data analysed, FRAP of pigment concentrate from mangosteen fruit rind disclosed significant variations (Table 6). However, the FRAP value of the pigment generated by all the five extraction methods ranged from 2.83±0.21 to 6.68±0.06 µl/ml. Highest antioxidant activity (2.83±0.21 µl/ml) was observed in MAE with acidified solvent which was followed by (4.68±0.068 µl/ml) acidified solvent extraction method, while lowest antioxidant activity (6.68±0.06 µl/ml) was observed in aqueous extraction method.

4.1.2.5.3 ABTS assay (µl/ml)

The values for ABTS assay of pigment concentrate from mangosteen fruit rind as affected by different extraction methods are illustrated in Table 6. Here, pigment extracted by different methods showed statistical differences. According to the data analysed, the ABTS radical scavenging activity of different methods ranged from 2.02±0.03 to 8.80±0.29 µl/ml. However, the highest antioxidant activity (2.02±0.03 µl/ml) was reported in MAE with acidified solvent, while lowest antioxidant activity (8.80±0.29 µl/ml) was recorded in aqueous extraction method followed by acidified aqueous extraction method (7.03±0.05 µl/ml).

4.1.2.6 Total phenolics (mg GAE/100 g)

The total phenolics of pigment concentrate from mangosteen fruit rind are displayed in Table 6. Total phenolic content differed significantly with extraction methods and varied from 12.30±1.53 to 32.25±0.30 mg GAE/100 g. Significantly highest total phenolics was recorded in MAE with acidified solvent (32.25±0.30 mg GAE/100 g) which was followed by acidified solvent extraction method (15.00±0.67 mg GAE/100 g) and acidified aqueous extraction method (14.90±0.67 mg GAE/100 g). However, the lowest value was observed in aqueous extraction method (12.30±1.53 mg GAE/100 g).

4.1.2.7 Total flavonoid content (mg QE/100 g)

The observations on total flavonoid content of pigment concentrate from mangosteen fruit rind as affected by the different extraction methods are depicted in Table 6. Statistical analysis of total flavonoid content showed noticeable differences with method of extraction. The range of total flavonoid content values varied from 15.47±0.02 to 40.02±3.52 mg QE/100 g. Irrespective of treatments, highest flavonoid content (40.02±3.52 mg QE/100 g) was registered in MAE with acidified solvent which was followed by acidified solvent extraction method (27.27±1.19 mg QE/100g), while lower value (15.47±0.02 mg QE/100 g) was observed in aqueous extraction method which was on par with acidified aqueous extraction method (15.87±1.39 mg QE/100 g).

4.1.2.8 Non-enzymatic browning

The observations with respect to non-enzymatic browning of the anthocyanin pigment concentrates from mangosteen fruit rind as affected by the different extraction methods were not detected in the samples (Table 6).

Table 6: Effect of different extraction methods on antioxidant properties of anthocyanin pigment concentrate from mangosteen fruit rind

Treatments	DPPH (µl/ml)	FRAP (µl/ml)	ABTS (µl/ml)	Phenols (mg GAE/100g)	Flavonoids (mg QE/100g)	Non-enzymatic browning
T_1	9.57±1.28^{a}	6.68±0.06^{a}	8.80±0.29^{a}	12.30±1.53^{c}	15.47±0.02^{c}	ND
T_2	4.32±0.09^{b}	4.68±0.08^{b}	7.03±0.05^{b}	14.90±0.67^{b}	15.87±1.39^{c}	ND
T_3	5.59±1.83^{b}	4.33±0.18^{b}	4.58±0.02^{c}	13.50±0.25^{bc}	23.70±1.65^{b}	ND
T_4	3.25±1.00^{b}	3.76±0.08^{bc}	3.04±0.03^{d}	15.00±0.67^{b}	27.27±1.19^{b}	ND
T_5	2.29±0.24^{b}	2.83±0.21^{c}	2.02±0.03^{e}	32.25±0.30^{a}	40.02±3.52^{a}	ND
S.Em±	1.10	0.33	0.13	0.82	1.92	
CD (0.05)	3.51	1.06	0.41	2.62	6.14	

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)
GAE: Gallic Acid Equivalent; **QE:** Quercetin Equivalent; **ND:** not detected

Treatments details:

T_1 Aqueous (distilled water) extraction
T_2 Acidified aqueous extraction (1 % citric acid)
T_3 Solvent extraction (50 % ethanol)
T_4 Acidified solvent extraction (50 % ethanol with 1 % citric acid)
T_5 Microwave assisted extraction with acidified solvent (50 % ethanol with 1 % citric acid)

4.2 Evaluation of anthocyanin pigment's stability in processed products

4.2.1 Guava squash coloured with anthocyanin pigment

4.2.1.1 Total monomeric anthocyanin content (mg/100 ml)

Total monomeric anthocyanin content of guava squash decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 7). The decrease was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, total monomeric anthocyanin content in the guava squash added with butterfly pea and mangosteen pigment was 0.60±0.02 mg/100 ml and 0.56±0.01 mg/100 ml, respectively. After three months of storage, the highest retention of total monomeric anthocyanin content (0.37±0.02 mg/100 ml) was noticed in guava squash added with butterfly pea pigment stored under refrigerated condition, whereas the lowest retention (0.19±0.01 mg/100 ml) was observed in guava squash added with mangosteen pigment stored under ambient condition.

4.2.1.2 Instrumental colour values

Instrumental colour values for L^* in guava squash decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 8). The decrease was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for L^* in guava squash without pigment, added with butterfly pea and mangosteen pigment was 93.61±0.04, 81.31±0.09 and 77.53±0.08, respectively. After three months of storage, the highest L^* value (81.68±0.23) was noticed in guava squash without pigment stored under refrigerated condition, whereas the lowest L^* value (62.61±0.13) was observed in guava squash added with mangosteen pigment stored under ambient condition.

Table 7: Changes in TMAC of guava squash incorporated with anthocyanin pigment during storage

Treatments	Initial	TMAC (mg/100 ml)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	NA	NA	NA	NA	NA	NA	NA
T_2 – Butterfly pea pigment	0.60±0.02[a]	0.51±0.01[a]	0.43±0.02[a]	0.35±0.01[a]	0.55±0.02[a]	0.46±0.01[a]	0.37±0.02[a]
T_3 – Mangosteen pigment	0.56±0.01[b]	0.42±0.02[b]	0.29±0.01[b]	0.19±0.01[b]	0.44±0.02[b]	0.34±0.03[b]	0.24±0.01[b]
S.Em±	0.01	0.01	0.01	0.01	0.02	0.02	0.01
C.D (0.05)	0.04	0.04	0.04	0.03	0.05	0.05	0.04

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)
NA = not available

Table 8: Changes in *L value of guava squash incorporated with anthocyanin pigment during storage**

Treatments	Initial	*L** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	93.61±0.04^{a}	79.44±0.12^{a}	71.31±0.07^{a}	61.10±0.08^{b}	91.71±0.12^{a}	88.01±0.27^{a}	81.68±0.23^{a}
T_2 – Butterfly pea pigment	81.31±0.09^{b}	75.99±0.03^{b}	69.65±0.05^{b}	64.70±0.09^{a}	78.54±0.14^{b}	75.93±0.11^{b}	67.71±0.19^{b}
T_3 – Mangosteen pigment	77.53±0.08^{c}	70.87±0.09^{c}	65.31±0.09^{c}	60.61±0.11^{c}	75.20±0.15^{c}	70.37±0.23^{c}	62.61±0.13^{c}
S.Em±	0.07	0.09	0.07	0.09	0.14	0.21	0.19
C.D (0.05)	0.23	0.28	0.22	0.29	0.43	0.67	0.58

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Instrumental colour values for *a** in guava squash decreased significantly throughout the storage period, irrespective of storage conditions except in guava squash added with butterfly pea pigment (Table 9). The decrease was rapid in the guava squash stored under refrigerated condition as compared to ambient condition. Before storage, instrumental colour value for *a** in guava squash without pigment, added with butterfly pea and mangosteen pigment was -28.05±0.06, 5.40±0.16 and 45.38±0.16, respectively. After three months of storage, the lowest *a** value (-22.33±0.12) was noticed in guava squash without pigment stored under refrigerated condition, whereas the highest *a** value (38.59±0.11) was observed in guava squash added with mangosteen pigment stored under ambient condition.

Instrumental colour values for *b** in guava squash decreased significantly throughout the storage period, irrespective of storage conditions except in guava squash added with butterfly pea pigment (Table 10). The decrease was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *b** in guava squash without pigment, added with butterfly pea and mangosteen pigment was 38.84±0.39, -37.05±0.19 and 45.15±0.09, respectively. After three months of storage, the lowest *b** value (-27.53±0.14) was noticed in guava squash added with butterfly pea pigment stored under refrigerated condition, whereas the highest *b** value (35.64±0.15) was observed in guava squash added with mangosteen pigment stored under refrigerated condition.

Instrumental colour values for *hue angle* (°) in guava squash increased significantly throughout the storage period, irrespective of storage conditions except in guava squash without pigment (Table 11). The increase was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *hue angle* in guava squash without pigment, added with butterfly pea and mangosteen pigment was 95.04±0.10°, 301.44±0.29° and 35.48±0.19°, respectively. After three months of storage, the highest *hue angle* value (321.01±0.11°) was noticed in guava squash added with butterfly pea pigment stored under ambient condition, whereas the lowest *hue angle* value (45.76±0.22°)

was observed in guava squash added with mangosteen pigment stored under refrigerated condition.

Instrumental colour values for *chroma* in guava squash decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 12). The decrease was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *chroma* in guava squash without pigment, added with butterfly pea and mangosteen pigment was 57.74±0.23, 63.69±0.17 and 55.74±0.08, respectively. After three months of storage, the highest *chroma* value (55.25±0.16) was noticed in guava squash added with butterfly pea pigment stored under refrigerated condition, whereas the lowest *chroma* value (39.98±0.14) was observed in guava squash added with mangosteen pigment stored under ambient condition.

4.2.1.3 Antioxidant properties

4.2.1.3.1 DPPH assay (µl/ml)

DPPH value of guava squash increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 13). The increase was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, DPPH value in the guava squash without pigment, added with butterfly pea and mangosteen pigment was 0.71±0.003, 0.59±0.009 and 0.64±0.004 µl/ml, respectively. After three months of storage, the highest DPPH radical scavenging activity (1.17±0.02 µl/ml) was observed in guava squash added with butterfly pea pigment stored under refrigerated condition, whereas the lowest radical scavenging activity (8.12±0.12 µl/ml) was noticed in guava squash without pigment stored under ambient condition.

Table 9: Changes in *a value of guava squash incorporated with anthocyanin pigment during storage**

Treatments	Initial	*a** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	-28.05±0.06^{c}	-25.84±0.25^{c}	-23.81±0.15^{c}	-20.67±0.18^{c}	-26.55±0.20^{c}	-24.29±0.08^{c}	-22.33±0.12^{c}
T_2 – Butterfly pea pigment	5.40±0.16^{b}	9.02±0.11^{b}	12.65±0.07^{b}	15.41±0.12^{b}	7.56±0.10^{b}	8.81±0.16^{b}	11.84±0.09^{b}
T_3 – Mangosteen pigment	45.38±0.16^{a}	40.94±0.28^{a}	38.12±0.12^{a}	34.49±0.13^{a}	43.02±0.04^{a}	41.15±0.06^{a}	38.59±0.11^{a}
S.Em±	0.14	0.23	0.12	0.15	0.13	0.11	0.11
C.D (0.05)	0.42	0.70	0.37	0.46	0.41	0.33	0.34

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 10: Changes in *b value of guava squash incorporated with anthocyanin pigment during storage**

Treatments	Initial	*b** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	38.84±0.39^{b}	35.42±0.22^{b}	31.90±0.32^{b}	28.99±0.09^{b}	35.98±0.37^{b}	34.07±0.09^{b}	31.91±0.06^{b}
T_2 – Butterfly pea pigment	-37.05±0.19^{c}	-33.39±0.11^{c}	-28.63±0.11^{c}	-24.99±0.10^{c}	-33.84±0.17^{c}	-30.96±0.09^{c}	-27.53±0.14^{c}
T_3 – Mangosteen pigment	45.15±0.09^{a}	41.56±0.10^{a}	36.35±0.07^{a}	30.74±0.10^{a}	43.10±0.07^{a}	40.73±0.11^{a}	35.64±0.15^{a}
S.Em±	0.26	0.15	0.20	0.10	0.24	0.10	0.12
C.D (0.05)	0.79	0.47	0.62	0.30	0.75	0.31	0.39

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 11: Changes in *hue angle* (°) value of guava squash incorporated with anthocyanin pigment during storage

Treatments	Initial	*Hue angle* (°) value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	95.04±0.10[b]	93.25±0.08[b]	90.54±0.12[b]	87.49±0.14[b]	94.13±0.08[b]	91.68±0.21[b]	89.40±0.11[b]
T_2 – Butterfly pea pigment	301.44±0.29[a]	307.68±0.20[a]	313.65±0.22[a]	321.01±0.11[a]	306.31±0.08[a]	311.51±0.10[a]	317.58±0.12[a]
T_3 – Mangosteen pigment	35.48±0.19[c]	41.38±0.10[c]	46.64±0.17[c]	51.55±0.12[c]	39.36±0.14[c]	43.25±0.16[c]	45.76±0.22[c]
S.Em±	0.21	0.14	0.18	0.12	0.10	0.16	0.16
C.D (0.05)	0.64	0.43	0.55	0.38	0.31	0.50	0.50

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 12: Changes in *chroma* value of guava squash incorporated with anthocyanin pigment during storage

Treatments	Initial	*Chroma* value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	57.74±0.23^{b}	53.80±0.19^{b}	50.53±0.07^{b}	46.32±0.16^{b}	55.07±0.09^{b}	52.26±0.18^{b}	49.39±0.13^{b}
T_2 – Butterfly pea pigment	63.69±0.17^{a}	59.51±0.15^{a}	55.46±0.16^{a}	50.31±0.09^{a}	61.54±0.10^{a}	59.27±0.29^{a}	55.25±0.16^{a}
T_3 – Mangosteen pigment	55.74±0.08^{c}	50.37±0.07^{c}	44.34±0.09^{c}	39.98±0.14^{c}	52.47±0.13^{c}	48.40±0.14^{c}	43.41±0.13^{c}
S.Em±	0.17	0.14	0.11	0.14	0.11	0.21	0.14
C.D (0.05)	0.53	0.45	0.36	0.43	0.33	0.66	0.44

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

4.2.1.3.2 ABTS assay (µl/ml)

ABTS value of guava squash increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 13). The increase was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, ABTS value in the guava squash without pigment, added with butterfly pea and mangosteen pigment was 0.69±0.004, 0.58±0.007 and 0.63±0.002 µl/ml, respectively. After three months of storage, the highest ABTS radical scavenging activity (1.06±0.02 µl/ml) was observed in guava squash added with butterfly pea pigment stored under refrigerated condition, whereas the lowest radical scavenging activity (4.77±0.17 µl/ml) was noticed in guava squash without pigment stored under ambient condition.

4.2.1.3.3 FRAP assay (µl/ml)

FRAP value of guava squash increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 13). The increase was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, FRAP value in the guava squash without pigment, added with butterfly pea and mangosteen pigment was 0.74±0.005, 0.61±0.004 and 0.71±0.006 µl/ml, respectively. After three months of storage, the highest ferric reducing antioxidant power (1.24±0.012 µl/ml) was observed in guava squash added with butterfly pea pigment stored under refrigerated condition, whereas lowest ferric reducing antioxidant power (6.59±0.081 µl/ml) was noticed in guava squash without pigment stored under ambient condition.

Table 13: Changes in DPPH, ABTS and FRAP antioxidant activity of guava squash incorporated with anthocyanin pigment during storage

Treatments	Initial	DPPH activity (µl/ml)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	0.71±0.003^a	0.74±0.006^a	1.41±0.03^a	8.12±0.12^a	0.72±0.002^a	1.05±0.011^a	4.94±0.23^a
T_2 – Butterfly pea pigment	0.59±0.009^c	0.60±0.002^c	0.75±0.01^c	2.22±0.09^c	0.59±0.002^c	0.72±0.004^c	1.17±0.02^c
T_3 – Mangosteen pigment	0.64±0.004^b	0.68±0.002^b	0.91±0.01^b	3.90±0.23^b	0.66±0.004^b	0.82±0.008^b	2.35±0.04^b
S.Em±	0.02	0.004	0.02	0.16	0.003	0.03	0.14
C.D (0.05)	0.01	0.012	0.05	0.49	0.01	0.01	0.42
	ABTS activity (µl/ml)						
T_1 – No pigment	0.69±0.004^a	0.72±0.004^a	1.25±0.027^a	4.77±0.17^a	0.69±0.002^a	0.97±0.007^a	2.93±0.08^a
T_2 – Butterfly pea pigment	0.58±0.007^c	0.59±0.002^c	0.72±0.004^c	1.75±0.06^c	0.58±0.002^c	0.70±0.004^c	1.06±0.02^c
T_3 – Mangosteen pigment	0.63±0.002^b	0.66±0.002^b	0.85±0.007^b	2.55±0.09^b	0.64±0.004^b	0.79±0.007^b	1.83±0.02^b
S.Em±	0.01	0.003	0.02	0.12	0.003	0.01	0.05
C.D (0.05)	0.02	0.01	0.05	0.36	0.01	0.02	0.14
	FRAP activity (µl/ml)						
T_1 – No pigment	0.74±0.005^a	1.03±0.011^a	3.19±0.032^a	6.59±0.081^a	0.76±0.003^a	2.07±0.039^a	5.39±0.277^a
T_2 – Butterfly pea pigment	0.61±0.004^c	0.65±0.005^c	0.74±0.008^c	2.35±0.051^c	0.64±0.006^c	0.70±0.005^c	1.24±0.012^c
T_3 – Mangosteen pigment	0.71±0.006^b	0.74±0.005^b	0.92±0.004^b	4.40±0.161^b	0.71±0.005^b	0.88±0.004^b	2.46±0.016^b
S.Em±	0.005	0.008	0.019	0.108	0.005	0.023	0.160
C.D (0.05)	0.016	0.025	0.059	0.337	0.015	0.070	0.499

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

4.2.1.4 Sensory evaluation of guava squash (9-point hedonic scale)

Data on mean sensory scores of guava squash without pigment, added with butterfly pea and mangosteen pigment during initial, first, second and three months after storage under ambient and refrigerated conditions are presented in Tables 14, 15, 16, respectively. Sensory scores of guava squash declined during storage period, irrespective of treatments and storage conditions. The rate of decrease was faster in the guava squash stored under ambient condition as compared to refrigerated condition. After three months of storage, guava squash added with butterfly pea pigment stored under refrigerated condition recorded highest sensory score (48.24), while the lowest (37.68) was noticed in the guava squash without pigment stored under ambient condition.

Note: Non-enzymatic browning in guava squash was not observed.

4.2.2 Guava jelly coloured with anthocyanin pigment

4.2.2.1 Total monomeric anthocyanin content (mg/100 g)

Total monomeric anthocyanin content of guava jelly decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 17). The decrease was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, total monomeric anthocyanin content in the guava jelly added with butterfly pea and mangosteen pigment was 0.49±0.014 mg/100 g and 0.42±0.008 mg/100 g, respectively. After three months of storage, the highest retention of total monomeric anthocyanin content (0.38±0.008 mg/100 g) was noticed in guava jelly added with butterfly pea pigment stored under refrigerated condition, whereas the lowest retention (0.17±0.005 mg/100 g) was observed in guava jelly added with mangosteen pigment stored under ambient condition.

Table 14: Sensory quality of guava squash incorporated with anthocyanin pigment (Initial)

Treatments	Initial						
	Appearance	**Colour**	**Flavour**	**Body and consistency**	**After-taste**	**Overall acceptability**	**Total score**
T_1 – No pigment	8.00	8.00	9.00	9.00	9.00	8.60	51.60
T_2 – Butterfly pea pigment	9.00	9.00	8.60	8.80	8.80	8.88	53.08
T_3 – Mangosteen pigment	8.60	9.00	8.80	9.00	8.80	8.84	53.04
Kendall's W test value	0.66	1.00	0.30	0.20	0.20	0.75	

Kendall's W value: Kendall's coefficient of concordance

Table 15: Changes in sensory quality of guava squash incorporated with anthocyanin pigment stored under ambient condition

Treatments	One MAS under ambient condition						
	Appearance	Colour	Flavour	Body and consistency	After-taste	Overall acceptability	Total score
T_1 – No pigment	7.20	6.80	8.00	8.00	8.20	7.60	45.80
T_2 – Butterfly pea pigment	8.80	8.80	8.20	8.00	8.20	8.40	50.40
T_3 – Mangosteen pigment	8.20	7.80	8.20	8.20	8.00	8.08	48.48
Kendall's W test value	0.86	0.90	0.20	0.20	0.20	0.77	
	Two MAS under ambient condition						
T_1 – No pigment	6.20	6.20	7.00	7.80	7.80	7.00	42.00
T_2 – Butterfly pea pigment	7.80	8.20	7.60	8.00	7.60	7.84	47.04
T_3 – Mangosteen pigment	7.40	7.20	7.40	8.00	7.60	7.52	45.12
Kendall's W test value	0.67	0.86	0.25	0.20	0.10	1.00	
	Three MAS under ambient condition						
T_1 – No pigment	5.40	5.40	6.40	6.80	7.40	6.28	37.68
T_2 – Butterfly pea pigment	7.40	7.20	6.80	7.20	7.20	7.16	42.96
T_3 – Mangosteen pigment	7.40	6.00	6.60	7.00	7.20	6.84	41.04
Kendall's W test value	1.00	0.77	0.20	0.20	0.10	0.96	

Kendall's W value: Kendall's coefficient of concordance

Table 16: Changes in sensory quality of guava squash incorporated with anthocyanin pigment stored under refrigerated condition

Treatments	One MAS under refrigerated condition						
	Appearance	**Colour**	**Flavour**	**Body and consistency**	**After-taste**	**Overall acceptability**	**Total score**
T_1 – No pigment	8.00	8.00	8.20	8.60	8.40	8.20	49.40
T_2 – Butterfly pea pigment	9.00	9.00	8.40	8.40	8.40	8.64	51.84
T_3 – Mangosteen pigment	8.60	8.60	8.60	8.40	8.20	8.48	50.88
Kendall's W test value	0.66	0.76	0.30	0.20	0.20	0.74	
	Two MAS under refrigerated condition						
T_1 – No pigment	8.00	7.40	8.00	8.20	8.20	8.00	47.80
T_2 – Butterfly pea pigment	8.80	8.80	8.20	8.40	8.00	8.44	50.64
T_3 – Mangosteen pigment	8.60	8.20	8.40	8.40	8.00	8.32	49.92
Kendall's W test value	0.65	0.76	0.30	0.20	0.20	0.65	
	Three MAS under refrigerated condition						
T_1 – No pigment	7.00	6.80	7.40	7.60	7.80	7.32	43.92
T_2 – Butterfly pea pigment	8.20	8.40	8.00	8.00	7.60	8.04	48.24
T_3 – Mangosteen pigment	8.20	8.00	7.60	7.80	7.60	7.84	47.04
Kendall's W test value	0.80	0.76	0.47	0.30	0.20	0.80	

Kendall's W value: Kendall's coefficient of concordance

Table 17: Changes in TMAC of guava jelly incorporated with anthocyanin pigment during storage

Treatments	Initial	TMAC (mg/100 g)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	NA	NA	NA	NA	NA	NA	NA
T_2 – Butterfly pea pigment	0.49±0.014[a]	0.44±0.006[a]	0.39±0.009[a]	0.31±0.007[a]	0.47±0.005[a]	0.44±0.006[a]	0.38±0.008[a]
T_3 – Mangosteen pigment	0.42±0.008[b]	0.39±0.009[b]	0.25±0.009[b]	0.17±0.005[b]	0.42±0.009[b]	0.32±0.005[b]	0.25±0.004[b]
S.Em±	0.009	0.006	0.007	0.005	0.006	0.005	0.005
C.D (0.05)	0.028	0.019	0.23	0.016	0.019	0.015	0.016

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)
NA = not available

4.2.2.2 Instrumental colour values

Instrumental colour values for *L** in guava jelly decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 18). The decrease was rapid in the guava jelly stored under refrigerated condition as compared to ambient condition. Before storage, instrumental colour value for *L** in guava jelly without pigment, added with butterfly pea and mangosteen pigment was 99.51±0.07, 71.51±0.13 and 79.41±0.09, respectively. After three months of storage, the highest *L** value (87.49±0.20) was noticed in guava jelly without pigment stored under refrigerated condition, whereas the lowest *L** value (44.52±0.13) was observed in guava jelly added with butterfly pea pigment stored under ambient condition.

Instrumental colour values for *a** in guava jelly decreased significantly throughout the storage period, irrespective of storage conditions except in guava jelly added with butterfly pea pigment (Table 19). The decrease was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *a** in guava jelly without pigment, added with butterfly pea and mangosteen pigment was 29.30±0.14, 1.60±0.16 and 45.49±0.12, respectively. After three months of storage, the lowest *a** value (10.39±0.16) was noticed in guava jelly with butterfly pea pigment stored under refrigerated condition, whereas the highest *a** value (38.59±0.11) was observed in guava jelly added with mangosteen pigment stored under refrigerated condition.

Instrumental colour values for *b** in guava jelly decreased significantly throughout the storage period, irrespective of storage conditions except in guava jelly added with butterfly pea pigment (Table 20). The decrease was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *b** in guava jelly without pigment, added with butterfly pea and mangosteen pigment was 40.45±0.14, -42.53±0.15 and 41.56±0.10, respectively. After three months of storage, the lowest *b** value (-32.53±0.14) was noticed in guava jelly added with butterfly pea pigment stored under refrigerated condition, whereas the highest *b** value (34.71±0.22) was

observed in guava jelly added with mangosteen pigment stored under refrigerated condition.

Instrumental colour values for *hue angle* (°) in guava jelly decreased significantly throughout the storage period, irrespective of storage conditions except in guava jelly added with mangosteen pigment (Table 21). The decrease was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *hue angle* in guava jelly without pigment, added with butterfly pea and mangosteen pigment was 84.46±0.15°, 330.33±0.18° and 30.45±0.16°, respectively. After three months of storage, the highest *hue angle* value (311.01±0.10°) was noticed in guava jelly added with butterfly pea pigment stored under refrigerated condition, whereas the lowest *hue angle* value (40.32±0.16°) was observed in guava jelly added with mangosteen pigment stored under refrigerated condition.

Instrumental colour values for *chroma* in guava jelly decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 22). The decrease was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *chroma* in guava jelly without pigment, added with butterfly pea and mangosteen pigment was 50.36±0.08, 63.77±0.12 and 59.29±0.30, respectively. After three months of storage, the highest *chroma* value (55.62±0.14) was noticed in guava jelly added with butterfly pea pigment stored under refrigerated condition, whereas the lowest *chroma* value (41.20±0.10) was observed in guava jelly without pigment stored under refrigerated condition.

4.2.2.3 Antioxidant properties

4.2.2.3.1 DPPH assay (µl/ml)

DPPH value of guava jelly increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 23). The increase was rapid in the guava squash stored under ambient condition as compared to refrigerated condition.

Table 18: Changes in *L value of guava jelly incorporated with anthocyanin pigment during storage**

Treatments	Initial	*L** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	99.51±0.07[a]	90.50±0.10[a]	86.49±0.14[a]	81.43±0.17[a]	98.30±0.10[a]	92.49±0.14[a]	87.49±0.20[a]
T_2 – Butterfly pea pigment	71.51±0.13[c]	65.33±0.14[c]	57.54±0.08[c]	44.52±0.13[c]	67.46±0.17[c]	62.29±0.20[c]	56.34±0.13[c]
T_3 – Mangosteen pigment	79.41±0.09[b]	72.61±0.15[b]	65.28±0.10[b]	56.52±0.13[b]	74.60±0.14[b]	67.60±0.12[b]	60.48±0.17[b]
S.Em±	0.10	0.13	0.11	0.14	0.14	0.16	0.17
C.D (0.05)	0.31	0.42	0.34	0.45	0.44	0.49	0.52

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 19: Changes in *a value of guava jelly incorporated with anthocyanin pigment during storage**

Treatments	Initial	*a** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	29.30±0.14^{b}	26.40±0.10^{b}	22.52±0.06^{b}	17.45±0.07^{b}	27.42±14^{b}	24.41±0.12^{b}	20.48±0.09^{b}
T_2 – Butterfly pea pigment	1.60±0.16^{c}	4.61±0.15^{c}	9.63±0.09^{c}	16.49±0.14^{c}	2.56±0.10^{c}	5.47±0.16^{c}	10.39±0.16^{c}
T_3 – Mangosteen pigment	45.49±0.12^{a}	42.55±0.19^{a}	39.43±0.18^{a}	35.45±0.13^{a}	43.51±0.12^{a}	40.75±0.28^{a}	37.50±0.14^{a}
S.Em±	0.14	0.15	0.12	0.12	0.12	0.20	0.13
C.D (0.05)	0.44	0.47	0.38	0.36	0.37	0.61	0.41

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 20: Changes in *b value of guava jelly incorporated with anthocyanin pigment during storage**

Treatments	Initial	*b** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	40.45±0.14^{b}	36.74±0.35^{b}	32.50±0.08^{b}	28.14±0.32^{b}	38.48±0.16^{b}	35.49±0.13^{b}	32.19±0.20^{b}
T_2 – Butterfly pea pigment	-42.53±0.15^{c}	-38.45±0.16^{c}	-33.51±0.15^{c}	-26.63±0.16^{c}	-40.46±0.15^{c}	-37.38±0.16^{c}	-32.53±0.14^{c}
T_3 – Mangosteen pigment	41.56±0.10^{a}	39.34±0.10^{a}	36.44±0.15^{a}	33.92±0.33^{a}	41.46±0.18^{a}	38.63±0.18^{a}	34.71±0.22^{a}
S.Em±	0.13	0.23	0.13	0.28	0.16	0.16	0.19
C.D (0.05)	0.41	0.71	0.41	0.87	0.5	0.49	0.59

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 21: Changes in *hue angle* (º) value of guava jelly incorporated with anthocyanin pigment during storage

Treatments	Initial	*Hue angle* (º) value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	84.46±0.15[b]	80.39±0.14[b]	75.31±0.08[b]	69.36±0.13[b]	82.51±0.09[b]	79.24±0.12[b]	74.17±0.05[b]
T_2 – Butterfly pea pigment	330.33±0.18[a]	325.07±0.18[a]	318.51±0.13[a]	309.16±0.24[a]	326.24±0.08[a]	320.39±0.15[a]	311.45±0.10[a]
T_3 – Mangosteen pigment	30.45±0.16[c]	33.50±0.10[c]	38.53±0.13[c]	43.46±0.10[c]	32.52±0.15[c]	35.32±0.17[c]	40.32±0.16[c]
S.Em±	0.16	0.15	0.12	0.17	0.11	0.15	0.11
C.D (0.05)	0.50	0.46	0.37	0.52	0.35	0.46	0.35

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 22: Changes in *chroma* value of guava jelly incorporated with anthocyanin pigment during storage

Treatments	Initial	*Chroma* value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	50.36±0.08^{c}	44.50±0.80^{c}	43.46±0.10^{c}	39.44±0.10^{c}	49.37±0.12^{c}	45.68±0.15^{c}	41.20±0.10^{c}
T_2 – Butterfly pea pigment	63.77±0.12^{a}	57.86±0.17^{a}	55.11±0.12^{a}	48.40±0.14^{a}	61.52±0.12^{a}	59.41±0.12^{a}	55.62±0.14^{a}
T_3 – Mangosteen pigment	59.29±0.30^{b}	55.33±0.18^{b}	52.35±0.10^{b}	46.32±0.16^{b}	56.34±0.16^{b}	53.74±0.20^{b}	50.43±0.09^{b}
S.Em±	0.19	0.48	0.11	0.14	0.14	0.16	0.11
C.D (0.05)	0.59	1.50	0.33	0.42	0.42	0.50	0.35

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Before storage, DPPH value in the guava jelly without pigment, added with butterfly pea and mangosteen pigment was 5.12±0.05, 3.86±0.05 and 4.76±0.004 µl/ml, respectively. After three months of storage, the highest DPPH radical scavenging activity (17.30±0.17 µl/ml) was observed in guava jelly added with butterfly pea pigment stored under refrigerated condition, whereas the lowest radical scavenging activity (28.67±0.25 µl/ml) was noticed in guava jelly without pigment stored under ambient condition.

4.2.2.3.2 ABTS assay (µl/ml)

ABTS value of guava jelly increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 23). The increase was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, ABTS value in the guava jelly without pigment, added with butterfly pea and mangosteen pigment was 4.14±0.11, 2.24±0.02 and 3.09±0.03 µl/ml, respectively. After three months of storage, the highest ABTS radical scavenging activity (10.59±0.13 µl/ml) was observed in guava jelly added with butterfly pea pigment stored under refrigerated condition, whereas the lowest radical scavenging activity (23.81±0.18 µl/ml) was noticed in guava jelly without pigment stored under ambient condition.

4.2.2.3.3 FRAP assay (µl/ml)

FRAP value of guava jelly increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 23). The increase was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, FRAP Value in the guava jelly without pigment, added with butterfly pea and mangosteen pigment was 2.58±0.01, 1.86±0.01 and 2.26±0.01 µl/ml, respectively. After three months of storage, the highest ferric reducing antioxidant power (6.19±0.03 µl/ml) was observed in guava jelly added with butterfly pea pigment stored under refrigerated condition, whereas lowest ferric reducing antioxidant power (16.16±0.07 µl/ml) was noticed in guava jelly without pigment stored under ambient condition.

Table 23: Changes in DPPH, ABTS and FRAP antioxidant activity of guava jelly incorporated with anthocyanin pigment during storage

Treatments	Initial	DPPH activity (µl/ml)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	5.12±0.05[a]	9.01±0.06[a]	18.62±0.21[a]	28.67±0.25[a]	6.45±0.02[a]	13.17±0.06[a]	21.37±0.09[a]
T_2 – Butterfly pea pigment	3.86±0.05[c]	5.34±0.04[c]	12.82±0.14[c]	21.42±0.19[c]	4.82±0.02[c]	9.89±0.03[c]	17.30±0.17[c]
T_3 – Mangosteen pigment	4.76±0.04[b]	7.89±0.12[b]	15.55±0.15[b]	23.50±0.10[b]	5.60±0.03[b]	10.97±0.03[b]	19.44±0.17[b]
S.Em±	0.04	0.08	0.17	0.19	0.02	0.04	0.14
C.D (0.05)	0.13	0.25	0.54	0.59	0.07	0.12	0.45
	ABTS activity (µl/ml)						
T_1 – No pigment	4.14±0.11[a]	9.27±0.09[a]	15.74±0.11[a]	23.81±0.18[a]	4.29±0.04[a]	8.25±0.10[a]	15.41±0.21[a]
T_2 – Butterfly pea pigment	2.24±0.02[c]	4.02±0.07[c]	8.64±0.05[c]	19.61±0.22[c]	3.25±0.03[c]	6.28±0.08[c]	10.59±0.13[c]
T_3 – Mangosteen pigment	3.09±0.03[b]	5.15±0.09[b]	10.02±0.20[b]	21.44±0.23[b]	3.95±0.07[b]	7.14±0.08[b]	13.66±0.17[b]
S.Em±	0.07	0.08	0.14	0.21	0.05	0.09	0.17
C.D (0.05)	0.21	0.26	0.42	0.66	0.15	0.28	0.53
	FRAP activity (µl/ml)						
T_1 – No pigment	2.58±0.01[a]	5.17±0.03[a]	9.31±0.07[a]	16.16±0.20[a]	4.76±0.01[a]	7.12±0.04[a]	12.15±0.12[a]
T_2 – Butterfly pea pigment	1.86±0.01[c]	2.46±0.01[c]	4.85±0.03[c]	10.76±0.09[c]	2.21±0.01[c]	3.70±0.04[c]	6.19±0.03[c]
T_3 – Mangosteen pigment	2.26±0.01[b]	4.00±0.02[b]	6.58±0.04[b]	12.68±0.06[b]	3.38±0.01[b]	5.12±0.04[b]	9.99±0.11[b]
S.Em±	0.01	0.02	0.05	0.13	0.01	0.04	0.10
C.D (0.05)	0.04	0.06	0.15	0.41	0.04	0.13	0.30

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

4.2.2.4 Sensory evaluation of guava jelly (9-point hedonic scale)

Data on mean sensory scores of guava jelly without pigment, added with butterfly pea and mangosteen pigment during initial, first, second and three months after storage under ambient and refrigerated conditions are presented in Tables 24, 25, 26, respectively. Sensory scores of guava jelly declined during storage period, irrespective of treatments and storage conditions. The rate of decrease was faster in the guava jelly stored under ambient condition as compared to refrigerated condition. After three months of storage, guava jelly added with mangosteen pigment stored under refrigerated condition recorded highest sensory score (40.00), while the lowest (36.05) was noticed in the guava jelly without pigment stored under ambient condition.

4.2.2.5 Non-enzymatic browning (OD value)

The data pertaining to non-enzymatic browning is presented in the Table 27. Non-significant variation was recorded with respect to non-enzymatic browning throughout the storage period, irrespective of treatments and storage conditions. Before storage, non-enzymatic browning in guava jelly without pigment, added with butterfly pea and mangosteen pigment was 0.167±0.013, 0.161±0.025 and 0.153±0.022, respectively. After three months of storage, the numerically highest value (0.379±0.052) was noticed in guava jelly without pigment stored under ambient condition, whereas the numerically lowest value (0.342±0.021) was observed in guava jelly added with butterfly pea pigment stored under refrigerated condition.

4.2.3 Ash gourd candy coloured with anthocyanin pigment

4.2.3.1 Total monomeric anthocyanin content (mg/100 g)

Total monomeric anthocyanin content of ash gourd candy decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 28). The decrease was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition.

Table 24: Sensory quality of guava jelly incorporated with anthocyanin pigment (Initial)

Treatments	Initial					
	Visual appearance and transparency	Colour	Flavour	After-taste	Overall acceptability	Total score
T_1 – No pigment	8.20	8.20	8.00	8.00	8.05	40.45
T_2 – Butterfly pea pigment	9.00	9.00	8.20	8.20	8.65	43.05
T_3 – Mangosteen pigment	8.80	9.00	8.20	8.20	8.55	42.75
Kendall's W test value	0.65	1.00	0.20	0.20	0.93	

Kendall's W value: Kendall's coefficient of concordance

Table 25: Changes in sensory quality of guava jelly incorporated with anthocyanin pigment stored under ambient condition

Treatments	One MAS under ambient condition					
	Visual appearance and transparency	Colour	Flavour	After-taste	Overall acceptability	Total score
T_1 – No pigment	8.00	7.80	7.80	7.80	7.85	39.25
T_2 – Butterfly pea pigment	8.60	8.80	8.00	8.00	8.35	41.75
T_3 – Mangosteen pigment	8.80	9.00	8.00	8.00	8.45	42.25
Kendall's W test value	0.65	0.95	0.20	0.20	0.93	
	Two MAS under ambient condition					
T_1 – No pigment	7.20	7.60	7.60	7.60	7.50	37.50
T_2 – Butterfly pea pigment	8.00	8.60	8.00	8.00	8.10	40.70
T_3 – Mangosteen pigment	7.80	8.60	8.00	8.00	8.05	40.45
Kendall's W test value	0.65	1.00	0.40	0.40	0.95	
	Three MAS under ambient condition					
T_1 – No pigment	6.60	7.00	7.60	7.60	7.25	36.05
T_2 – Butterfly pea pigment	7.20	8.40	7.80	7.80	7.80	39.00
T_3 – Mangosteen pigment	7.20	8.60	7.80	7.80	7.85	39.25
Kendall's W test value	0.60	0.95	0.20	0.20	0.95	

Kendall's W value: Kendall's coefficient of concordance

Table 26: Changes in sensory quality of guava jelly incorporated with anthocyanin pigment stored under refrigerated condition

Treatments	One MAS under refrigerated condition					
	Visual appearance and transparency	Colour	Flavour	After-taste	Overall acceptability	Total score
T_1 – No pigment	8.20	8.20	7.80	7.80	7.95	39.95
T_2 – Butterfly pea pigment	9.00	9.00	8.00	8.00	8.50	42.50
T_3 – Mangosteen pigment	8.80	9.00	8.00	8.00	8.45	42.25
Kendall's W test value	0.65	1.00	0.20	0.20	0.95	
	Two MAS under refrigerated condition					
T_1 – No pigment	7.40	7.80	7.80	7.80	7.70	38.50
T_2 – Butterfly pea pigment	8.20	9.00	8.00	8.00	8.30	41.50
T_3 – Mangosteen pigment	7.80	9.00	8.00	8.00	8.25	41.05
Kendall's W test value	0.60	1.00	0.20	0.20	0.95	
	Three MAS under refrigerated condition					
T_1 – No pigment	7.00	7.60	7.60	7.60	7.45	37.25
T_2 – Butterfly pea pigment	7.60	8.60	7.80	7.80	7.95	39.75
T_3 – Mangosteen pigment	7.60	8.80	7.80	7.80	8.00	40.00
Kendall's W test value	0.60	0.95	0.20	0.20	0.95	

Kendall's W value: Kendall's coefficient of concordance

Table 27: Changes in non-enzymatic browning of guava jelly incorporated with anthocyanin pigment during storage

Treatments	Initial	Non-enzymatic browning (OD value)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	0.167±0.013	0.275±0.003	0.374±0.029	0.379±0.052	0.241±0.007	0.341±0.035	0.364±0.030
T_2 – Butterfly pea pigment	0.161±0.025	0.254±0.008	0.352±0.028	0.360±0.015	0.231±0.014	0.329±0.029	0.342±0.021
T_3 – Mangosteen pigment	0.153±0.022	0.256±0.006	0.336±0.010	0.347±0.027	0.231±0.012	0.324±0.038	0.353±0.021
S.Em±	0.020	0.006	0.024	0.035	0.011	0.034	0.022
C.D (0.05)	NS	NS	NS	NS	NS	NS	NS

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

The decrease was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, total monomeric anthocyanin content in the ash gourd candy added with butterfly pea and mangosteen pigment was 0.43±0.008 mg/100 g and 0.37±0.007 mg/100 g, respectively. After three months of storage, the highest retention of total monomeric anthocyanin content (0.31±0.008 mg/100 g) was noticed in ash gourd candy added with butterfly pea pigment stored under refrigerated condition, whereas the lowest retention (0.11±0.006 mg/100 g) was observed in ash gourd candy added with mangosteen pigment stored under ambient condition.

4.2.3.2 Instrumental colour values

Instrumental colour values for L^* in ash gourd candy decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 29). The decrease was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for L^* in ash gourd candy without pigment, added with butterfly pea and mangosteen pigment was 98.88±0.10, 95.88±0.04 and 97.50±0.04, respectively. After three months of storage, the highest L^* value (80.58±0.07) was noticed in ash gourd candy without pigment stored under refrigerated condition, whereas the lowest L^* value (73.47±0.14) was observed in ash gourd candy added with butterfly pea pigment stored under ambient condition.

Instrumental colour values for a^* in ash gourd candy decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 30). The decrease was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for a^* in ash gourd candy without pigment, added with butterfly pea and mangosteen pigment was -0.06±0.004, 10.59±0.079 and 50.80±0.112, respectively. After three months of storage, the lowest a^* value (-5.56±0.12) was noticed in ash gourd candy without pigment stored under ambient condition, whereas the highest a^* value (44.50±0.14) was observed in ash gourd candy added with mangosteen pigment stored under refrigerated condition.

Table 28: Changes in TMAC of ash gourd candy incorporated with anthocyanin pigment during storage

Treatments	Initial	TMAC (mg/100 g)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	NA	NA	NA	NA	NA	NA	NA
T_2 – Butterfly pea pigment	0.43±0.008[a]	0.33±0.005[a]	0.26±0.006[a]	0.22±0.005[a]	0.39±0.004[a]	0.34±0.004[a]	0.31±0.008[a]
T_3 – Mangosteen pigment	0.37±0.007[b]	0.28±0.005[b]	0.22±0.002[b]	0.11±0.006[b]	0.34±0.006[b]	0.29±0.006[b]	0.26±0.006[b]
S.Em±	0.006	0.004	0.004	0.005	0.004	0.004	0.006
C.D (0.05)	0.019	0.013	0.011	0.015	0.012	0.013	0.018

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)
NA = not available

Table 29: Changes in *L value of ash gourd candy incorporated with anthocyanin pigment during storage**

Treatments	Initial	*L** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	98.88±0.10[a]	95.45±0.13[a]	87.55±0.08[a]	76.71±0.11[a]	96.64±0.05[a]	88.48±0.10[a]	80.58±0.07[a]
T_2 – Butterfly pea pigment	95.88±0.04[c]	92.42±0.03[c]	82.48±0.16[c]	73.47±0.14[c]	93.49±0.07[c]	84.38±0.30[c]	75.97±0.29[c]
T_3 – Mangosteen pigment	97.50±0.04[b]	94.64±0.12[b]	84.47±0.06[b]	75.35±0.11[b]	95.44±0.13[b]	86.42±0.08[b]	78.47±0.07[b]
S.Em±	0.06	0.10	0.11	0.12	0.09	0.19	0.18
C.D (0.05)	0.20	0.32	0.33	0.37	0.28	0.59	0.56

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 30: Changes in *a value of ash gourd candy incorporated with anthocyanin pigment during storage**

Treatments	Initial	*a** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	-0.06±0.004^{c}	-1.24±0.01^{c}	-2.98±0.01^{c}	-5.56±0.12^{c}	-0.95±0.01^{c}	-1.89±0.03^{c}	-3.07±0.12^{c}
T_2 – Butterfly pea pigment	10.59±0.079^{b}	8.51±0.13^{b}	6.26±0.02^{b}	3.60±0.20^{b}	9.20±0.19^{b}	7.63±0.14^{b}	5.41±0.15^{b}
T_3 – Mangosteen pigment	50.80±0.112^{a}	48.04±0.03^{a}	45.74±0.17^{a}	41.75±0.20^{a}	49.35±0.11^{a}	47.21±0.45^{a}	44.50±0.14^{a}
S.Em±	0.08	0.08	0.10	0.16	0.13	0.27	0.14
C.D (0.05)	0.25	0.24	0.30	0.49	0.39	0.85	0.43

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Instrumental colour values for *b** in ash gourd candy increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 31). The increase was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *b** in ash gourd candy without pigment, added with butterfly pea and mangosteen pigment was 15.51±0.12, -52.16±0.03 and 10.37±0.17, respectively. After three months of storage, the lowest *b** value (-46.53±0.14) was noticed in ash gourd candy added with butterfly pea pigment stored under refrigerated condition, whereas the highest *b** value (24.56±0.14) was observed in ash gourd candy without pigment stored under ambient condition.

Instrumental colour values for *hue angle* (°) in ash gourd candy increased significantly throughout the storage period, irrespective of storage conditions except in ash gourd candy without pigment (Table 32). The increase was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *hue angle* in ash gourd candy without pigment, added with butterfly pea and mangosteen pigment was 106.41±0.14°, 305.38±0.11° and 25.48±0.12°, respectively. After three months of storage, the highest *hue angle* value (318±0.10°) was noticed in ash gourd candy added with butterfly pea pigment stored under ambient condition, whereas the lowest *hue angle* value (35.45±0.17°) was observed in ash gourd candy added with mangosteen pigment stored under refrigerated condition.

Instrumental colour values for *chroma* in ash gourd candy decreased significantly throughout the storage period, irrespective of storage conditions except ash gourd candy without pigment (Table 33). The decrease was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *chroma* in ash gourd candy without pigment, added with butterfly pea and mangosteen pigment was 38.38±0.04, 52.31±0.15 and 56.44±0.10, respectively. After three months of storage, the highest *chroma* value (50.75±0.08) was noticed in ash gourd candy added with mangosteen pigment stored under refrigerated condition, whereas the lowest *chroma* value (42.57±0.11) was

observed in ash gourd candy added with butterfly pea pigment stored under ambient condition.

4.2.3.3 Antioxidant properties

4.2.3.3.1 DPPH assay (µl/ml)

DPPH value of ash gourd candy increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 34). The increase was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, DPPH value in the ash gourd candy without pigment, added with butterfly pea and mangosteen pigment was 5.03±0.04, 3.46±0.02 and 4.19±0.08 µl/ml, respectively. After three months of storage, the highest DPPH radical scavenging activity (9.69±0.05 µl/ml) was observed in ash gourd candy added with butterfly pea pigment stored under refrigerated condition, whereas the lowest radical scavenging activity (22.51±0.12 µl/ml) was noticed in ash gourd candy without pigment stored under ambient condition.

4.2.3.3.2 ABTS assay (µl/ml)

ABTS value of ash gourd candy increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 34). The increase was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, ABTS value in the ash gourd candy without pigment, added with butterfly pea and mangosteen pigment was 3.45±0.04, 1.96±0.02 and 2.50±0.02 µl/ml, respectively. After three months of storage, the highest ABTS radical scavenging activity (8.34±0.09 µl/ml) was observed in ash gourd candy added with butterfly pea pigment stored under refrigerated condition, whereas the lowest radical scavenging activity (18.22±0.33 µl/ml) was noticed in ash gourd candy without pigment stored under ambient condition.

4.2.3.3.3 FRAP assay (µl/ml)

FRAP value of ash gourd candy increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 34).

Table 31: Changes in *b value of ash gourd candy incorporated with anthocyanin pigment during storage**

Treatments	Initial	*b** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	15.51±0.12[a]	17.29±0.15[a]	20.50±0.07[a]	24.56±0.14[a]	16.54±0.14[a]	18.79±0.21[a]	20.59±0.21[a]
T_2 – Butterfly pea pigment	-52.16±0.03[c]	-50.52±0.14[c]	-47.39±0.13[c]	-44.57±0.14[c]	-51.09±0.08[c]	-48.25±0.05[c]	-46.53±0.14[c]
T_3 – Mangosteen pigment	10.37±0.17[b]	13.48±0.12[b]	16.58±0.09[b]	22.56±0.11[b]	11.61±0.17[b]	14.51±0.13[b]	17.40±0.12[b]
S.Em±	0.12	0.14	0.10	0.13	0.14	0.15	0.16
C.D (0.05)	0.37	0.42	0.32	0.41	0.42	0.46	0.51

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 32: Changes in *hue angle* (º) value of ash gourd candy incorporated with anthocyanin pigment during storage

Treatments	Initial	*Hue angle* (º) value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	106.41±0.14^{b}	103.48±0.15^{b}	99.91±0.03^{b}	93.22±0.14^{b}	104.40±0.09^{b}	101.68±0.16^{b}	98.07±0.07^{b}
T_2 – Butterfly pea pigment	305.38±0.11^{a}	307.50±0.14^{a}	313.15±0.12^{a}	318.34±0.10^{a}	306.75±0.11^{a}	308.61±0.19^{a}	311.45±0.10^{a}
T_3 – Mangosteen pigment	25.48±0.12^{c}	33.57±0.07^{c}	46.61±0.01^{c}	60.45±0.17^{c}	28.58±0.08^{c}	31.51±0.16^{c}	35.45±0.17^{c}
S.Em±	0.12	0.13	0.07	0.14	0.09	0.17	0.12
C.D (0.05)	0.38	0.39	0.23	0.45	0.29	0.53	0.38

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 33: Changes in *chroma* value of ash gourd candy incorporated with anthocyanin pigment during storage

Treatments	Initial	*Chroma* value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	38.38±0.04^{c}	40.71±0.15^{c}	43.45±0.12^{c}	42.57±0.11^{c}	39.24±0.06^{c}	41.69±0.12^{c}	43.00±0.16^{c}
T_2 – Butterfly pea pigment	52.31±0.15^{b}	50.36±0.12^{b}	47.75±0.09^{b}	44.36±0.14^{b}	51.81±0.07^{b}	49.43±0.09^{b}	46.62±0.14^{b}
T_3 – Mangosteen pigment	56.44±0.10^{a}	53.41±0.15^{a}	49.23±0.08^{a}	46.35±0.13^{a}	55.23±0.08^{a}	53.77±0.15^{a}	50.75±0.08^{a}
S.Em±	0.11	0.14	0.10	0.13	0.07	0.12	0.13
C.D (0.05)	0.33	0.43	0.30	0.39	0.21	0.38	0.41

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 34: Changes in DPPH, ABTS and FRAP antioxidant activity of ash gourd candy incorporated with anthocyanin pigment during storage

Treatments	Initial	DPPH activity (µl/ml)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	5.03±0.04^{a}	7.48±0.05^{a}	13.05±0.09^{a}	22.51±0.12^{a}	5.62±0.01^{a}	8.91±0.03^{a}	17.14±0.05^{a}
T_2 – Butterfly pea pigment	3.46±0.02^{c}	4.90±0.02^{c}	8.08±0.07^{c}	12.65±0.16^{c}	4.03±0.01^{c}	6.33±0.01^{c}	9.69±0.05^{c}
T_3 – Mangosteen pigment	4.19±0.08^{b}	5.86±0.03^{b}	10.34±0.16^{b}	17.65±0.17^{b}	4.92±0.03^{b}	7.32±0.01^{b}	13.57±0.10^{b}
S.Em±	0.06	0.03	0.12	0.15	0.02	0.02	0.07
C.D (0.05)	0.16	0.10	0.36	0.47	0.06	0.05	0.21
	ABTS activity (µl/ml)						
T_1 – No pigment	3.45±0.04^{a}	6.09±0.18^{a}	10.51±0.25^{a}	18.±0.33^{a}	4.48±0.01^{a}	7.53±0.03^{a}	11.09±0.19^{a}
T_2 – Butterfly pea pigment	1.96±0.02^{c}	3.20±0.05^{c}	6.72±0.10^{c}	12.51±0.09^{c}	2.36±0.01^{c}	4.60±0.04^{c}	8.34±0.09^{c}
T_3 – Mangosteen pigment	2.50±0.02^{b}	4.02±0.05^{b}	8.85±0.07^{b}	15.09±0.20^{b}	3.08±0.03^{b}	6.05±0.06^{b}	10.16±0.09^{b}
S.Em±	0.03	0.11	0.16	0.23	0.02	0.05	0.14
C.D (0.05)	0.08	0.35	0.49	0.72	0.06	0.14	0.42
	FRAP activity (µl/ml)						
T_1 – No pigment	2.43±0.01^{a}	4.62±0.02^{a}	7.67±0.05^{a}	13.08±0.06^{a}	3.91±0.01^{a}	6.75±0.01^{a}	10.76±0.19^{a}
T_2 – Butterfly pea pigment	1.75±0.01^{c}	2.33±0.01^{c}	4.37±0.02^{c}	8.61±0.05^{c}	2.11±0.03^{c}	3.41±0.03^{c}	6.14±0.05^{c}
T_3 – Mangosteen pigment	2.09±0.02^{b}	3.66±0.02^{b}	5.39±0.02^{b}	10.88±0.08^{b}	2.92±0.01^{b}	4.58±0.04^{b}	7.67±0.04^{b}
S.Em±	0.01	0.02	0.03	0.07	0.02	0.03	0.11
C.D (0.05)	0.04	0.05	0.10	0.20	0.07	0.08	0.36

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

The increase was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, FRAP Value in the ash gourd candy without pigment, added with butterfly pea and mangosteen pigment was 2.43±0.01, 1.75±0.01 and 2.09±0.02 µl/ml, respectively. After three months of storage, the highest ferric reducing antioxidant power (6.14±0.05 µl/ml) was observed in ash gourd candy added with butterfly pea pigment stored under refrigerated condition, whereas lowest ferric reducing antioxidant power (13.08±0.06 µl/ml) was noticed in ash gourd candy without pigment stored under ambient condition.

4.2.3.4 Sensory evaluation of guava jelly (9-point hedonic scale)

Data on mean sensory scores of ash gourd candy without pigment, added with butterfly pea and mangosteen pigment during initial, first, second and three months after storage ambient and refrigerated conditions are presented in Tables 35, 36, 37, respectively. Sensory scores of ash gourd candy declined during storage period, irrespective of treatments and storage conditions. The rate of decrease was faster in the ash gourd candy stored under ambient condition as compared to refrigerated condition. After three months of storage, ash gourd candy added with butterfly pea pigment stored under refrigerated condition recorded highest sensory score (48.72), while the lowest (41.28) was noticed in the ash gourd candy without pigment stored under ambient condition.

Note: Non-enzymatic browning in ash gourd was not observed.

4.3 Standardization of extraction method for betalain from basella berries and amaranthus

4.3.1 Basella berries

4.3.1.1 Recovery percentage of pigment concentrate (%)

The data on per cent recovery of betalain pigment of basella berries concentrate as affected by different extraction methods is given in the Table 38. Considerable variation in per cent recovery of betalain pigment was recorded and it ranged between 40.37±0.38 to 53.13±0.41 per cent.

Table 35: Sensory quality of ash gourd candy incorporated with anthocyanin pigment (Initial)

Treatments	Initial						
	Visual appearance	**Colour**	**Flavour**	**Texture**	**After-taste**	**Overall acceptability**	**Total score**
T_1 – No pigment	8.40	8.20	8.40	8.60	8.00	8.32	49.92
T_2 – Butterfly pea pigment	9.00	9.00	8.60	8.80	8.40	8.76	52.56
T_3 – Mangosteen pigment	9.00	9.00	8.60	8.80	8.40	8.76	52.56
Kendall's W test value	0.60	0.80	0.20	0.20	0.40	0.69	

Kendall's W value: Kendall's coefficient of concordance

Table 36: Changes in sensory quality of ash gourd candy incorporated with anthocyanin pigment stored under ambient condition

Treatments	One MAS under ambient condition						
	Visual appearance	Colour	Flavour	Texture	After-taste	Overall acceptability	Total score
T_1 – No pigment	8.40	8.20	8.20	8.20	7.80	8.32	49.12
T_2 – Butterfly pea pigment	9.00	9.00	8.40	8.40	8.00	8.76	51.56
T_3 – Mangosteen pigment	9.00	9.00	8.40	8.40	8.00	8.76	51.56
Kendall's W test value	0.60	0.80	0.20	0.20	0.20	0.69	
	Two MAS under ambient condition						
T_1 – No pigment	7.80	7.80	7.40	7.40	7.60	7.68	45.68
T_2 – Butterfly pea pigment	8.60	8.60	7.80	7.80	7.80	8.12	48.72
T_3 – Mangosteen pigment	8.40	8.20	7.60	7.60	7.80	7.92	47.52
Kendall's W test value	0.65	0.60	0.30	0.30	0.20	0.93	
	Three MAS under ambient condition						
T_1 – No pigment	6.60	6.20	7.00	7.00	7.60	6.88	41.28
T_2 – Butterfly pea pigment	7.60	7.60	7.40	7.40	7.80	7.56	45.36
T_3 – Mangosteen pigment	7.00	6.80	7.20	7.20	7.80	7.20	43.20
Kendall's W test value	0.60	0.86	0.30	0.30	0.20	0.90	

Kendall's W value: Kendall's coefficient of concordance

Table 37: Changes in sensory quality of ash gourd candy incorporated with anthocyanin pigment stored under refrigerated condition

Treatments	One MAS under refrigerated condition						
	Visual appearance	Colour	Flavour	Texture	After-taste	Overall acceptability	Total score
T_1 – No pigment	8.40	8.40	8.40	8.40	7.80	8.24	49.64
T_2 – Butterfly pea pigment	9.00	9.00	8.60	8.60	8.00	8.64	51.84
T_3 – Mangosteen pigment	9.00	9.00	8.60	8.60	8.00	8.64	51.84
Kendall's W test value	0.60	0.60	0.20	0.20	0.20	0.80	
	Two MAS under refrigerated condition						
T_1 – No pigment	8.20	8.00	8.20	8.20	7.80	8.08	48.48
T_2 – Butterfly pea pigment	9.00	9.00	8.40	8.40	8.00	8.56	51.36
T_3 – Mangosteen pigment	8.80	9.00	8.40	8.40	8.00	8.52	51.12
Kendall's W test value	0.65	0.80	0.20	0.20	0.20	0.75	
	Three MAS under refrigerated condition						
T_1 – No pigment	7.00	7.40	7.60	7.60	7.60	7.44	44.64
T_2 – Butterfly pea pigment	8.60	8.60	7.80	7.80	7.80	8.12	48.72
T_3 – Mangosteen pigment	8.20	8.40	7.80	7.80	7.80	8.00	48.00
Kendall's W test value	0.93	0.95	0.20	0.20	0.20	0.93	

Kendall's W value: Kendall's coefficient of concordance

Significantly higher per cent recovery of betalain pigment was recorded in acidified aqueous extraction method (53.13±0.41 %) which was followed by aqueous extraction method (49.53±0.33 %) and the lowest per cent recovery was observed in MAE method (40.37±0.38 %).

4.3.1.2 Moisture content (%)

The results on the moisture content percentage in the pigment concentrate of basella berries are presented in the Table 38. Non-significant variation was recorded in the moisture content as influenced by different extraction methods that had a range from 15.50±0.01 to 15.55±0.02 per cent.

4.3.1.3 Betacyanin content (mg/g)

The observation on the betacyanin content present in the basella berries pigment concentrate is presented in the Table 38. Significant variation was recorded in the betacyanin content as influenced by different extraction methods which ranged from 128.15±4.50 to 605.83±4.10 mg/g. Highest betacyanin content was registered in MAE method (605.83±4.10 mg/g) followed by solvent extraction method (459.80±3.10 mg/g) and the lowest value was reported in acidified aqueous extraction method (128.15±4.50 mg/g).

4.3.1.4 Betaxanthin content (mg/g)

Betaxanthin content of basella berries pigment concentrate as influenced by different extraction methods showed significant variations among the treatments as displayed in Table 38. Betaxanthin content of basella berries pigment concentrate was in the range of 22.00±0.99 to 86.35±1.67 mg/g. Significantly highest betaxanthin content (86.35±1.67 mg/g) was noticed in MAE method which was followed by solvent extraction method (68.20±1.67 mg/g) and the lowest content (22.00±0.99 mg/g) was recorded in acidified aqueous extraction method.

4.3.1.5 Total betalain content (mg/g)

According to the data analyzed, betalain content of basella berries pigment concentrate is displayed in Table 38. However, the betalain content of basella berries

pigment concentrate ranged from 150.15±3.75 to 692.18±2.52 mg/g. Statistically highest value was recorded in MAE method (692.18±2.52 mg/g) which was followed by solvent extraction method (528.00±4.15 mg/g) and the lowest value was noticed in acidified aqueous extraction method (150.15±3.75 mg/g).

4.3.1.6 Colour properties

4.3.1.6.1 Instrumental colour values

The observations pertaining to *L** value of basella berries pigment concentrate are given in the Table 39. All the different treatments showed significant variations as influenced by different extraction methods. Irrespective of the different extraction methods, all the five treatments were in the range of 50.98±0.23 to 69.93±0.05. However, significantly highest *L** value was recorded in acidified aqueous extraction method (69.93±0.05) followed by acidified solvent extraction method (65.53±0.21) and the lowest value was registered in solvent extraction method (50.98±0.23).

The results on instrumental *a** value of basella berries pigment concentrate is presented in the Table 39. Instrumental *a** value varied significantly among different treatments which ranged from 10.48±0.23 to 52.27±0.24. However, significantly highest *a** value was recorded in MAE method (52.27±0.24) which was followed by solvent extraction method (23.45±0.24) and the lower value was recorded in acidified aqueous extraction method (10.48±0.23).

The data with respect to instrumental *b** values of basella berries pigment concentrate varied significantly among different treatments as influenced by extraction methods are displayed in Table 39. The *b** values of basella berries pigment values ranged from 23.24±0.16 to 32.17±0.18. Significantly highest *b** value was recorded in aqueous extraction method (32.17±0.18) which was followed by solvent extraction method (28.35±0.36), whereas the lowest *b** value was recorded in MAE method (23.24±0.16).

Table 38: Effect of different extraction methods on recovery percentage, moisture content and pigment content of betalain pigment concentrate from basella berries

Treatments		Recovery percentage	Moisture content (%)	Betacyanin content (mg/g)	Betaxanthin content (mg/g)	Total betalain content (mg/g)
T_1	Aqueous (distilled water) extraction	49.53±0.33^{b}	15.54±0.01	314.33±9.09^{c}	46.20±0.95^{d}	360.53±8.73^{c}
T_2	Acidified aqueous extraction (1 % citric acid)	53.13±0.41^{a}	15.55±0.02	128.15±4.50^{e}	22.00±0.99^{e}	150.15±3.75^{e}
T_3	Solvent extraction (50% ethanol)	42.13±0.39^{d}	15.50±0.01	459.80±3.10^{b}	68.20±1.67^{b}	528.00±4.15^{b}
T_4	Acidified solvent extraction (50 % ethanol with 1 % citric acid)	44.13±0.26^{c}	15.52±0.01	214.50±4.83^{d}	62.70±1.43^{c}	277.20±6.25^{d}
T_5	Microwave assisted extraction with solvent medium (50 % ethanol)	40.37±0.38^{e}	15.52±0.02	605.83±4.10^{a}	86.35±1.67^{a}	692.18±2.52^{a}
S.Em±		0.36	0.01	5.53	1.38	5.53
CD (0.05)		1.14	NS	17.64	4.40	17.64

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 39: Effect of different extraction methods on instrumental colour values of betalain pigment concentrate from basella berries

Treatments		*L** value	*a** value	*b** value	*Chroma*	*Hue angle*
T_1	Aqueous (distilled water) extraction	64.68±0.12^{c}	10.83±0.39^{d}	32.17±0.18^{a}	30.78±0.40^{c}	68.41±0.23^{b}
T_2	Acidified aqueous extraction (1 % citric acid)	69.93±0.05^{a}	10.48±0.23^{d}	26.22±0.14^{c}	26.65±0.31^{e}	62.51±0.15^{c}
T_3	Solvent extraction (50% ethanol)	50.98±0.23^{e}	23.45±0.24^{b}	28.35±0.36^{b}	39.66±0.17^{b}	54.33±0.18^{d}
T_4	Acidified solvent extraction (50 % ethanol with 1 % citric acid)	65.53±0.21^{b}	12.11±0.11^{c}	25.83±0.36^{c}	28.89±0.12^{d}	70.63±0.17^{a}
T_5	Microwave assisted extraction with solvent medium (50 % ethanol)	63.75±0.40^{d}	52.27±0.24^{a}	23.24±0.16^{d}	58.75±0.19^{a}	27.40±0.17^{e}
S.Em±		0.23	0.26	0.23	0.26	0.18
CD (0.05)		0.75	0.82	0.74	0.82	0.59

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

The data related to *chroma* values of basella berries pigment concentrate are displayed in Table 39. *Chroma* values of all the five treatments showed significant differences. *Chroma* values of different treatments as influenced by different extraction methods had a range from 26.65±0.31 to 58.75±0.19. Significantly highest *chroma* value was reported in MAE method (58.75±0.19) followed by solvent extraction method (39.66±0.17) and the lowest value was reported in acidified aqueous extraction method (26.65±0.31).

The data with respect to *hue angle* of basella berries pigment concentrate varied significantly among the treatments as influenced by different extraction methods (Table 39). *Hue angle* values of different treatments were in the range of 27.40±0.17 to 70.63±0.17. However, statistically highest *hue angle* value (70.63±0.17) was recorded in acidified solvent extraction method which was followed by aqueous extraction method (68.41±0.23) and the lowest value was registered in MAE method (27.40±0.17).

4.3.1.7 Antioxidant properties

4.3.1.7.1 DPPH assay (µl/ml)

According to the data analyzed, antioxidant activity of basella berries betalain pigment concentrate showed significant difference among the treatments as influenced by different extraction methods (Table 40). Antioxidant activity of basella berries betalain pigment concentrate as depicted by DPPH method had a range from 3.40±0.04 to 8.58±0.23 µl/ml. Significantly highest antioxidant activity (3.40±0.04 µl/ml) was recorded in MAE method, whereas the lowest antioxidant activity (8.58±0.23 µl/ml) was noticed in aqueous extraction method which was followed by acidified solvent extraction method (5.68±0.15 µl/ml)

4.3.1.7.2 FRAP assay (µl/ml)

The results with respect to antioxidant activity of basella berries betalain pigment concentrate obtained through FRAP method showed statistical variation among different treatments is displayed in the Table 40. Antioxidant activity of basella berries pigment concentrate was in the range of 3.69±0.19 to 13.08±0.41

µl/ml. However, significantly highest antioxidant activity (3.69±0.19 µl/ml) was recorded in MAE method, while lowest antioxidant activity was registered in acidified aqueous extraction method (13.08±0.41 µl/ml) which was followed by acidified solvent extraction method (10.34±0.42 µl/ml).

4.3.1.7.3 ABTS assay (µl/ml)

The results for ABTS antioxidant activity of basella berries betalain pigment as affected by different extraction methods are illustrated in Table 40. Different extraction methods showed statistical differences. According to the data analyzed, the ABTS antioxidant activity of different methods had range from 2.99±0.04 to 9.26±0.27 µl/ml. Among the different treatments, significantly highest antioxidant activity (2.99±0.04 µl/ml) was reported in MAE method, whereas the lowest antioxidant activity (9.26±0.27 µl/ml) was noticed in acidified aqueous extraction method followed by acidified solvent extraction method (7.59±0.22 µl/ml).

4.3.1.7.4 Total phenolics (mg GAE/100 g)

The results on the total phenol content of basella berries pigment concentrate are demonstrated in the Table 40. The total phenol content of five treatments as influenced by different extraction methods showed significant variations and it ranged from 127.90±0.29 to 211.37±0.29 mg GAE/100 g. However, highest total phenol content was reported in MAE method (211.37±0.29 mg GAE/100 g) which was followed by solvent extraction method (200.37±0.94 mg GAE/100 g), whereas the lowest value was noticed in acidified aqueous extraction method (127.90±0.29 mg GAE/100 g).

4.3.1.7.5 Total flavonoid content (mg QE/100 g)

The observations pertaining to total flavonoid content of basella berries pigment concentrate as influenced by different extraction methods showed significant variations among different treatments. The data with regard to total flavonoid content is displayed in the Table 40. The flavonoid content of different treatments was in the range of 46.70±0.26 to 124.07±2.53 mg QE/100 g.

Table 40: Effect of different extraction methods on antioxidant properties of betalain pigment concentrate from basella berries

Treatments	DPPH (µl/ml)	FRAP (µl/ml)	ABTS (µl/ml)	Phenols (mg GAE/100g)	Flavonoids (mg QE/100g)	Non-enzymatic browning
T_1	5.23±0.04^{c}	6.80±0.38^{c}	5.27±0.15^{c}	147.57±0.96^{c}	66.67±1.19^{c}	ND
T_2	8.58±0.23^{a}	13.08±0.41^{a}	9.26±0.27^{a}	127.90±0.29^{e}	46.70±0.26^{d}	ND
T_3	4.79±0.06^{d}	4.33±0.16^{d}	3.63±0.06^{d}	200.37±0.94^{b}	119.19±0.93^{b}	ND
T_4	5.68±0.15^{b}	10.34±0.42^{b}	7.59±0.22^{b}	143.67±0.32^{d}	62.53±0.35^{c}	ND
T_5	3.40±0.04^{e}	3.69±0.19^{d}	2.99±0.04^{e}	211.37±0.29^{a}	124.07±2.53^{a}	ND
SE(m)	0.13	0.33	0.17	0.64	1.33	
CD (5%)	0.4	1.05	0.56	2.05	4.25	

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$);
GAE: Gallic Acid Equivalent; **QE:** Quercetin Equivalent; **ND:** not detected

Treatments details:

T_1 Aqueous (distilled water) extraction

T_2 Acidified aqueous extraction (1% citric acid)

T_3 Solvent extraction (50% ethanol)

T_4 Acidified solvent extraction (50% ethanol with 1% citric acid)

T_5 Microwave assisted extraction with solvent medium (50 % ethanol)

However, the highest value for total flavonoid was recorded in MAE method (124.07±2.53 mg QE/100g) followed by solvent extraction method (119.19±0.93 mg QE/100g), while the lowest value for total flavonoids (46.70±0.26 mg QE/100g) was recorded in acidified aqueous extraction method.

4.3.1.7.5 Non-enzymatic browning

The observations with respect to non-enzymatic browning of the betalain pigment concentrates from basella berries as affected by the different extraction methods were not detected in the samples (Table 40).

4.3.2 Amaranthus

4.3.2.1 Recovery percentage of pigment concentrate (%)

The per cent recovery of betalain pigment concentrates of amaranthus as affected by the different extraction methods are presented in Table 41. Per cent recovery of betalain pigment concentrates showed significant differences among the methods and it ranged from 24.17±0.24 to 58.73±0.43 per cent. Statistically highest betalain pigment per cent recovery was observed in acidified aqueous extraction method (58.73±0.43 %) followed by acidified aqueous extraction method (40.04±0.29 %), whereas the lowest value in comparison to all other methods was registered in aqueous extraction method (24.17±0.24 %).

4.3.2.2 Moisture content (mg/g)

The results on the moisture content percentage in the pigment concentrate of red amaranthus leaves are presented in the Table 41. Non-significant variation was recorded in the moisture content as influenced by different extraction methods that had a range from 15.52±0.06 to 15.60±0.04 per cent.

4.3.2.3 Betacyanin content (mg/g)

Significant variations pertaining to betacyanin content of amaranthus as influenced by different extraction methods were observed (Table 41). However, the betacyanin content of amaranthus varied from 177.93±1.98 to 601.15±2.25 mg/g.

Statistically highest betacyanin content (601.15±2.25 mg/g) was registered in MAE method (50 % Ethanol) followed by solvent extraction method (456.23±5.04 mg/g). However, the lowest betacyanin content (177.93±1.98 mg/g) was recorded in acidified solvent extraction method.

4.3.2.4 Betaxanthin content (mg/g)

Table 41 represents the results of betaxanthin content observed in amaranthus as affected by different extraction methods, which showed significant differences between treatments. The betaxanthin content of amaranthus was in the range of 24.20±1.67 to 75.63±0.55 mg/g. Statistically highest betaxanthin content compared to all other methods was noticed in MAE method (75.63±0.55 mg/g) followed by solvent extraction method (59.68±1.46 mg/g). However, the lowest value was noticed in acidified solvent extraction method (24.20±1.67 mg/g).

4.3.2.5 Total betalain content (mg/g)

The results recorded on total betalain content of amaranthus as influenced by different extraction methods demonstrated noticeable differences (Table 41). Total betalain content of amaranthus ranged from 202.13±2.97 to 676.78±2.79 mg/g. Total betalain content (676.78±2.79 mg/g) registered in MAE method was found to be significantly highest, whereas the lowest value was reported in acidified solvent extraction method (202.13±2.97 mg/g).

4.3.2.6 Colour properties

4.3.2.6.1 Instrumental colour values

The observations pertaining to L^* value of betalain pigment concentrate from amaranthus are given in the Table 42. All the treatments showed significant variations as influenced by different extraction methods. Irrespective of the different extraction methods, instrumental L^* value of all the five treatments were in the range of 41.94±0.06 to 81.57±0.20. However, significantly highest L^* value was recorded in acidified solvent extraction method (81.57±0.20) followed by solvent extraction

method (66.35±0.16), whereas the lowest value was registered in MAE method (41.94±0.06).

The results on instrumental *a** values of betalain pigment concentrate from amaranthus is given in the Table 42. Instrumental *a** value varied significantly among different treatments which ranged from 4.34±0.18 to 33.37±0.22. However, significantly highest instrumental *a** value was reported in MAE method (33.37±0.22) followed by solvent extraction method (26.49±0.14), whereas lowest value was recorded in acidified aqueous extraction method (4.34±0.18).

The data with respect to instrumental *b** values of betalain pigment concentrate from amaranthus varied significantly among different treatments as influenced by extraction methods which is displayed in Table 42. The values ranged from 20.50±0.01 to 56.76±0.02. Significantly highest instrumental *b** value was recorded in solvent extraction method (56.76±0.02) followed by aqueous extraction method (54.59±0.21), whereas the lowest *b** value was recorded in acidified aqueous extraction method (20.50±0.01).

The *chroma* values of betalain pigment concentrate from amaranthus of all the five treatments showed significant differences. The data related to *chroma* values is displayed in Table 42. *Chroma* values of different treatments as influenced by different extraction methods had a range from 20.89±0.03 to 63.37±0.17. Significantly highest *chroma* value was recorded in MAE method (63.37±0.17) followed by solvent extraction method (62.53±0.17). However, the lowest value was recorded in acidified aqueous extraction method (20.89±0.03).

The data with respect to *hue angle* values of betalain pigment concentrate from amaranthus varied significantly among the treatments as influenced by different extraction methods (Table 42). The *Hue angle* values of different treatments were in the range of 58.41±0.02 to 82.61±0.07. However, statistically highest *hue angle* value (82.61±0.07) was reported in aqueous extraction method followed by acidified aqueous extraction method (78.74±0.03) and acidified solvent extraction method (78.64±0.17), whereas lowest value was registered in MAE method (58.41±0.02).

Table 41: Effect of different extraction methods on recovery percentage, moisture content and pigment content of betalain pigment concentrate from red amaranthus leaves

Treatments		Recovery percentage	Moisture content (%)	Betacyanin content (mg/g)	Betaxanthin content (mg/g)	Total betalain content (mg/g)
T_1	Aqueous (distilled water) extraction	24.17±0.24^{d}	15.60±0.04	233.75±1.98^{c}	41.53±0.73^{c}	275.28±1.67^{c}
T_2	Acidified aqueous extraction (1 % citric acid)	58.73±0.43^{a}	15.52±0.06	222.75±1.72^{d}	33.28±0.73^{d}	256.03±1.10^{d}
T_3	Solvent extraction (50% ethanol)	30.23±0.61^{c}	15.55±0.02	456.23±5.04^{b}	59.68±1.46^{b}	515.90±6.34^{b}
T_4	Acidified solvent extraction (50 % ethanol with 1 % citric acid)	40.04±0.29^{b}	15.57±0.02	177.93±1.98^{e}	24.20±1.67^{e}	202.13±2.97^{e}
T_5	Microwave assisted extraction with solvent medium (50 % ethanol)	29.23±0.29^{c}	15.58±0.03	601.15±2.25^{a}	75.63±0.55^{a}	676.78±2.79^{a}
S.Em±		0.40	0.04	2.87	1.12	3.49
CD (0.05)		1.26	NS	9.17	3.58	11.14

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 42: Effect of different extraction methods on instrumental colour values of betalain pigment concentrate from red amaranthus leaves

Treatments		*L** value	*a** value	*b** value	*Chroma*	*Hue angle*
T_1	Aqueous (distilled water) extraction	66.35±0.16^{b}	7.42±0.26^{d}	54.59±0.21^{b}	54.56±0.25^{c}	82.61±0.07^{a}
T_2	Acidified aqueous extraction (1 % citric acid)	62.58±0.27^{c}	4.34±0.18^{e}	20.50±0.01^{e}	20.89±0.03^{e}	78.64±0.17^{b}
T_3	Solvent extraction (50 % ethanol)	48.31±0.03^{d}	26.49±0.14^{b}	53.67±0.19^{c}	62.53±0.17^{b}	65.59±0.18^{c}
T_4	Acidified solvent extraction (50 % ethanol with 1 % citric acid)	81.57±0.20^{a}	14.34±0.22^{c}	22.78±0.06^{d}	26.78±0.03^{d}	78.74±0.03^{b}
T_5	Microwave assisted extraction with solvent medium (50 % ethanol)	41.94±0.06^{e}	33.37±0.22^{a}	56.76±0.02^{a}	63.37±0.17^{a}	58.41±0.02^{d}
S.Em±		0.17	0.21	0.13	0.16	0.12
CD (0.05)		0.54	0.67	0.41	0.50	0.37

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

4.3.2.7 Antioxidant properties

4.3.2.7.1 DPPH assay (µl/ml)

According to the data analyzed, antioxidant activity of betalain pigment concentrate from amaranthus showed significant difference among the treatments as influenced by different extraction methods (Table 43). Antioxidant activity of betalain pigment concentrate from amaranthus as depicted by DPPH method had a range from 1.34±0.01 to 2.27±0.01µl/ml. However, significantly highest antioxidant activity (1.34±0.01 µl/ml) was recorded in MAE method, whereas lowest antioxidant activity (2.27±0.01µl/ml) was noticed in acidified solvent extraction method followed by aqueous extraction method (1.92±0.01 µl/ml).

4.3.2.7.2 FRAP assay (µl/ml)

The data with respect to antioxidant activity of betalain pigment concentrate from amaranthus obtained through FRAP method showed statistical variation among different treatments as displayed in the Table 43. Radical scavenging activities of the antioxidants present in the betalain pigment concentrate from amaranthus were in the range of 3.69±0.19 to 13.08±0.41 µl/ml. However, significantly highest antioxidant activity (3.69±0.19 µl/ml) was recorded in MAE method, whereas lowest antioxidant activity was registered in acidified solvent extraction method (13.08±0.41 µl/ml) followed by acidified aqueous extraction method (10.34±0.42 µl/ml).

4.3.2.7.3 ABTS assay (µl/ml)

The values for ABTS antioxidant activity of betalain pigment concentrate from amaranthus as affected by different extraction methods are illustrated in Table 43. Here, different extraction methods showed statistical differences. According to the data analyzed, the ABTS antioxidant activity of different methods ranged from 0.81±0.01 to 1.09±0.01 µl/ml. Among the different treatments, significantly highest antioxidant activity (0.81±0.01 µl/ml) was recorded in MAE method, whereas lowest antioxidant activity (1.09±0.01 µl/ml) was noticed in acidified solvent extraction method followed by aqueous extraction method (1.00±0.01 µl/ml).

4.3.2.7.4 Total phenolics (mg GAE/100 g)

The results on the phenol content of the betalain pigment concentrate from amaranthus are demonstrated in the Table 43. Total phenol content of five treatments as influenced by different extraction methods showed significant variations and it ranged from 139.17±0.29 to 190.03±0.22 mg GAE/100 g. However, highest phenol content was reported in MAE method (190.03±0.22 mg GAE/100 g) which was on par with solvent extraction method (189.20±0.27 mg GAE/100 g), whereas the lowest total phenol content was noticed in acidified solvent extraction method (7.87±0.09 mg/100 g).

4.3.2.7.5 Total flavonoid content (mg QE/100 g)

The observations pertaining to total flavonoid content of betalain pigment concentrate from amaranthus as influenced by different extraction methods showed significant variations among different treatments. The data with regard to total flavonoid content is displayed in the Table 43. Although the flavonoid content of different treatments were in the range of 94.60±0.27 to 179.07±0.49 mg QE/100 g, the highest total flavonoid content was recorded in MAE method (179.07±0.49 mg QE/100 g) followed by solvent extraction method (165.27±1.04 mg QE/100 g), while the lowest total flavonoid content (94.60±0.27 mg QE/100g) was recorded in acidified solvent extraction method.

4.3.2.7.6 Non-enzymatic browning

The observations with respect to non-enzymatic browning of the betalain pigment concentrates from red amaranthus leaves as affected by the different extraction methods were not detected in the samples (Table 43).

Table 43: Effect of different extraction methods on antioxidant properties of betalain pigment concentrate from red amaranthus leaves

Treatments	DPPH (µl/ml)	FRAP (µl/ml)	ABTS (µl/ml)	Phenols (mg GAE/100g)	Flavonoids (mg QE/100g)	Non-enzymatic browning
T_1	1.92±0.01^{b}	6.80±0.38^{c}	1.00±0.01^{b}	144.73±0.48^{c}	107.70±0.52^{c}	ND
T_2	1.69±0.01^{c}	10.34±0.42^{b}	0.93±0.01^{c}	146.13±0.70^{b}	103.13±0.99^{d}	ND
T_3	1.36±0.01^{d}	4.33±0.16^{d}	0.82±0.01^{d}	189.20±0.27^{a}	165.27±1.04^{b}	ND
T_4	2.27±0.01^{a}	13.08±0.41^{a}	1.09±0.01^{a}	139.17±0.29^{d}	94.60±0.27^{e}	ND
T_5	1.34±0.01^{d}	3.69±0.19^{d}	0.81±0.01^{d}	190.03±0.22^{a}	179.07±0.49^{a}	ND
S.Em±	0.01	0.33	0.01	0.43	0.73	
CD (0.05)	0.03	1.05	0.03	1.37	2.32	

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$);
GAE: Gallic Acid Equivalent; **QE:** Quercetin Equivalent; **ND:** not detected

Treatments details:

T_1 Aqueous (distilled water) extraction

T_2 Acidified aqueous extraction (1 % citric acid)

T_3 Solvent extraction (50 % ethanol)

T_4 Acidified solvent extraction (50 % ethanol with 1 % citric acid)

T_5 Microwave assisted extraction with solvent medium (50 % ethanol)

4.4 Evaluation of betalain pigment's stability in processed products

4.4.1 Guava squash coloured with betalain pigment

4.4.1.1 Total betalain content (mg/100 ml)

Total betalain content of guava squash decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 44). The decrease was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, total betalain content in the guava squash added with basella and amaranthus pigment was 0.86±0.008 mg/100 ml and 0.72±0.011 mg/100 ml, respectively. After three months of storage, the highest retention of total betalain content (0.55±0.012 mg/100 ml) was noticed in guava squash added with basella pigment stored under refrigerated condition, whereas the lowest retention (0.23±0.006 mg/100 ml) was observed in guava squash added with amaranthus pigment stored under ambient condition.

4.4.1.2 Instrumental colour values

Instrumental colour values of L^* in guava squash decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 45). The decrease was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for L^* in guava squash without pigment, added with basella and amaranthus pigment was 93.61±0.04, 76.57±0.11 and 78.54±0.14, respectively. After three months of storage, the highest L^* value (81.68±0.23) was noticed in guava squash without pigment stored under refrigerated condition, whereas the lowest L^* value (61.10±0.08) was observed in guava squash without pigment stored under ambient condition.

Instrumental colour values of a^* in guava squash decreased significantly throughout the storage period, irrespective of storage conditions except guava squash without pigment (Table 46). The decrease was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for a^* in guava squash without pigment, added with basella and

amaranthus pigment was -28.05±0.06, 24.47±0.16 and 21.47±0.13, respectively. After three months of storage, the lowest *a** value (-22.33±0.12) was noticed in guava squash without pigment stored under refrigerated condition, whereas the highest *a** value (15.57±0.09) was observed in guava squash added with basella pigment stored under refrigerated condition.

Instrumental colour values of *b** in guava squash increased significantly throughout the storage period, irrespective of storage conditions except in guava squash without pigment (Table 47). The increase was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *b** in guava squash without pigment, added with basella and amaranthus pigment was 38.84±0.39, 6.27±0.09 and 10.33±0.11, respectively. After three months of storage, the lowest *b** value (21.39±0.14) was noticed in guava squash added with basella pigment stored under refrigerated condition, whereas the highest *b** value (31.91±0.06) was observed in guava squash without pigment stored under refrigerated condition.

Instrumental colour values for *hue angle* (°) in guava squash increased significantly throughout the storage period, irrespective of storage conditions except in guava squash without pigment (Table 48). The increase was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *hue angle* in guava squash without pigment, added with basella and amaranthus pigment was 95.04±0.10°, 1.49±0.14° and 3.69±0.24°, respectively. After three months of storage, the highest *hue angle* value (89.40±0.11°) was noticed in guava squash without pigment stored under refrigerated condition, whereas the lowest *hue angle* value (7.47±0.15°) was observed in guava squash added with basella pigment stored under refrigerated condition.

Instrumental colour values for *chroma* in guava squash decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 49). The decrease was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, instrumental

colour value for *chroma* in guava squash without pigment, added with basella and amaranthus pigment was 57.74±0.23, 34.39±0.15 and 25.74±0.08, respectively. After three months of storage, the highest *chroma* value (49.39±0.13) was noticed in guava squash without pigment stored under refrigerated condition, whereas the lowest *chroma* value (10.04±0.12) was observed in guava squash added with amaranthus pigment stored under ambient condition.

4.4.1.3 Antioxidant properties

4.4.1.3.1 DPPH assay (µl/ml)

DPPH value of guava squash increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 50). The increase was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, DPPH value in the guava squash without pigment, added with basella and amaranthus pigment was 0.71±0.003, 0.66±0.002 and 0.68±0.002 µl/ml, respectively. After three months of storage, the highest DPPH radical scavenging activity (1.82±0.053 µl/ml) was observed in guava squash added with basella pigment stored under refrigerated condition, whereas the lowest radical scavenging activity (8.12±0.124 µl/ml) was noticed in guava squash without pigment stored under ambient condition.

4.4.1.3.2 ABTS assay (µl/ml)

ABTS value of guava squash increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 50). The increase was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, ABTS value in the guava squash without pigment, added with basella and amaranthus pigment was 0.69±0.004, 0.65±0.004 and 0.67±0.003 µl/ml, respectively. After three months of storage, the highest ABTS radical scavenging activity (1.72±0.024 µl/ml) was observed in guava squash added with basella pigment stored under refrigerated condition, whereas the lowest radical scavenging activity (4.77±0.169 µl/ml) was noticed in guava squash without pigment stored under ambient condition.

Table 44: Changes in total betalain content of guava squash incorporated with betalain pigment during storage

Treatments	Initial	Total betalain content (mg/100 ml)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	NA	NA	NA	NA	NA	NA	NA
T_2 – Basella pigment	0.86±0.008[a]	0.73±0.002[a]	0.60±0.004[a]	0.35±0.005[a]	0.77±0.008[a]	0.66±0.007[a]	0.55±0.012[a]
T_3 – Amaranthus pigment	0.72±0.011[b]	0.57±0.004[b]	0.43±0.004[b]	0.23±0.006[b]	0.64±0.004[b]	0.49±0.004[b]	0.41±0.004[b]
S.Em±	0.008	0.003	0.003	0.005	0.005	0.005	0.007
C.D (0.05)	0.025	0.008	0.01	0.014	0.016	0.015	0.023

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)
NA = not-available

Table 45: Changes in *L value of guava squash incorporated with betalain pigment during storage**

Treatments	Initial	*L** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	93.61±0.04^{a}	79.44±0.12^{a}	71.31±0.07^{a}	61.10±0.08^{c}	91.71±0.12^{a}	88.01±0.27^{a}	81.68±0.23^{a}
T_2 – Basella pigment	76.57±0.11^{c}	74.57±0.10^{c}	70.65±0.07^{b}	65.40±0.14^{a}	75.62±0.12^{c}	72.68±0.14^{b}	69.76±0.03^{b}
T_3 – Amaranthus pigment	78.54±0.14^{b}	76.42±0.21^{b}	68.81±0.32^{c}	63.69±0.08^{b}	76.09±0.04^{b}	70.87±0.09^{c}	65.32±0.08^{c}
S.Em±	0.10	0.15	0.19	0.10	0.10	0.18	0.14
C.D (0.05)	0.33	0.48	0.61	0.32	0.32	0.57	0.44

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 46: Changes in *a value of guava squash incorporated with betalain pigment during storage**

Treatments	Initial	*a** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	-28.05±0.06^{c}	-25.84±0.25^{c}	-23.81±0.15^{c}	-20.67±0.18^{c}	-26.55±0.20^{c}	-24.29±0.08^{c}	-22.33±0.12^{c}
T_2 – Basella pigment	24.47±0.16^{a}	20.94±0.21^{a}	17.03±0.05^{a}	11.22±0.06^{a}	22.45±0.16^{a}	19.29±0.16^{a}	15.57±0.09^{a}
T_3 – Amaranthus pigment	21.47±0.13^{b}	17.52±0.15^{b}	13.59±0.06^{b}	7.56±0.10^{b}	18.81±0.16^{b}	16.41±0.14^{b}	11.77±0.24^{b}
S.Em±	0.13	0.21	0.10	0.12	0.17	0.13	0.16
C.D (0.05)	0.39	0.65	0.31	0.38	0.54	0.42	0.51

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 47: Changes in *b value of guava squash incorporated with betalain pigment during storage**

Treatments	Initial	*b** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	38.84±0.39[a]	35.42±0.22[a]	31.90±0.32[a]	28.99±0.09[a]	35.98±0.37[a]	34.07±0.09[a]	31.91±0.06[a]
T_2 – Basella pigment	6.27±0.09[c]	9.98±0.35[c]	16.27±0.37[c]	27.28±0.09[c]	8.28±0.12[c]	14.56±0.10[c]	21.39±0.14[c]
T_3 – Amaranthus pigment	10.33±0.11[b]	15.06±0.28[b]	20.73±0.11[b]	27.54±0.27[b]	13.26±0.10[b]	17.54±0.15[b]	23.52±0.13[b]
S.Em±	0.24	0.29	0.29	0.17	0.23	0.11	0.12
C.D (0.05)	0.74	0.90	0.90	0.53	0.73	0.36	0.37

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 48: Changes in *hue angle* (°) value of guava squash incorporated with betalain pigment during storage

Treatments	Initial	*Hue angle* (°) value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	95.04±0.10[a]	93.25±0.08[a]	90.54±0.12[a]	87.49±0.14[a]	94.13±0.08[a]	91.68±0.21[a]	89.40±0.11[a]
T_2 – Basella pigment	1.49±0.14[c]	3.44±0.14[c]	6.39±0.06[c]	10.36±0.09[c]	2.57±0.15[c]	5.19±0.16[c]	7.47±0.15[c]
T_3 – Amaranthus pigment	3.60±0.24[b]	7.51±0.18[b]	9.36±0.14[b]	13.23±0.15[b]	5.92±0.32[b]	8.37±0.11[b]	11.48±0.12[b]
S.Em±	0.17	0.14	0.11	0.13	0.21	0.16	0.13
C.D (0.05)	0.53	0.43	0.35	0.39	0.65	0.51	0.39

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 49: Changes in *chroma* value of guava squash incorporated with betalain pigment during storage

Treatments	Initial	*Chroma* value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	57.74±0.23[a]	53.80±0.19[a]	50.53±0.07[a]	46.32±0.16[a]	55.07±0.09[a]	52.26±0.18[a]	49.39±0.13[a]
T_2 – Basella pigment	34.39±0.15[b]	29.35±0.14[b]	25.26±0.13[b]	19.49±0.12[b]	32.14±0.19[b]	29.63±0.10[b]	25.25±0.16[b]
T_3 – Amaranthus pigment	25.74±0.08[c]	21.37±0.07[c]	15.34±0.09[c]	10.04±0.12[c]	23.00±0.13[c]	18.40±0.14[c]	13.55±0.14[c]
S.Em±	0.17	0.14	0.10	0.14	0.14	0.14	0.15
C.D (0.05)	0.51	0.44	0.32	0.42	0.45	0.44	0.46

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 50: Changes in DPPH, ABTS and FRAP antioxidant activity of guava squash incorporated with betalain pigment during storage

Treatments	Initial	DPPH activity (µl/ml)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	0.71±0.003^{a}	0.74±0.006^{a}	1.41±0.027^{a}	8.12±0.124^{a}	0.72±0.002^{a}	1.05±0.011^{a}	4.94±0.232^{a}
T_2 – Basella pigment	0.66±0.002^{c}	0.70±0.002^{c}	1.10±0.025^{c}	3.89±0.027^{c}	0.63±0.004^{c}	0.76±0.004^{c}	1.82±0.053^{c}
T_3 – Amaranthus pigment	0.68±0.002^{b}	0.71±0.004^{b}	1.33±0.002^{b}	5.23±0.044^{b}	0.67±0.004^{b}	0.89±0.005^{b}	2.88±0.021^{b}
S.Em±	0.003	0.004	0.021	0.077	0.003	0.008	0.138
C.D (0.05)	0.008	0.013	0.066	0.241	0.010	0.023	0.430
	ABTS activity (µl/ml)						
T_1 – No pigment	0.69±0.004^{a}	0.72±0.004^{a}	1.25±0.027^{a}	4.77±0.169^{a}	0.69±0.002^{a}	0.97±0.007^{a}	2.93±0.075^{a}
T_2 – Basella pigment	0.65±0.004^{b}	0.67±0.003^{c}	0.85±0.005^{c}	2.78±0.132^{c}	0.65±0.002^{c}	0.82±0.006^{c}	1.72±0.024^{c}
T_3 – Amaranthus pigment	0.67±0.003^{a}	0.69±0.005^{b}	1.02±0.010^{b}	3.47±0.016^{b}	0.68±0.004^{b}	0.87±0.004^{b}	2.20±0.020^{b}
S.Em±	0.004	0.004	0.017	0.124	0.003	0.006	0.047
C.D (0.05)	0.011	0.013	0.053	0.387	0.009	0.018	0.146
	FRAP activity (µl/ml)						
T_1 – No pigment	0.74±0.005^{a}	1.03±0.011^{a}	3.19±0.032^{a}	6.59±0.081^{a}	0.76±0.003^{a}	2.07±0.039^{a}	5.39±0.277^{a}
T_2 – Basella pigment	0.69±0.004^{c}	0.85±0.014^{c}	1.34±0.021^{c}	4.52±0.043^{c}	0.70±0.006^{c}	1.02±0.011^{c}	1.72±0.024^{c}
T_3 – Amaranthus pigment	0.72±0.002^{b}	0.93±0.004^{b}	2.12±0.037^{b}	5.62±0.093^{b}	0.72±0.002^{b}	1.40±0.079^{b}	3.02±0.053^{b}
S.Em±	0.004	0.011	0.03	0.075	0.004	0.051	0.163
C.D (0.05)	0.012	0.033	0.095	0.234	0.013	0.159	0.509

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

4.4.1.3.3 FRAP assay (µl/ml)

FRAP value of guava squash increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 50). The increase was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, FRAP value in the guava squash without pigment, added with basella and amaranthus pigment was 0.74±0.005, 0.69±0.004 and 0.72±0.002 µl/ml, respectively. After three months of storage, the highest ferric reducing antioxidant power (1.72±0.024 µl/ml) was observed in guava squash added with basella pigment stored under refrigerated condition, whereas the lowest ferric reducing antioxidant power (6.59±0.081 µl/ml) was noticed in guava squash without pigment stored under ambient condition.

4.4.1.4 Sensory evaluation of guava squash (9-point hedonic scale)

Data on mean sensory scores of guava squash without pigment, added with basella and amaranthus pigment during initial, first, second and three months after storage under ambient and refrigerated conditions are presented in Tables 51, 52, 33, respectively. Sensory scores of guava squash declined during storage period, irrespective of treatments and storage conditions. The rate of decrease was faster in the guava squash stored under ambient condition as compared to refrigerated condition. After three months of storage, guava squash added with basella pigment stored under refrigerated condition recorded highest sensory score (46.80), while the lowest (37.68) was noticed in the guava squash without pigment stored under ambient condition.

Note: Non-enzymatic browning in guava squash was not observed.

Table 51: Sensory quality of guava squash incorporated with betalain pigment (Initial)

Treatments	Initial						
	Appearance	**Colour**	**Flavour**	**Body and consistency**	**After-taste**	**Overall acceptability**	**Total score**
T_1 – No pigment	8.00	8.00	9.00	9.00	9.00	8.60	51.60
T_2 – Basella pigment	8.40	8.60	8.80	9.00	8.80	8.72	52.32
T_3 – Amaranthus pigment	8.20	8.20	8.80	8.80	8.80	8.56	51.36
Kendall's W test value	0.30	0.47	0.20	0.20	0.20	0.29	

Kendall's W value: Kendall's coefficient of concordance

Table 52: Changes in sensory quality of guava squash incorporated with betalain pigment stored under ambient condition

Treatments	One MAS under ambient condition						
	Appearance	Colour	Flavour	Body and consistency	After-taste	Overall acceptability	Total score
T_1 – No pigment	7.20	6.80	8.00	8.00	8.20	7.64	45.84
T_2 – Basella pigment	7.40	7.60	8.00	8.00	8.20	7.84	47.04
T_3 – Amaranthus pigment	7.20	7.00	7.80	7.80	8.00	7.56	45.36
Kendall's W test value	0.10	0.65	0.20	0.20	0.20	0.84	
	Two MAS under ambient condition						
T_1 – No pigment	6.20	6.20	7.00	7.80	7.80	7.00	42.00
T_2 – Basella pigment	6.80	7.20	7.60	7.80	7.60	7.40	44.40
T_3 – Amaranthus pigment	6.40	6.40	7.20	7.60	7.60	7.00	42.20
Kendall's W test value	0.24	0.61	0.35	0.20	0.10	0.61	
	Three MAS under ambient condition						
T_1 – No pigment	5.40	5.40	6.40	6.80	7.40	6.28	37.68
T_2 – Basella pigment	6.40	7.20	6.80	7.00	7.00	6.88	41.28
T_3 – Amaranthus pigment	6.20	5.60	6.60	6.80	7.00	6.44	38.64
Kendall's W test value	0.75	0.95	0.20	0.20	0.27	0.96	

Kendall's W value: Kendall's coefficient of concordance

Table 53: Changes in sensory quality of guava squash incorporated with betalain pigment stored under refrigerated condition

Treatments	One MAS under refrigerated condition						
	Appearance	Colour	Flavour	Body and consistency	After-taste	Overall acceptability	Total score
T_1 – No pigment	8.00	8.00	8.20	8.40	8.40	8.20	49.20
T_2 – Basella pigment	8.40	8.60	8.60	8.40	8.40	8.48	50.88
T_3 – Amaranthus pigment	8.00	8.20	8.40	8.20	8.20	8.20	49.20
Kendall's W test value	0.40	0.47	0.30	0.10	0.20	0.57	
	Two MAS under refrigerated condition						
T_1 – No pigment	8.00	7.40	8.00	8.20	8.20	7.96	47.76
T_2 – Basella pigment	8.20	8.40	8.20	8.40	8.00	8.24	49.44
T_3 – Amaranthus pigment	8.00	7.60	8.00	8.20	8.00	7.96	47.76
Kendall's W test value	0.10	0.75	0.10	0.20	0.20	0.53	
	Three MAS under refrigerated condition						
T_1 – No pigment	7.00	6.80	7.40	7.60	7.80	7.32	43.92
T_2 – Basella pigment	7.60	7.80	8.00	7.80	7.80	7.80	46.80
T_3 – Amaranthus pigment	7.40	6.80	7.60	7.60	7.60	7.40	44.40
Kendall's W test value	0.20	0.65	0.47	0.20	0.20	0.66	

Kendall's W value: Kendall's coefficient of concordance

4.4.2 Guava jelly coloured with betalain pigment

4.4.2.1 Total betalain content (mg/100 g)

Total betalain content of guava jelly decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 54). The decrease was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, total betalain content in the guava jelly added with basella and amaranthus pigment was 0.70±0.004 mg/100 g and 0.54±0.002 mg/100 g, respectively. After three months of storage, the highest retention of total betalain content (0.37±0.011 mg/100 g) was noticed in guava jelly added with basella pigment stored under refrigerated condition, whereas the lowest retention (0.11±0.007 mg/100 g) was observed in guava jelly added with amaranthus pigment stored at ambient condition.

4.4.2.2 Instrumental colour values

Instrumental colour values for L^* in guava jelly decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 55). The decrease was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for L^* in guava jelly without pigment, added with basella and amaranthus pigment was 99.51±0.07, 71.63±0.11 and 73.59±0.14, respectively. After three months of storage, the highest L^* value (87.49±0.20) was noticed in guava jelly without pigment stored under refrigerated condition, whereas the lowest L^* value (58.92±0.08) was observed in guava jelly added amaranthus pigment stored under ambient condition.

Instrumental colour values for a^* in guava jelly decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 56). The decrease was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for a^* in guava jelly without pigment, added with basella and amaranthus pigment was 29.30±0.14, 26.92±0.16 and 23.92±0.13, respectively. After three months of storage, the lowest a^* value (10.01±0.10) was noticed in guava jelly added with amaranthus

pigment stored under ambient condition, whereas the highest *a** value (20.48±0.09) was observed in guava jelly without pigment stored under refrigerated condition.

Instrumental colour values for *b** in guava jelly increased significantly throughout the storage period, irrespective of storage conditions except in guava jelly without pigment (Table 57). The increase was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *b** in guava jelly without pigment, added with basella and amaranthus pigment was 40.45±0.14, 7.77±0.09 and 11.83±0.11, respectively. After three months of storage, the lowest *b** value (22.89±0.14) was noticed in guava jelly added with basella pigment stored under refrigerated condition, whereas the highest *b** value (32.19±0.20) was observed in guava jelly without pigment stored under refrigerated condition.

Instrumental colour values for *hue angle* (°) in guava jelly decreased significantly throughout the storage period, irrespective of storage conditions except in guava jelly without pigment (Table 58). The increase was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *hue angle* in guava jelly without pigment, added with basella and amaranthus pigment was 95.04±0.10°, 1.49±0.14° and 3.69±0.24°, respectively. After three months of storage, the highest *hue angle* value (89.40±0.11°) was noticed in guava jelly without pigment stored under refrigerated condition, whereas the lowest *hue angle* value (7.47±0.15°) was observed in guava jelly added with basella pigment stored under refrigerated condition.

Instrumental colour values for *chroma* in guava jelly decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 59). The decrease was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *chroma* in guava jelly without pigment, added with basella and amaranthus pigment was 50.36±0.08, 33.97±0.15 and 25.32±0.08, respectively.

Table 54: Changes in total betalain content of guava jelly incorporated with betalain pigment during storage

Treatments	Initial	Total betalain content (mg/100 g)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	NA	NA	NA	NA	NA	NA	NA
T_2 – Basella pigment	0.70±0.004[a]	0.51±0.005[a]	0.42±0.004[a]	0.20±0.005[a]	0.58±0.007[a]	0.48±0.009[a]	0.37±0.011[a]
T_3 – Amaranthus pigment	0.54±0.002[b]	0.36±0.004[b]	0.28±0.007[b]	0.11±0.006[b]	0.46±0.004[b]	0.31±0.004[b]	0.23±0.007[b]
S.Em±	0.003	0.004	0.004	0.004	0.005	0.005	0.008
C.D (0.05)	0.008	0.012	0.014	0.014	0.015	0.017	0.024

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)
NA = not available

Table 55: Changes in *L value of guava jelly incorporated with betalain pigment during storage**

Treatments	Initial	*L** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	99.51±0.07^{a}	90.50±0.10^{a}	86.49±0.14^{a}	81.43±0.17^{a}	98.30±0.10^{a}	92.49±0.14^{a}	87.49±0.20^{a}
T_2 – Basella pigment	71.63±0.11^{c}	69.80±0.10^{c}	65.71±0.07^{b}	59.43±0.12^{b}	70.68±0.12^{c}	67.91±0.14^{b}	64.82±0.03^{b}
T_3 – Amaranthus pigment	73.59±0.14^{b}	71.65±0.21^{b}	63.87±0.32^{c}	58.92±0.08^{c}	71.14±0.04^{b}	66.10±0.09^{c}	60.38±0.08^{c}
S.Em±	0.11	0.15	0.21	0.13	0.10	0.13	0.12
C.D (0.05)	0.34	0.46	0.64	0.40	0.30	0.39	0.39

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 56: Changes in *a value of guava jelly incorporated with betalain pigment during storage**

Treatments	Initial	*a** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	29.30±0.14[a]	26.40±0.10[a]	22.52±0.06[a]	17.45±0.07[a]	27.42±0.14[a]	24.41±0.12[a]	20.48±0.09[a]
T_2 – Basella pigment	26.92±0.16[b]	23.39±0.21[b]	19.48±0.05[b]	13.67±0.06[b]	24.90±0.16[b]	21.74±0.16[b]	18.02±0.09[b]
T_3 – Amaranthus pigment	23.92±0.13[c]	19.97±0.15[c]	16.04±0.06[c]	10.01±0.10[c]	21.26±0.16[c]	18.86±0.14[c]	14.22±0.24[c]
S.Em±	0.14	0.16	0.06	0.08	0.15	0.14	0.16
C.D (0.05)	0.45	0.50	0.18	0.24	0.47	0.45	0.49

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 57: Changes in *b value of guava jelly incorporated with betalain pigment during storage**

Treatments	Initial	*b** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	40.45±0.14^{a}	36.74±0.35^{a}	32.50±0.08^{a}	28.14±0.32^{b}	38.48±0.16^{a}	35.43±0.13^{a}	32.19±0.20^{a}
T_2 – Basella pigment	7.77±0.09^{c}	11.48±0.35^{c}	17.77±0.37^{c}	25.78±0.09^{c}	9.78±0.12^{c}	16.06±0.10^{c}	22.89±0.14^{c}
T_3 – Amaranthus pigment	11.83±0.11^{b}	16.56±0.28^{b}	22.23±0.11^{b}	29.04±0.27^{a}	14.76±0.10^{b}	19.04±0.15^{b}	25.02±0.13^{b}
S.Em±	0.12	0.33	0.23	0.25	0.13	0.13	0.16
C.D (0.05)	0.36	1.03	0.71	0.77	0.40	0.39	0.5

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 58: Changes in *hue angle* (º) value of guava jelly incorporated with betalain pigment during storage

Treatments	Initial	*Hue angle* (º) value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	84.46±0.15[a]	80.39±0.14[a]	75.31±0.08[a]	69.36±0.13[a]	82.51±0.09[a]	79.24±0.12[a]	74.17±0.05[a]
T_2 – Basella pigment	6.63±0.14[c]	8.58±0.14[c]	11.53±0.06[c]	15.50±0.09[c]	7.71±0.15[c]	10.33±0.16[c]	12.61±0.05[c]
T_3 – Amaranthus pigment	8.74±0.24[b]	12.65±0.18[b]	14.50±0.14[b]	18.37±0.15[b]	11.06±0.32[b]	13.51±0.11[b]	16.62±0.12[b]
S.Em±	0.18	0.15	0.10	0.12	0.21	0.13	0.11
C.D (0.05)	0.56	0.48	0.30	0.38	0.65	0.41	0.35

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 59: Changes in *chroma* value of guava jelly incorporated with betalain pigment during storage

Treatments	Initial	*Chroma* value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	50.36±0.08[a]	47.70±0.10[a]	43.46±0.10[a]	39.44±0.10[a]	49.37±0.12[a]	45.68±0.15[a]	41.20±0.10[a]
T_2 – Basella pigment	33.97±0.15[b]	28.93±0.14[b]	24.84±0.13[b]	19.07±0.12[b]	31.72±0.19[b]	29.21±0.10[b]	24.83±0.16[b]
T_3 – Amaranthus pigment	25.32±0.08[c]	20.95±0.07[c]	14.92±0.09[c]	9.62±0.12[c]	22.58±0.13[c]	17.98±0.14[c]	13.13±0.14[c]
S.Em±	0.11	0.11	0.11	0.11	0.15	0.13	0.14
C.D (0.05)	0.33	0.34	0.34	0.35	0.47	0.41	0.43

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

After three months of storage, the highest *chroma* value (41.20±0.10) was noticed in guava jelly without pigment stored under refrigerated condition, whereas the lowest *chroma* value (9.62±0.12) was observed in guava jelly added with amaranthus pigment stored under ambient condition.

4.4.2.3.1 DPPH assay (µl/ml)

DPPH value of guava jelly increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 60). The increase was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, DPPH value in the guava jelly without pigment, added with basella and amaranthus pigment was 5.12±0.047, 4.23±0.054 and 4.79±0.002 µl/ml, respectively. After three months of storage, the highest DPPH radical scavenging activity (18.45±0.014 µl/ml) was observed in guava jelly added with basella pigment stored under refrigerated condition, whereas the lowest radical scavenging activity (28.67±0.249 µl/ml) was noticed in guava jelly without pigment stored under ambient condition.

4.4.2.3.2 ABTS assay (µl/ml)

ABTS value of guava jelly increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 60). The increase was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, ABTS value in the guava jelly without pigment, added with basella and amaranthus pigment was 4.14±0.11, 2.40±0.02 and 3.15±0.04 µl/ml, respectively. After three months of storage, the highest ABTS radical scavenging activity (11.52±0.12 µl/ml) was observed in guava jelly added with basella pigment stored under refrigerated condition, whereas the lowest radical scavenging activity (23.81±0.18 µl/ml) was noticed in guava jelly without pigment stored under ambient condition.

4.4.2.3.3 FRAP assay (µl/ml)

FRAP value of guava jelly increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 60). The increase was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, FRAP value in the guava jelly without pigment, added with basella and amaranthus pigment was 2.58±0.013, 2.01±0.012 and 2.38±0.002 µl/ml, respectively. After three months of storage, the highest ferric reducing antioxidant power (7.21±0.048 µl/ml) was observed in guava jelly added with basella pigment stored under refrigerated condition, whereas the lowest ferric reducing antioxidant power (16.16±0.201 µl/ml) was noticed in guava jelly without pigment stored under ambient condition.

4.4.2.4 Sensory evaluation of guava jelly (9-point hedonic scale)

Data on mean sensory scores of guava jelly without pigment, added with basella and amaranthus pigment during initial, first, second and three months after storage under ambient and refrigerated conditions are presented in Tables 61, 62, 63, respectively. Sensory scores of guava jelly declined during storage period, irrespective of treatments and storage conditions. The rate of decrease was faster in the guava jelly stored under ambient condition as compared to refrigerated condition. After three months of storage, guava jelly added with basella pigment stored under refrigerated condition recorded highest sensory score (39.45), while the lowest (36.05) was noticed in the guava jelly without pigment stored under ambient condition.

4.4.2.5 Non-enzymatic browning

The data pertaining to non-enzymatic browning is presented in the Table 64. Non-significant variation was recorded with respect to non-enzymatic browning throughout the storage period, irrespective of treatments and storage conditions. Before storage, non-enzymatic browning in guava jelly without pigment, added with basella and red amaranthus pigment was 0.167±0.013, 0.163±0.024 and 0.160±0.014, respectively.

Table 60: Changes in DPPH, ABTS and FRAP antioxidant activity of guava jelly incorporated with betalain pigment during storage

Treatments	Initial	DPPH activity (µl/ml)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	5.12±0.047^{a}	9.01±0.063^{a}	18.62±0.214^{a}	28.67±0.249^{a}	6.45±0.018^{a}	13.17±0.057^{a}	21.37±0.087^{a}
T_2 – Basella pigment	4.23±0.054^{c}	5.55±0.040^{c}	13.29±0.115^{c}	23.23±0.216^{c}	4.94±0.018^{c}	10.13±0.086^{c}	18.45±0.014^{c}
T_3 – Amaranthus pigment	4.79±0.002^{b}	8.14±0.058^{b}	16.13±0.111^{b}	24.63±0.258^{b}	5.69±0.013^{b}	11.59±0.115^{b}	19.77±0.139^{b}
S.Em±	0.04	0.06	0.15	0.24	0.017	0.089	0.095
C.D (0.05)	0.13	0.17	0.48	0.75	0.052	0.277	0.297
	ABTS activity (µl/ml)						
T_1 – No pigment	4.14±0.11^{a}	9.27±0.09^{a}	15.74±0.11^{a}	23.81±0.18^{a}	4.29±0.04^{a}	8.25±0.10^{a}	15.41±0.21^{a}
T_2 – Basella pigment	2.40±0.02^{c}	4.76±0.08^{c}	9.50±0.04^{c}	19.68±0.29^{c}	3.41±0.01^{c}	6.46±0.06^{c}	11.52±0.12^{c}
T_3 – Amaranthus pigment	3.15±0.04^{b}	5.41±0.07^{b}	10.45±0.17^{b}	21.45±0.17^{b}	4.13±0.01^{b}	7.39±0.03^{b}	13.99±0.17^{b}
S.Em±	0.07	0.08	0.12	0.22	0.03	0.07	0.17
C.D (0.05)	0.22	0.25	0.36	0.68	0.08	0.22	0.53
	FRAP activity (µl/ml)						
T_1 – No pigment	2.58±0.013^{a}	5.17±0.025^{a}	9.31±0.070^{a}	16.16±0.201^{a}	4.76±0.013^{a}	7.12±0.044^{a}	12.15±0.116^{a}
T_2 – Basella pigment	2.01±0.012^{c}	2.63±0.019^{c}	5.46±0.034^{c}	11.09±0.121^{c}	2.32±0.018^{c}	4.05±0.042^{c}	7.21±0.048^{c}
T_3 – Amaranthus pigment	2.38±0.002^{b}	4.40±0.021^{b}	7.24±0.103^{b}	13.83±0.013^{b}	3.50±0.029^{b}	5.74±0.159^{b}	10.63±0.177^{b}
S.Em±	0.01	0.02	0.07	0.14	0.02	0.10	0.13
C.D (0.05)	0.03	0.07	0.23	0.42	0.07	0.31	0.39

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 61: Sensory quality of guava jelly incorporated with betalain pigment (Initial)

Treatments	Initial					
	Visual appearance and transparency	**Colour**	**Flavour**	**After-taste**	**Overall acceptability**	**Total score**
T_1 – No pigment	8.20	8.20	8.00	8.00	8.05	40.45
T_2 – Basella pigment	8.60	8.60	8.20	8.40	8.45	42.25
T_3 – Amaranthus pigment	8.60	8.60	8.20	8.20	8.40	42.00
Kendall's W test value	0.40	0.40	0.20	0.30	0.75	

Kendall's W value: Kendall's coefficient of concordance

Table 62: Changes in sensory quality of guava jelly incorporated with betalain pigment stored under ambient condition

Treatments	One MAS under ambient condition					
	Visual appearance and transparency	Colour	Flavour	After-taste	Overall acceptability	Total score
T_1 – No pigment	8.00	7.80	7.80	7.80	7.85	39.25
T_2 – Basella pigment	8.60	8.40	8.00	8.00	8.25	41.25
T_3 – Amaranthus pigment	8.40	8.40	8.00	8.00	8.20	41.00
Kendall's W test value	0.47	0.60	0.20	0.20	0.65	
	Two MAS under ambient condition					
T_1 – No pigment	7.20	7.60	7.60	7.60	7.50	37.50
T_2 – Basella pigment	8.20	8.20	7.80	7.80	8.00	40.00
T_3 – Amaranthus pigment	8.00	8.00	7.80	7.80	7.90	39.50
Kendall's W test value	0.84	0.47	0.20	0.20	0.84	
	Three MAS under ambient condition					
T_1 – No pigment	6.60	7.00	7.60	7.60	7.25	36.05
T_2 – Basella pigment	7.20	7.60	7.80	7.80	7.60	38.00
T_3 – Amaranthus pigment	7.00	7.20	7.80	7.80	7.45	37.25
Kendall's W test value	0.35	0.47	0.20	0.20	0.54	

Kendall's W value: Kendall's coefficient of concordance

Table 63: Changes in sensory quality of guava jelly incorporated with betalain pigment stored under refrigerated condition

Treatments	One MAS under refrigerated condition					
	Visual appearance and transparency	Colour	Flavour	After-taste	Overall acceptability	Total score
T_1 – No pigment	8.20	8.20	7.80	7.80	7.95	39.95
T_2 – Basella pigment	8.40	8.60	8.00	8.00	8.25	41.25
T_3 – Amaranthus pigment	8.20	8.40	8.00	8.00	8.15	40.75
Kendall's W test value	0.20	0.30	0.20	0.20	0.50	
	Two MAS under refrigerated condition					
T_1 – No pigment	7.40	7.80	7.80	7.80	7.70	38.50
T_2 – Basella pigment	8.40	8.20	8.00	8.00	8.15	40.75
T_3 – Amaranthus pigment	8.20	8.20	8.00	8.00	8.10	40.50
Kendall's W test value	0.84	0.40	0.20	0.20	0.84	
	Three MAS under refrigerated condition					
T_1 – No pigment	7.00	7.60	7.60	7.60	7.45	37.25
T_2 – Basella pigment	7.80	8.00	8.00	7.80	7.85	39.45
T_3 – Amaranthus pigment	7.60	7.80	7.80	7.80	7.70	38.70
Kendall's W test value	0.65	0.30	0.30	0.20	0.84	

Kendall's W value: Kendall's coefficient of concordance

Table 64: Changes in non-enzymatic browning of guava jelly incorporated with betalain pigment during storage

Treatments	Initial	Non-enzymatic browning (OD value)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	0.167±0.013	0.275±0.003	0.374±0.029	0.379±0.052	0.241±0.007	0.341±0.035	0.364±0.030
T_2 – Basella pigment	0.163±0.024	0.262±0.002	0.348±0.002	0.353±0.024	0.234±0.009	0.328±0.036	0.349±0.014
T_3 – Amaranthus pigment	0.160±0.014	0.264±0.005	0.357±0.023	0.364±0.013	0.236±0.009	0.333±0.032	0.361±0.007
S.Em±	0.018	0.003	0.021	0.034	0.008	0.034	0.019
C.D (0.05)	NS	NS	NS	NS	NS	NS	NS

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

After three months of storage, the numerically highest value (0.379±0.052) was noticed in guava jelly without pigment stored under ambient condition, whereas the numerically lowest value (0.349±0.014) was observed in guava jelly added with basella pigment stored under refrigerated condition.

4.4.3 Ash gourd candy coloured with betalain pigment

4.4.3.1 Total betalain content (mg/100 g)

Total betalain content of ash gourd candy decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 65). The decrease was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, total betalain content in the ash gourd candy added with basella and amaranthus pigment was 0.75±0.007 mg/100 g and 0.61±0.011 mg/100 g, respectively. After three months of storage, the highest retention of total betalain content (0.44±0.012 mg/100 g) was noticed in ash gourd candy added with basella pigment stored under refrigerated condition, whereas the lowest retention (0.15±0.006 mg/100 g) was observed in ash gourd candy added with amaranthus pigment stored under ambient condition.

4.4.3.2 Instrumental colour values

Instrumental colour values for L^* in ash gourd candy decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 66). The decrease was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for L^* in ash gourd candy without pigment, added with basella and amaranthus pigment was 98.88±0.10, 93.94±0.11 and 95.91±0.14, respectively. After three months of storage, the highest L^* value (87.13±0.03) was noticed in ash gourd candy added with basella pigment stored under refrigerated condition, whereas the lowest L^* value (76.71±0.11) was observed in ash gourd candy without pigment stored under ambient condition. Instrumental colour values for a^* in ash gourd candy decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 67). The decrease was rapid in the ash gourd

candy stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *a** in ash gourd candy without pigment, added with basella and amaranthus pigment was -0.06±0.004, 25.80±0.16 and 22.80±0.13, respectively. After three months of storage, the lowest *a** value (-5.56±0.12) was noticed in ash gourd candy without pigment stored under ambient condition, whereas the highest *a** value (16.90±0.09) was observed in ash gourd candy added with basella pigment stored under refrigerated condition.

Instrumental colour values for *b** in ash gourd candy increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 68). The increase was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *b** in ash gourd candy without pigment, added with basella and amaranthus pigment was 15.51±0.12, 4.51±0.09 and 8.57±0.11, respectively. After three months of storage, the lowest *b** value (19.63±0.14) was noticed in ash gourd candy added with basella pigment stored under refrigerated condition, whereas the highest *b** value (24.56±0.14) was observed in ash gourd candy without pigment stored under ambient condition.

Instrumental colour values for *hue angle* (°) in ash gourd candy increased significantly throughout the storage period, irrespective of storage conditions except in ash gourd candy without pigment (Table 69). The increase was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *hue angle* in ash gourd candy without pigment, added with basella and amaranthus pigment was 106.41±0.14°, 4.16±0.14° and 6.27±0.24°, respectively. After three months of storage, the highest *hue angle* value (98.07±0.07°) was noticed in ash gourd candy without pigment stored under refrigerated condition, whereas the lowest *hue angle* value (10.14±0.15°) was observed in ash gourd candy added with basella pigment stored under refrigerated condition.

Table 65: Changes in total betalain content of ash gourd candy incorporated with betalain pigment during storage

Treatments	Initial	Total betalain content (mg/100 g)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	NA	NA	NA	NA	NA	NA	NA
T_2 – Basella pigment	0.75±0.007[a]	0.62±0.004[a]	0.49±0.004[a]	0.24±0.005[a]	0.65±0.007[a]	0.54±0.008[a]	0.44±0.012[a]
T_3 – Amaranthus pigment	0.61±0.011[b]	0.45±0.002[b]	0.32±0.002[b]	0.15±0.006[b]	0.53±0.006[b]	0.37±0.006[b]	0.30±0.007[b]
S.Em±	0.008	0.003	0.003	0.005	0.005	0.006	0.008
C.D (0.05)	0.024	0.008	0.008	0.014	0.017	0.018	0.025

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)
NA = not available

Table 66: Changes in *L value of ash gourd candy incorporated with betalain pigment during storage**

Treatments	Initial	*L** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	98.88±0.10^{a}	95.45±0.13^{a}	87.55±0.08^{a}	76.71±0.11^{c}	96.64±0.05^{a}	88.48±0.10^{b}	80.58±0.07^{c}
T_2 – Basella pigment	93.94±0.11^{c}	91.94±0.10^{c}	88.02±0.06^{a}	82.77±0.14^{a}	92.99±0.13^{c}	90.05±0.14^{a}	87.13±0.03^{a}
T_3 – Amaranthus pigment	95.91±0.14^{b}	93.79±0.21^{b}	86.18±0.32^{b}	81.06±0.08^{b}	93.46±0.04^{b}	88.24±0.09^{b}	82.69±0.08^{b}
S.Em±	0.12	0.15	0.20	0.11	0.08	0.11	0.06
C.D (0.05)	0.36	0.48	0.61	0.35	0.25	0.35	0.20

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 67: Changes in *a value of ash gourd candy incorporated with betalain pigment during storage**

Treatments	Initial	*a** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	-0.06±0.04^{c}	-1.24±0.01^{c}	-2.98±0.01^{c}	-5.56±0.12^{c}	-0.95±0.01^{c}	-1.89±0.03^{c}	-3.07±0.12^{c}
T_2 – Basella pigment	25.80±0.16^{a}	22.27±0.21^{a}	18.36±0.05^{a}	12.55±0.06^{a}	23.78±0.16^{a}	20.62±0.16^{a}	16.90±0.09^{a}
T_3 – Amaranthus pigment	22.80±0.13^{b}	18.85±0.15^{b}	14.92±0.06^{b}	8.89±0.10^{b}	20.14±0.16^{b}	17.74±0.14^{b}	13.10±0.24^{b}
S.Em±	0.12	0.15	0.04	0.10	0.13	0.13	0.17
C.D (0.05)	0.37	0.47	0.14	0.30	0.41	0.39	0.52

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 68: Changes in *b value of ash gourd candy incorporated with betalain pigment during storage**

Treatments	Initial	*b** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	15.51±0.12^{a}	17.29±0.15^{a}	20.50±0.07^{a}	24.56±0.14^{b}	16.54±0.14^{a}	18.79±0.21^{a}	20.59±0.21^{b}
T_2 – Basella pigment	4.51±0.09^{c}	8.22±0.35^{c}	14.51±0.37^{c}	22.52±0.09^{c}	6.52±0.12^{c}	12.80±0.10^{c}	19.63±0.14^{c}
T_3 – Amaranthus pigment	8.57±0.11^{b}	13.30±0.28^{b}	18.97±0.11^{b}	25.78±0.27^{a}	11.50±0.10^{b}	15.78±0.15^{b}	21.76±0.13^{a}
S.Em±	0.11	0.27	0.23	0.18	0.12	0.16	0.17
C.D (0.05)	0.33	0.85	0.71	0.56	0.37	0.49	0.52

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 69: Changes in *hue angle* (º) value of ash gourd candy incorporated with betalain pigment during storage

Treatments	Initial	*Hue angle* (º) value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	106.41±0.14[a]	103.48±0.15[a]	99.91±0.03[a]	93.22±0.14[a]	104.40±0.09[a]	101.68±0.16[a]	98.07±0.07[a]
T_2 – Basella pigment	4.16±0.14[c]	6.11±0.14[c]	9.06±0.06[c]	13.03±0.09[c]	5.24±0.15[c]	7.86±0.16[c]	10.14±0.15[c]
T_3 – Amaranthus pigment	6.27±0.24[b]	10.18±0.18[b]	12.03±0.14[b]	15.90±0.15[b]	8.59±0.32[b]	11.04±0.11[b]	14.15±0.12[b]
S.Em±	0.18	0.15	0.09	0.13	0.21	0.14	0.12
C.D (0.05)	0.56	0.48	0.27	0.40	0.65	0.45	0.36

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 70: Changes in *chroma* value of ash gourd candy incorporated with betalain pigment during storage

Treatments	Initial	*Chroma* value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	38.38±0.04[a]	40.71±0.15[a]	43.45±0.12[a]	46.35±0.13[a]	39.24±0.06[a]	41.69±0.12[a]	43.00±0.16[a]
T_2 – Basella pigment	32.82±0.15[b]	27.78±0.14[b]	23.69±0.13[b]	17.92±0.12[b]	30.57±0.19[b]	28.06±0.10[b]	23.68±0.16[b]
T_3 – Amaranthus pigment	24.17±0.08[c]	19.80±0.07[c]	13.77±0.09[c]	8.47±0.12[c]	21.43±0.13[c]	16.83±0.14[c]	11.98±0.14[c]
S.Em±	0.1	0.13	0.12	0.12	0.14	0.12	0.16
C.D (0.05)	0.31	0.39	0.36	0.38	0.43	0.38	0.48

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Instrumental colour values for *chroma* in ash gourd candy decreased significantly throughout the storage period, irrespective of storage conditions except in ash gourd candy without pigment (Table 70). The decrease was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *chroma* in ash gourd candy without pigment, added with basella and amaranthus pigment was 38.38±0.04, 32.82±0.15 and 24.17±0.08, respectively. After three months of storage, the highest *chroma* value (46.35±0.13) was noticed in ash gourd candy without pigment stored under ambient condition, whereas the lowest *chroma* value (8.47±0.12) was observed in ash gourd candy added with amaranthus pigment stored under ambient condition.

4.4.3.3 Antioxidant properties

4.4.3.3.1 DPPH assay (µl/ml)

DPPH value of ash gourd candy increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 71). The increase was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, DPPH value in the ash gourd candy without pigment, added with basella and amaranthus pigment was 5.03±0.04, 3.75±0.02 and 4.63±0.09 µl/ml, respectively. After three months of storage, the highest DPPH radical scavenging activity (9.93±0.05 µl/ml) was observed in ash gourd candy added with basella pigment stored under refrigerated condition, whereas the lowest radical scavenging activity (22.51±0.12 µl/ml) was noticed in ash gourd candy without pigment stored under ambient condition.

4.4.3.3.2 ABTS assay (µl/ml)

ABTS value of ash gourd candy increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 71). The increase was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, ABTS value in the ash gourd candy without pigment, added with basella and amaranthus pigment was 3.45±0.04, 2.01±0.03 and 2.68±0.02 µl/ml, respectively. After three months of storage, the highest ABTS

radical scavenging activity (8.82±0.08 µl/ml) was observed in ash gourd candy added with basella pigment stored under refrigerated condition, whereas the lowest radical scavenging activity (18.22±0.33 µl/ml) was noticed in ash gourd candy without pigment stored under ambient condition.

4.4.3.3.3 FRAP assay (µl/ml)

FRAP value of ash gourd candy increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 71). The increase was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, FRAP value in the ash gourd candy without pigment, added with basella and amaranthus pigment was 2.43±0.01, 1.83±0.01 and 2.19±0.03 µl/ml, respectively. After three months of storage, the highest ferric reducing antioxidant power (7.17±0.07 µl/ml) was observed in ash gourd candy added with basella pigment stored under refrigerated condition, whereas the lowest ferric reducing antioxidant power (13.08±0.06 µl/ml) was noticed in ash gourd candy without pigment stored under ambient condition.

4.4.3.4 Sensory evaluation of ash gourd candy (9-point hedonic scale)

Data on mean sensory scores of ash gourd candy without pigment, added with basella and amaranthus pigment during initial, first, second and three months after storage under ambient and refrigerated conditions are presented in Tables 72, 73, 74, respectively. Sensory scores of ash gourd candy declined during storage period, irrespective of treatments and storage conditions. The rate of decrease was faster in the ash gourd candy stored under ambient condition as compared to refrigerated condition. After three months of storage, ash gourd candy added with basella pigment stored under refrigerated condition recorded highest sensory score (47.28), while the lowest (42.88) was noticed in the ash gourd candy without pigment stored under ambient condition.

Note: Non-enzymatic browning in ash gourd candy was not observed.

Table 71: Changes in DPPH, ABTS and FRAP antioxidant activity of ash gourd candy incorporated with betalain pigment during storage

Treatments	Initial	DPPH activity (µl/ml)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	5.03±0.04^{a}	7.48±0.05^{a}	13.05±0.09^{a}	22.51±0.12^{a}	5.62±0.01^{a}	8.91±0.03^{a}	17.14±0.05^{a}
T_2 – Basella pigment	3.75±0.02^{c}	5.74±0.03^{c}	8.93±0.09^{c}	13.30±0.18^{c}	4.63±0.01^{c}	6.55±0.01^{c}	9.93±0.05^{c}
T_3 – Amaranthus pigment	4.63±0.09^{b}	7.11±0.04^{b}	11.78±0.21^{b}	18.94±0.19^{b}	5.15±0.04^{b}	7.61±0.01^{b}	14.05±0.10^{b}
S.Em±	0.06	0.04	0.14	0.17	0.02	0.02	0.07
C.D (0.05)	0.19	0.12	0.44	0.52	0.07	0.06	0.23
	ABTS activity (µl/ml)						
T_1 – No pigment	3.45±0.04^{a}	6.09±0.18^{a}	10.51±0.25^{a}	18.22±0.33^{a}	4.48±0.01^{a}	7.53±0.03^{a}	12.17±0.27^{a}
T_2 – Basella pigment	2.01±0.03^{c}	3.75±0.08^{c}	7.09±0.20^{c}	12.81±0.15^{c}	2.55±0.02^{c}	4.96±0.20^{c}	8.82±0.08^{c}
T_3 – Amaranthus pigment	2.68±0.02^{b}	4.86±0.07^{b}	9.54±0.06^{b}	16.84±0.04^{b}	3.15±0.01^{b}	6.42±0.08^{b}	10.48±0.09^{b}
S.Em±	0.03	0.12	0.19	0.21	0.02	0.12	0.17
C.D (0.05)	0.10	0.38	0.58	0.66	0.05	0.39	0.53
	FRAP activity (µl/ml)						
T_1 – No pigment	2.43±0.01^{a}	4.62±0.02^{a}	7.67±0.05^{a}	13.08±0.06^{a}	3.91±0.01^{a}	6.75±0.01^{a}	10.76±0.19^{a}
T_2 – Basella pigment	1.83±0.01^{c}	2.68±0.01^{c}	5.68±0.04^{c}	9.58±0.07^{c}	2.14±0.04^{c}	4.06±0.04^{c}	7.17±0.07^{c}
T_3 – Amaranthus pigment	2.19±0.03^{b}	3.39±0.06^{b}	6.29±0.13^{b}	12.01±0.42^{b}	2.99±0.01^{b}	5.10±0.07^{b}	8.48±0.02^{b}
S.Em±	0.02	0.03	0.08	0.25	0.02	0.05	0.12
C.D (0.05)	0.05	0.11	0.25	0.78	0.07	0.15	0.36

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 72: Sensory quality of ash gourd candy incorporated with betalain pigment (Initial)

Treatments	Initial						
	Visual appearance	**Colour**	**Flavour**	**Texture**	**After-taste**	**Overall acceptability**	**Total score**
T_1 – No pigment	8.40	8.20	8.40	8.60	8.00	8.32	49.92
T_2 – Basella pigment	8.80	8.80	8.80	8.80	8.40	8.72	52.32
T_3 – Amaranthus pigment	8.60	8.60	8.80	8.80	8.40	8.64	51.84
Kendall's W test value	0.30	0.47	0.40	0.20	0.27	0.65	

Kendall's W value: Kendall's coefficient of concordance

Table 73: Changes in sensory quality of ash gourd candy incorporated with betalain pigment stored under ambient condition

Treatments	One MAS under ambient condition						
	Visual appearance	Colour	Flavour	Texture	After-taste	Overall acceptability	Total score
T_1 – No pigment	8.40	8.20	8.20	8.20	7.80	8.16	48.96
T_2 – Basella pigment	8.60	8.60	8.00	8.40	8.00	8.32	49.92
T_3 – Amaranthus pigment	8.40	8.40	7.80	8.40	8.00	8.20	49.20
Kendall's W test value	0.20	0.20	0.30	0.20	0.20	0.27	
	Two MAS under ambient condition						
T_1 – No pigment	7.80	7.80	7.40	7.40	7.60	7.68	45.68
T_2 – Basella pigment	8.20	8.20	7.60	7.80	7.80	7.92	47.52
T_3 – Amaranthus pigment	8.00	8.00	7.20	7.60	7.80	7.72	46.32
Kendall's W test value	0.30	0.30	0.30	0.30	0.20	0.61	
	Three MAS under ambient condition						
T_1 – No pigment	6.60	7.80	7.00	7.00	7.60	6.88	42.88
T_2 – Basella pigment	7.40	8.20	6.80	7.40	7.80	7.40	45.00
T_3 – Amaranthus pigment	7.20	8.00	6.60	7.20	7.80	7.20	44.00
Kendall's W test value	0.65	0.30	0.30	0.30	0.20	0.80	

Kendall's W value: Kendall's coefficient of concordance

Table 74: Changes in sensory quality of ash gourd candy incorporated with betalain pigment stored under refrigerated condition

Treatments	One MAS under refrigerated condition						
	Visual appearance	Colour	Flavour	Texture	After-taste	Overall acceptability	Total score
T_1 – No pigment	8.40	8.20	8.40	8.40	7.80	8.24	49.44
T_2 – Basella pigment	8.40	8.80	8.60	8.60	8.00	8.48	50.88
T_3 – Amaranthus pigment	8.20	8.20	8.40	8.60	8.00	8.28	49.68
Kendall's W test value	0.20	0.60	0.20	0.20	0.20	0.58	
	Two MAS under refrigerated condition						
T_1 – No pigment	8.20	8.00	8.20	8.20	7.80	8.08	48.48
T_2 – Basella pigment	8.40	8.40	8.20	8.40	8.00	8.28	49.68
T_3 – Amaranthus pigment	8.20	8.20	8.00	8.40	8.00	8.16	48.96
Kendall's W test value	0.20	0.30	0.20	0.20	0.20	0.26	
	Three MAS under refrigerated condition						
T_1 – No pigment	7.00	7.40	7.60	7.60	7.60	7.44	44.64
T_2 – Basella pigment	7.80	8.00	8.00	7.80	7.80	7.88	47.28
T_3 – Amaranthus pigment	7.60	7.60	7.60	7.80	7.80	7.68	46.08
Kendall's W test value	0.65	0.48	0.40	0.20	0.20	0.87	

Kendall's W value: Kendall's coefficient of concordance

4.5 Standardization of extraction method for carotenoid pigment concentrate from gac fruit seed aril and marigold petals

4.5.1 Gac fruit seed aril

4.5.1.1 Recovery percentage of pigment concentrate (%)

The data on per cent recovery of carotenoid pigment concentrate from gac fruit seed aril as affected by different extraction methods is given in the Table 75. Considerable variation in per cent recovery of carotenoid pigment was recorded and it ranged between 73.33±0.12 to 89.53±0.13 per cent. Significantly highest per cent recovery of carotenoid pigment was recorded in MAE with ethanol method (89.53±0.13 %) which was followed by ethanol extraction method (88.43±0.19 %), while the lowest per cent recovery was observed in MAE with ethanol and ethyl lactate extraction method (73.33±0.12 %).

4.5.1.2 Moisture content (%)

The results on the moisture content percentage in the pigment concentrate of gac fruit seed aril are presented in the Table 75. Non-significant variation was recorded in the moisture content as influenced by different extraction methods. The values ranged from 15.58±0.03 to 15.51±0.05 per cent.

4.5.1.3 Total carotenoid content (µg/g)

The observation on the total carotenoid content present in the carotenoid pigment concentrate from gac fruit seed aril is presented in the Table 75. Significant variation was recorded in the total carotenoid content as influenced by different extraction methods that had a range from 1488.81±5.91 to 2069.83±4.38 µg/g. Statistically highest total carotenoid content was registered in ethyl lactate extraction method (2069.83±4.38 µg/g) followed by MAE with ethanol and ethyl lactate extraction method (1897.76±5.98 µg/g), while the lowest value was reported in MAE with ethanol extraction method (1488.81±5.91 µg/g).

4.5.1.4 β-carotene content (µg/g)

β-carotene content of carotenoid pigment concentrate from gac fruit seed aril as influenced by different extraction methods showed significant variations among the treatments and are displayed in Table 75. β-carotene content of pigment concentrate from gac fruit seed aril were in the range of 536.64±0.65 to 689.43±1.04 µg/g. Significantly highest value (689.43±1.04 µg/g) for β-carotene content was noticed in ethyl lactate extraction method which was followed by MAE with ethanol and ethyl lactate extraction method (648.67±0.96 µg/g), whereas the lowest value (536.64±0.65 µg/g) was recorded in ethanol extraction method.

4.5.1.5 Lycopene content (µg/g)

According to the data analyzed, lycopene content of carotenoid pigment concentrate from gac fruit seed aril is given in Table 75. However, the lycopene content of pigment concentrate from gac fruit seed aril ranged from 791.07±0.25 to 1052.31±0.87 µg/g. Statistically highest value was recorded in ethyl lactate extraction method (1052.31±0.87 µg/g) followed by MAE with ethanol and ethyl lactate extraction method (981.15±0.74 µg/g), while the lowest value was noticed in ethanol extraction method (791.07±0.25 µg/g).

4.5.1.6 Lutein content (µg/g)

According to the data analyzed, lutein content of carotenoid pigment concentrate from gac fruit seed aril is recorded in Table 75. However, the lutein content of pigment concentrate from gac fruit seed aril ranged from 38.47±1.64 to 216.96±0.22 µg/g. Statistically highest value was recorded in ethyl lactate extraction method (216.96±0.22 µg/g) followed by MAE with ethanol and ethyl lactate extraction method (179.68±2.27 µg/g), while the lowest value was noticed in ethanol extraction method (38.47±1.64 µg/g).

Table 75: Effect of different extraction methods on recovery percentage, moisture content and pigment content of carotenoid pigment concentrate from gac fruit seed aril

Treatments		Recovery percentage	Moisture content (%)	Total carotenoid content (µg/g)	β-carotene content (µg/g)	Lycopene content (µg/g)	Lutein content (µg/g)
T_1	Ethanol	88.43±0.19^{b}	15.51±0.05	1488.81±5.91^{e}	536.64±0.65^{e}	791.07±0.25^{e}	38.47±1.64^{e}
T_2	Ethyl lactate	81.40±0.23^{c}	15.57±0.02	2069.83±4.38^{a}	689.43±1.04^{a}	1052.31±0.87^{a}	216.96±0.22^{a}
T_3	Ethanol and ethyl lactate (1:1 ratio)	77.27±0.35^{d}	15.56±0.03	1764.56±5.71^{c}	601.59±0.87^{c}	971.42±0.98^{c}	160.93±0.91^{c}
T_4	MAE with ethanol	89.53±0.13^{a}	15.57±0.01	1531.64±4.54^{d}	553.14±0.69^{d}	831.42±0.57^{d}	84.79±2.43^{d}
T_5	MAE with ethanol and ethyl lactate (1:1 ratio)	73.33±0.12^{e}	15.58±0.03	1897.76±5.98^{b}	648.67±0.96^{b}	981.15±0.74^{b}	179.68±2.27^{b}
S.Em±		0.22	0.03	5.35	0.86	0.73	1.71
CD (0.05)		0.67	NS	16.28	2.60	2.21	5.21

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

4.5.1.7 Colour properties

4.5.1.7.1 Instrumental colour values

The observations pertaining to *L** value of carotenoid pigment concentrate from gac fruit seed aril are given in the Table 76. All the different treatments showed significant variations as influenced by different extraction methods. Irrespective of the different extraction methods, all the five treatments were in the range of 60.44±0.12 to 67.49±2.05. However, significantly higher *L** value was recorded in ethanol extraction method (67.49±2.05) followed by ethanol and ethyl lactate extraction method (66.38±0.03), while the lowest value was registered in ethyl lactate extraction method (60.44±0.12).

The results on instrumental *a** value of carotenoid pigment concentrate from gac fruit seed aril is displayed in the Table 76. Instrumental *a** value varied significantly among different treatments which ranged from 50.84±0.02 to 61.35±0.20. However, significantly highest *a** value was reported in ethyl lactate extraction method (61.35±0.20) followed by MAE with ethanol and ethyl lactate extraction method (58.60±0.16), while the lowest value was recorded in ethanol extraction method (50.84±0.02).

The data with respect to instrumental *b** values of carotenoid pigment concentrate from gac fruit seed aril varied significantly among different treatments as influenced by extraction methods are displayed in Table 76. The *b** values of pigment concentrate from gac fruit seed aril ranged from 36.62±0.08 to 43.24±0.10. Significantly highest *b** value was recorded in ethyl lactate extraction method (43.24±0.10) followed by MAE with ethanol and ethyl lactate extraction method (42.25±0.10), whereas the lowest *b** value was recorded in ethanol extraction method (36.62±0.08).

The data related to *chroma* values of carotenoid pigment concentrate from gac fruit seed aril are displayed in Table 76. *Chroma* values of all the five treatments showed significant differences. *Chroma* values of different treatments as influenced by different extraction methods ranged from 57.29±0.09 to 65.62±0.10. Significantly highest *chroma* value was recorded in ethyl lactate extraction method (65.62±0.10)

followed by MAE with ethanol and ethyl lactate extraction method (62.41±0.14), while the lowest value was reported in ethanol extraction method (57.29±0.09).

The data with respect to *hue angle* of carotenoid pigment concentrate from gac fruit seed aril varied significantly among the treatments as influenced by different extraction methods are displayed in (Table 76). *Hue angle* values of different treatments were in the range of 36.64±0.18 to 47.91±0.30. However, statistically highest *hue angle* value (47.91±0.30) was reported in ethanol extraction method followed by MAE with ethanol extraction method (44.56±0.10), whereas the lowest value was registered in ethyl lactate extraction method (36.64±0.18).

4.5.1.8 Antioxidant properties

4.5.1.8.1 DPPH assay (µl/ml)

According to the data analyzed, antioxidant activity of carotenoid pigment concentrate from gac fruit seed aril showed significant difference among the treatments as influenced by different extraction methods (Table 77). Antioxidant activity of pigment concentrate from gac fruit seed aril as depicted by DPPH method ranged from 0.59±0.05 to 2.19±0.17 µl/ml. Significantly highest antioxidant activity (0.59±0.05 µl/ml) was recorded in ethyl lactate extraction method, whereas the lowest activity (2.19±0.17 µl/ml) was noticed in ethanol extraction method followed by MAE with ethanol extraction method (1.63±0.011 µl/ml).

4.5.1.8.2 ABTS assay (µl/ml)

The results for ABTS antioxidant activity of carotenoid pigment concentrate from gac fruit seed aril as affected by different extraction methods are illustrated in Table 77. Different extraction methods showed statistical differences. According to the data analyzed, the ABTS antioxidant activity of different methods ranged from 0.58±0.005 to 2.02±0.018 µl/ml. Among the different treatments, significantly highest antioxidant activity of 0.58±0.005 µl/ml was reported in ethyl lactate extraction method, whereas lowest activity (2.02±0.018 µl/ml) was noticed in ethanol extraction method followed by MAE with ethanol extraction method (1.52±0.006 µl/ml).

4.5.1.8.3 FRAP assay (µl/ml)

The results with respect to antioxidant activity of carotenoid pigment concentrate from gac fruit seed aril obtained through FRAP method showed statistical variation among different treatments are displayed in Table 77. Antioxidant activity of pigment concentrate from gac fruit seed aril was in the range of 0.63±0.004 to 2.30±0.020 µl/ml. However, significantly highest antioxidant activity (0.63±0.004 µl/ml) was recorded in ethyl lactate extraction method, while the lowest activity was registered in ethanol extraction method (2.30±0.020 µl/ml) followed by MAE with ethanol extraction method (1.42±0.014 µl/ml).

4.5.1.9 Total phenolics (mg GAE/100 g)

The results on the total phenolics of carotenoid pigment concentrate from gac fruit seed aril are demonstrated in the Table 77. The total phenolics of five treatments as influenced by different extraction methods showed significant variations and it ranged from 127.90±0.20 to 211.57±0.13 mg GAE/100 g. However, highest total phenolics was reported in ethyl lactate extraction method (211.57±0.13 mg GAE/100 g) followed by MAE with ethanol and ethyl lactate extraction method (203.50±0.16 mg GAE/100 g), whereas the lowest value was noticed in ethanol extraction method (127.90±0.20 mg GAE/100 g).

4.5.1.10 Non-enzymatic browning

The observations with respect to non-enzymatic browning of the carotenoid pigment concentrates from gac fruit seed aril as affected by the different extraction methods were not detected in the samples (Table 77).

Table 76: Effect of different extraction methods on instrumental colour values of carotenoid pigment concentrate from gac fruit seed aril

Treatments		*L** value	*a** value	*b** value	*Chroma*	*Hue angle*
T_1	Ethanol	67.49±2.05^{a}	50.84±0.02^{e}	36.62±0.08^{e}	57.29±0.09^{e}	47.91±0.30^{a}
T_2	Ethyl lactate	60.44±0.12^{c}	61.35±0.20^{a}	43.24±0.10^{a}	65.62±0.10^{a}	36.64±0.18^{e}
T_3	Ethanol and ethyl lactate (1:1 ratio)	66.38±0.03^{ab}	56.17±0.10^{c}	40.38±0.13^{c}	60.68±0.30^{c}	42.58±0.11^{c}
T_4	MAE with ethanol	65.96±1.64^{ab}	53.70±0.13^{d}	38.38±0.26^{d}	58.90±0.12^{d}	44.56±0.10^{b}
T_5	MAE with ethanol and ethyl lactate (1:1 ratio)	63.94±0.01^{bc}	58.60±0.16^{b}	42.25±0.10^{b}	62.41±0.14^{b}	39.71±0.03^{d}
S.Em±		0.18	0.13	0.15	0.17	0.17
CD (0.05)		3.57	0.41	0.45	0.51	0.52

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 77: Effect of different extraction methods on antioxidant properties of carotenoid pigment concentrate from gac fruit seed aril

Treatments	DPPH (µl/ml)	ABTS (µl/ml)	FRAP (µl/ml)	Phenols (mg GAE/100g)	Non-enzymatic browning
T_1	2.19±0.017^{a}	2.02±0.018^{a}	2.30±0.020^{a}	127.90±0.20^{e}	ND
T_2	0.59±0.005^{e}	0.58±0.005^{e}	0.63±0.004^{e}	211.57±0.13^{a}	ND
T_3	1.56±0.005^{c}	1.40±0.013^{c}	1.01±0.011^{c}	145.50±0.15^{c}	ND
T_4	1.63±0.011^{b}	1.52±0.006^{b}	1.42±0.014^{b}	143.93±0.10^{d}	ND
T_5	0.76±0.005^{d}	0.69±0.004^{d}	0.77±0.006^{d}	203.50±0.16^{b}	ND
SE(m)	0.010	0.011	0.013	0.15	
CD (5%)	0.029	0.033	0.038	0.47	

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$);
GAE: Gallic Acid Equivalent; **ND:** not detected

Treatments details:

T_1 Ethanol

T_2 Ethyl lactate

T_3 Ethanol and ethyl lactate (1:1 ratio)

T_4 Microwave assisted extraction with ethanol

T_5 Microwave assisted extraction with ethanol and ethyl lactate (1:1 ratio)

4.5.2 Marigold petals

4.5.2.1 Recovery percentage of pigment concentrate (%)

The per cent recovery of carotenoid pigment concentrates from marigold petals as affected by the different extraction methods are presented in Table 78. Per cent recovery of carotenoid pigment concentrates showed significant differences among the methods and it ranged from 77.30±0.48 to 93.33±0.27 per cent. Statistically highest carotenoid pigment per cent recovery was observed in ethyl lactate extraction method (93.33±0.27 %) followed by MAE with ethanol extraction method (86.80±0.29 %), whereas the lowest value in comparison to all other methods was registered in ethanol and ethyl lactate extraction method (77.30±0.48 %).

4.5.2.2 Moisture content (%)

The results on the moisture content percentage in the pigment concentrate of marigold flower petals are presented in the Table 78. Non-significant variation was recorded in the moisture content as influenced by different extraction methods. The values ranged from 15.43±0.02 to 15.47±0.01 per cent.

4.5.2.3 Total carotenoid content (µg/g)

The observation on the total carotenoid content present in the pigment concentrates from marigold petals is presented in the Table 78. Significant variation was recorded in the total carotenoid content as influenced by different extraction methods that ranged from 1311.03±0.50 to 2276.93±3.61 µg/g. Statistically highest total carotenoid content was registered in ethyl lactate extraction method (2276.93±3.61 µg/g) followed by MAE with ethanol and ethyl lactate extraction method (2116.98±0.49 µg/g), while the lowest value was reported in ethanol extraction method (1311.03±0.50 µg/g).

4.5.2.4 Lutein content (µg/g)

According to the data analyzed, lutein content of carotenoid pigment concentrates from marigold petals is recorded in Table 78. However, the lutein content of pigment concentrates from marigold petals ranged from 1009.36±1.61 to

1310.02±0.27 µg/g. Statistically highest value was recorded in ethyl lactate extraction method (1310.02±0.27 µg/g) followed by MAE with ethanol and ethyl lactate extraction method (1231.14±0.23 µg/g), while the lowest value was noticed in ethanol extraction method (1009.36±1.61 µg/g).

4.5.2.5 β-carotene content (µg/g)

β-carotene content of carotenoid pigment concentrates from marigold petals as influenced by different extraction methods showed significant variations among the treatments and are displayed in Table 78. β-carotene content of pigment concentrates from marigold petals were in the range of 154.65±0.71 to 491.03±0.85 µg/g. Significantly highest value (491.03±0.85 µg/g) for β-carotene content was noticed in ethyl lactate extraction method which was followed by MAE with ethanol and ethyl lactate extraction method (472.65±1.97 µg/g), whereas the lowest value (154.65±0.71 µg/g) was recorded in ethanol extraction method.

4.5.2.6 Lycopene content (µg/g)

According to the data analyzed, lycopene content of carotenoid pigment concentrates from marigold petals is displayed in Table 78. However, the lycopene content of pigment concentrates from marigold petals ranged from 61.98±0.25 to 236.96±0.10 µg/g. Statistically highest value was recorded in ethyl lactate extraction method (236.96±0.10 µg/g) followed by ethanol and ethyl lactate extraction method (220.88±1.14 µg/g), while the lowest value was noticed in ethanol extraction method (61.98±0.25 µg/g).

4.5.2.7 Colour properties

4.5.2.7.1 Instrumental colour values

The observations pertaining to L^* value of carotenoid pigment concentrates from marigold petals are given in the Table 79. All the different treatments showed significant variations as influenced by different extraction methods.

Table 78: Effect of different extraction methods on recovery percentage, moisture content and pigment content of carotenoid pigment concentrate from marigold flower petals

Treatments		Recovery percentage	Moisture content (%)	Total carotenoid content (µg/g)	Lutein content (µg/g)	β-carotene content (µg/g)	Lycopene content (µg/g)
T_1	Ethanol	80.87±0.13^{d}	15.45±0.02	1311.03±0.50^{e}	1009.36±1.61^{e}	154.65±0.71^{e}	61.98±0.25^{e}
T_2	Ethyl lactate	93.33±0.27^{a}	15.44±0.03	2276.93±3.61^{a}	1310.02±0.27^{a}	491.03±0.85^{a}	236.96±0.10^{a}
T_3	Ethanol and ethyl lactate (1:1 ratio)	77.30±0.48^{e}	15.47±0.01	1986.42±5.99^{c}	1211.34±1.30^{c}	432.69±1.40^{c}	220.88±1.14^{b}
T_4	MAE with ethanol	86.80±0.29^{b}	15.43±0.02	1488.81±3.55^{d}	1082.08±0.92^{d}	184.95±0.29^{d}	130.55±1.13^{d}
T_5	MAE with ethanol and ethyl lactate (1:1 ratio)	83.77±0.28^{c}	15.44±0.03	2116.98±0.49^{b}	1231.14±0.23^{b}	472.65±1.97^{b}	203.96±0.31^{c}
S.Em±		0.31	0.02	3.52	1.03	1.20	0.74
CD (0.05)		0.94	NS	10.72	3.12	3.64	2.25

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Irrespective of the different extraction methods, all the five treatments were in the range of 70.20±0.12 to 75.36±0.23. However, significantly highest *L** value was recorded in ethyl lactate extraction method (75.36±0.23) followed by ethanol and ethyl lactate extraction method (74.58±0.06), while the lowest value was registered in ethanol extraction method (70.20±0.12).

The results on instrumental *a** value of carotenoid pigment concentrates from marigold petals is displayed in the Table 79. Instrumental *a** value varied significantly among different treatments which ranged from 10.48±0.14 to 21.45±0.19. However, significantly highest *a** value was reported in ethyl lactate extraction method (21.45±0.19) followed by MAE with ethanol and ethyl lactate extraction method (20.66±0.12), while the lowest value was recorded in ethanol extraction method (10.48±0.14).

The data with respect to instrumental *b** values of carotenoid pigment concentrates from marigold petals varied significantly among different treatments as influenced by extraction methods are displayed in Table 79. The *b** values of pigment concentrates from marigold petals ranged from 61.70±0.16 to 76.49±0.15. Significantly highest *b** value was recorded in ethanol extraction method (76.49±0.15) followed by MAE with ethanol extraction method (69.68±0.10), whereas the lowest *b** value was recorded in ethanol and ethyl lactate extraction method (61.70±0.16).

The data related to *chroma* values of carotenoid pigment concentrates from marigold petals are displayed in Table 79. *Chroma* values of all the five treatments showed significant differences. *Chroma* values of different treatments as influenced by different extraction methods ranged from 75.36±0.21 to 77.44±0.18. Significantly highest *chroma* value was reported in MAE with ethanol extraction method (77.44±0.18) followed by ethyl lactate extraction method (77.29±0.11), while the lowest value was reported in MAE with ethanol and ethyl lactate extraction method (75.36±0.21).

The data with respect to *hue angle* of carotenoid pigment concentrates from marigold petals varied significantly among the treatments as influenced by different extraction methods are displayed in (Table 79). *Hue angle* values of different

treatments were in the range of 63.57±0.29 to 82.44±0.19. However, statistically highest *hue angle* value (82.44±0.19) was reported in ethanol extraction method followed by MAE with ethanol extraction method (78.36±0.05), whereas the lowest value was registered in ethyl lactate extraction method (63.57±0.29).

4.5.2.8 Antioxidant properties

4.5.2.8.1 DPPH assay (µl/ml)

According to the data analyzed, antioxidant activity of carotenoid pigment concentrates from marigold petals showed significant difference among the treatments as influenced by different extraction methods (Table 80). Antioxidant activity of pigment concentrates from marigold petals as depicted by DPPH method ranged from 0.355±0.03 to 0.642±0.003 µl/ml. Significantly highest antioxidant activity (0.355±0.03 µl/ml) was recorded in ethyl lactate extraction method, whereas the lowest activity (0.642±0.003 µl/ml) was noticed in ethanol extraction method followed by MAE with ethanol extraction method (0.514±0.002 µl/ml).

4.5.2.8.2 ABTS assay (µl/ml)

The results for ABTS antioxidant activity of carotenoid pigment concentrates from marigold petals as affected by different extraction methods are illustrated in Table 80. Different extraction methods showed statistical differences. According to the data analyzed, the ABTS antioxidant activity of different methods ranged from 0.201±0.002 to 0.610±0.003 µl/ml. Among the different treatments, significantly highest antioxidant activity (0.201±0.002 µl/ml) was reported in ethyl lactate extraction method, whereas the lowest activity (0.610±0.003 µl/ml) was noticed in ethanol extraction method followed by MAE with ethanol extraction method (0.501±0.001 µl/ml).

4.5.2.8.3 FRAP assay (µl/ml)

The results with respect to antioxidant activity of carotenoid pigment concentrates from marigold petals obtained through FRAP method showed statistical variation among different treatments are displayed in Table 80. Antioxidant activity of

pigment concentrates from marigold petals was in the range of 0.557±0.003 to 1.470±0.010 µl/ml. However, significantly highest antioxidant activity (0.557±0.003 µl/ml) was recorded in ethyl lactate extraction method, while the lowest activity was registered in ethanol extraction method (1.470±0.010 µl/ml) followed by MAE with ethanol extraction method (1.138±0.009 µl/ml).

4.5.2.9 Total phenolics (mg GAE/100 g)

The results on the total phenolics of carotenoid pigment concentrates from marigold petals are displayed in the Table 80. The total phenolics of five treatments as influenced by different extraction methods showed significant variations and it ranged from 254.24±0.41 to 337.61±0.23 mg GAE/100 g. However, highest total phenolics was reported in ethyl lactate extraction method (337.61±0.23 mg GAE/100 g) followed by MAE with ethanol and ethyl lactate extraction method (327.98±0.43 mg GAE/100 g), whereas the lowest value was noticed in ethanol extraction method (254.24±0.41 mg GAE/100 g).

4.5.2.10 Non-enzymatic browning

The observations with respect to non-enzymatic browning of the carotenoid pigment concentrates from marigold flower petals as affected by the different extraction methods were not detected in the samples (Table 80).

4.6 Evaluation of carotenoid pigment's stability in processed products

4.6.1 Guava squash coloured with carotenoid pigment

4.6.1.1 Lycopene content (µg/100 ml)

Lycopene content of guava squash decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 81). The decrease was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, lycopene content in the guava squash added with gac fruit and marigold pigment was 128.98±0.06 µg/100 ml and 27.91±0.41 µg/100 ml, respectively.

Table 79: Effect of different extraction methods on instrumental colour values of carotenoid pigment concentrate from marigold flower petals

Treatments		*L** value	*a** value	*b** value	*Chroma*	*Hue angle*
T_1	Ethanol	70.20±0.12^{e}	10.48±0.14^{e}	76.49±0.15^{a}	76.36±0.04^{b}	82.44±0.19^{a}
T_2	Ethyl lactate	75.36±0.23^{a}	21.45±0.19^{a}	63.57±0.15^{c}	77.44±0.18^{a}	63.57±0.29^{e}
T_3	Ethanol and ethyl lactate (1:1 ratio)	74.58±0.06^{b}	19.70±0.11^{c}	61.70±0.16^{e}	75.43±0.18^{c}	68.25±0.04^{c}
T_4	MAE with ethanol	72.04±0.08^{d}	13.53±0.09^{d}	69.68±0.10^{b}	77.29±0.11^{a}	78.36±0.05^{b}
T_5	MAE with ethanol and ethyl lactate (1:1 ratio)	73.80±0.21^{c}	20.66±0.12^{b}	62.92±0.18^{d}	75.36±0.21^{c}	64.43±0.14^{d}
S.Em±		0.16	0.13	0.15	0.16	0.17
CD (0.05)		0.48	0.41	0.46	0.48	0.52

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 80: Effect of different extraction methods on antioxidant properties of carotenoid pigment concentrate from marigold flower petals

Treatments	DPPH (µl/ml)	ABTS (µl/ml)	FRAP (µl/ml)	Phenols (mg GAE/100g)	Non-enzymatic browning
T_1	0.642±0.003^{a}	0.610±0.003^{a}	1.470±0.010^{a}	254.24±0.41^{e}	ND
T_2	0.355±0.003^{e}	0.201±0.002^{e}	0.557±0.003^{e}	337.61±0.23^{a}	ND
T_3	0.474±0.003^{c}	0.301±0.002^{c}	0.775±0.008^{c}	289.13±0.42^{c}	ND
T_4	0.514±0.002^{b}	0.501±0.001^{b}	1.138±0.009^{b}	269.48±0.26^{d}	ND
T_5	0.458±0.002^{d}	0.243±0.002^{d}	0.675±0.005^{d}	327.98±0.43^{b}	ND
SE(m)	0.003	0.002	0.008	0.36	
CD (5%)	0.008	0.007	0.023	1.100	

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$);
GAE: Gallic Acid Equivalent; **ND:** not detected

Treatments details:

T_1 Ethanol
T_2 Ethyl lactate
T_3 Ethanol and ethyl lactate (1:1 ratio)
T_4 Microwave assisted extraction with ethanol
T_5 Microwave assisted extraction with ethanol and ethyl lactate (1:1 ratio)

After three months of storage, the highest retention of lycopene content (111.31±0.23 µg/100 g) was noticed in guava squash added with gac fruit pigment stored under refrigerated condition, whereas the lowest retention (12.09±0.27 µg/100 g) was observed in guava squash added with marigold pigment stored under ambient condition.

4.6.1.2 β-carotene content (µg/100 ml)

β-carotene content of guava squash decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 82). The decrease was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, β-carotene content in the guava squash added with gac fruit and marigold pigment was 81.15±0.39 µg/100 ml and 58.68±0.22 µg/100 ml, respectively. After three months of storage, the highest retention of β-carotene content (64.30±0.28 µg/100 ml) was noticed in guava squash added with gac fruit pigment stored under refrigerated condition, whereas the lowest retention (35.87±0.09 µg/100 ml) was observed in guava squash added with marigold pigment stored under ambient condition.

4.6.1.3 Lutein content (µg/100 ml)

Lutein content of guava squash decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 83). The decrease was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, lutein content in the guava squash added with gac fruit and marigold pigment was 26.45±0.09 µg/100 ml and 154.05±0.08 µg/100 ml, respectively. After three months of storage, the highest retention of lutein content (139.63±0.19 µg/100 ml) was noticed in guava squash added with marigold pigment stored under refrigerated condition, whereas the lowest retention (4.36±0.16 µg/100 ml) was observed in guava squash added with gac fruit pigment stored under ambient condition.

4.6.1.4 Total carotenoid content (µg/100 ml)

Total carotenoid content of guava squash decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 84). The decrease was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, total carotenoid content in the guava squash added with gac fruit and marigold pigment was 239.61±0.45 µg/100 ml and 246.21±0.45 µg/100 ml, respectively. After three months of storage, the highest retention of total carotenoid content (203.24±0.28 µg/100 ml) was noticed in guava squash added with marigold pigment stored under refrigerated condition, whereas the lowest retention (175.35±0.35 µg/100 ml) was observed in guava squash added with gac fruit pigment stored at ambient condition.

4.6.1.5 Colour properties

4.6.1.5.1 Instrumental colour values

Instrumental colour values for L^* in guava squash decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 85). The decrease was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for L^* in guava squash without pigment, added with gac fruit and marigold pigment was 93.61±0.04, 83.67±0.11 and 80.65±0.17, respectively. After three months of storage, the highest L^* value (81.68±0.23) was noticed in guava squash without pigment stored under refrigerated condition, whereas the lowest L^* value (61.10±0.08) was observed in guava squash without pigment stored under ambient condition. Instrumental colour values for a^* in guava squash decreased significantly throughout the storage period, irrespective of storage conditions except in guava squash without pigment (Table 86). The decrease was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for a^* in guava squash without pigment, added with gac fruit and marigold pigment was -28.05±0.06, 33.81±0.13 and 28.35±0.16, respectively.

Table 81: Changes in lycopene content of guava squash incorporated with carotenoid pigment during storage

Treatments	Initial	Lycopene content (µg/100 ml)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	NA	NA	NA	NA	NA	NA	NA
T_2 – Gac fruit pigment	128.98±0.06^{a}	121.27±0.33^{a}	113.23±0.17^{a}	103.44±0.15^{a}	123.10±0.22^{a}	119.71±0.16^{a}	111.31±0.23^{a}
T_3 – Marigold pigment	27.91±0.41^{b}	22.15±0.34^{b}	16.74±0.18^{b}	12.09±0.27^{b}	24.15±0.27^{b}	19.92±0.24^{b}	13.44±0.18^{b}
S.Em±	0.24	0.27	0.14	0.18	0.63	0.17	0.53
C.D (0.05)	0.74	0.85	0.44	0.56	0.20	0.52	0.17

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)
NA = not available

Table 82: Changes in β-carotene content of guava squash incorporated with carotenoid pigment during storage

Treatments	Initial	β-carotene content (µg/100 ml)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	NA	NA	NA	NA	NA	NA	NA
T_2 – Gac fruit pigment	81.15±0.39[a]	74.08±0.28[a]	67.42±0.22[a]	59.26±0.28[a]	76.33±0.26[a]	71.45±0.22[a]	64.30±0.28[a]
T_3 – Marigold pigment	58.68±0.22[b]	53.03±0.10[b]	44.94±0.09[b]	35.87±0.09[b]	56.05±0.10[b]	50.00±0.36[b]	44.74±0.32[b]
S.Em±	0.26	0.17	0.14	0.17	0.16	0.24	0.24
C.D (0.05)	0.81	0.53	0.42	0.52	0.51	0.75	0.75

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)
NA = not available

Table 83: Changes in lutein content of guava squash incorporated with carotenoid pigment during storage

Treatments	Initial	Lutein content (µg/100 ml)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	NA	NA	NA	NA	NA	NA	NA
T_2 – Gac fruit pigment	26.45±0.09^{b}	17.37±0.05^{b}	12.18±0.14^{b}	4.36±0.16^{b}	24.61±0.19^{b}	19.07±0.15^{b}	13.47±0.14^{b}
T_3 – Marigold pigment	154.05±0.08^{a}	143.83±0.09^{a}	138.19±0.16^{a}	129.57±0.23^{a}	150.21±0.15^{a}	144.48±0.22^{a}	139.63±0.19^{a}
S.Em±	0.07	0.06	0.13	0.16	0.14	0.15	0.14
C.D (0.05)	0.22	0.19	0.39	0.50	0.44	0.47	0.43

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)
NA = not available

Table 84: Changes in total carotenoid content of guava squash incorporated with carotenoid pigment during storage

Treatments	Initial	Total carotenoid content (µg/100 ml)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	NA	NA	NA	NA	NA	NA	NA
T_2 – Gac fruit pigment	239.61±0.45^{b}	216.52±0.56^{b}	193.69±0.52^{b}	175.35±0.35^{b}	230.27±0.29^{b}	212.12±0.50^{b}	195.20±0.37^{b}
T_3 – Marigold pigment	246.21±0.45^{a}	227.43±0.39^{a}	201.45±0.54^{a}	190.60±0.30^{a}	240.31±0.44^{a}	215.36±0.44^{a}	203.24±0.28^{a}
S.Em±	0.37	0.40	0.43	0.27	0.30	0.38	0.27
C.D (0.05)	1.15	1.23	1.35	0.83	0.94	1.19	0.84

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)
NA = not available

After three months of storage, the lowest *a** value (-22.33±0.12) was noticed in guava squash without pigment stored under refrigerated condition, whereas the highest *a** value (23.44±0.14) was observed in guava squash added with gac fruit pigment stored under refrigerated condition.

Instrumental colour values for *b** in guava squash decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 87). The decrease was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *b** in guava squash without pigment, added with gac fruit and marigold pigment was 38.84±0.39, 28.98±0.15 and 36.94±0.15, respectively. After three months of storage, the lowest *b** value (15.81±0.10) was noticed in guava squash added with gac fruit pigment stored under ambient condition, whereas the highest *b** value (31.91±0.06) was observed in guava squash without pigment stored under refrigerated condition.

Instrumental colour values for *hue angle* (°) in guava squash increased significantly throughout the storage period, irrespective of storage conditions except in guava squash without pigment (Table 88). The increase was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *hue angle* in guava squash without pigment, added with gac fruit and marigold pigment was 95.04±0.10°, 61.49±0.14° and 83.20±0.06°, respectively. After three months of storage, the highest *hue angle* value (93.23±0.15°) was noticed in guava squash added with marigold pigment stored under ambient condition, whereas the lowest *hue angle* value (67.47±0.15°) was observed in guava squash added with gac fruit pigment stored under refrigerated condition.

Instrumental colour values for *chroma* in guava squash decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 89). The decrease was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *chroma* in guava squash without pigment, added with gac fruit and

marigold pigment was 57.74±0.23, 34.59±0.11 and 55.14±0.08, respectively. After three months of storage, the highest *chroma* value (49.39±0.13) was noticed in guava squash without pigment stored under refrigerated condition, whereas the lowest *chroma* value (18.70±0.16) was observed in guava squash added with gac fruit pigment stored under ambient condition.

4.6.1.6 Antioxidant properties

4.6.1.6.1 DPPH assay (µl/ml)

DPPH value of guava squash increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 90). The increase was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, DPPH value in the guava squash without pigment, added with gac fruit and marigold pigment was 0.710±0.004, 0.582±0.005 and 0.541±0.001 µl/ml, respectively. After three months of storage, highest DPPH radical scavenging activity (0.832±0.011 µl/ml) was observed in guava squash added with marigold pigment stored under refrigerated condition, whereas lowest activity (8.118±0.123 µl/ml) was noticed in guava squash without pigment stored under ambient condition.

4.6.1.6.2 ABTS assay (µl/ml)

ABTS value of guava squash increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 90). The increase was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, ABTS value in the guava squash without pigment, added with gac fruit and marigold pigment was 0.687±0.003, 0.543±0.004 and 0.515±0.005 µl/ml, respectively. After three months of storage, the highest ABTS radical scavenging activity (0.937±0.011 µl/ml) was observed in guava squash added with marigold pigment stored under refrigerated condition, whereas lowest activity (4.771±0.170 µl/ml) was noticed in guava squash without pigment stored under ambient condition.

Table 85: Changes in *L value of guava squash incorporated with carotenoid pigment during storage**

Treatments	Initial	*L** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	93.61±0.04^{a}	79.44±0.12^{b}	71.31±0.07^{c}	61.10±0.08^{c}	91.71±0.12^{a}	88.01±0.27^{a}	81.68±0.23^{a}
T_2 – Gac fruit pigment	83.67±0.11^{b}	80.12±0.11^{a}	74.24±0.29^{a}	67.76±0.14^{a}	81.45±0.16^{b}	78.30±0.18^{b}	72.12±0.32^{b}
T_3 – Marigold pigment	80.65±0.17^{c}	77.17±0.09^{c}	73.10±0.13^{b}	65.49±0.24^{b}	78.45±0.13^{c}	75.03±0.14^{c}	68.32±0.13^{c}
S.Em±	0.12	0.11	0.19	0.17	0.14	0.20	0.24
C.D (0.05)	0.38	0.34	0.58	0.52	0.42	0.63	0.75

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 86: Changes in *a value of guava squash incorporated with carotenoid pigment during storage**

Treatments	Initial	*a** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	-28.05±0.06^{c}	-25.84±0.25^{c}	-23.81±0.15^{c}	-20.67±0.18^{c}	-26.55±0.20^{c}	-24.29±0.08^{c}	-22.33±0.12^{c}
T_2 – Gac fruit pigment	33.81±0.13^{a}	30.39±0.16^{a}	26.48±0.18^{a}	18.42±0.17^{a}	31.59±0.16^{a}	26.71±1.85^{a}	23.44±0.14^{a}
T_3 – Marigold pigment	28.35±0.16^{b}	24.53±0.14^{b}	18.36±0.12^{b}	11.49±0.14^{b}	26.36±0.16^{b}	21.74±0.14^{b}	15.72±0.12^{b}
S.Em±	0.13	0.19	0.15	0.16	0.18	1.07	0.13
C.D (0.05)	0.39	0.59	0.47	0.51	0.55	3.34	0.39

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 87: Changes in *b value of guava squash incorporated with carotenoid pigment during storage**

Treatments	Initial	*b** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	38.84±0.39[a]	35.42±0.22[a]	31.90±0.32[a]	28.99±0.09[a]	35.98±0.37[a]	34.07±0.09[a]	31.91±0.06[a]
T_2 – Gac fruit pigment	28.98±0.15[c]	26.10±0.15[c]	23.28±0.11[c]	15.81±0.10[c]	26.83±0.09[c]	23.94±0.14[c]	18.82±0.37[c]
T_3 – Marigold pigment	36.94±0.15[b]	31.90±0.14[b]	27.81±0.13[b]	22.04±0.12[b]	34.69±0.19[b]	32.18±0.10[b]	27.80±0.16[b]
S.Em±	0.26	0.17	0.21	0.10	0.25	0.11	0.24
C.D (0.05)	0.80	0.54	0.65	0.33	0.77	0.36	0.73

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 88: Changes in *hue angle* (º) value of guava squash incorporated with carotenoid pigment during storage

Treatments	Initial	*Hue angle* (º) value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	95.04±0.10^{a}	93.25±0.08^{a}	90.54±0.12a	87.49±0.14^{b}	94.13±0.08^{a}	91.68±0.21^{a}	89.40±0.11^{b}
T_2 – Gac fruit pigment	61.49±0.14^{c}	63.44±0.14^{c}	66.39±0.06^{c}	70.36±0.09^{c}	62.57±0.15^{c}	65.19±0.16^{c}	67.47±0.15^{c}
T_3 – Marigold pigment	83.20±0.06^{b}	87.51±0.18^{b}	89.36±0.14^{b}	93.23±0.15^{a}	85.92±0.32^{b}	88.37±0.11^{b}	91.48±0.12^{a}
S.Em±	0.10	0.14	0.11	0.13	0.21	0.16	0.13
C.D (0.05)	0.32	0.43	0.35	0.39	0.65	0.51	0.40

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 89: Changes in *chroma* value of guava squash incorporated with carotenoid pigment during storage

Treatments	Initial	*Chroma* value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	57.74±0.23[a]	53.80±0.19[a]	50.53±0.07[a]	46.32±0.16[a]	55.07±0.09[a]	52.26±0.18[a]	49.39±0.13[a]
T_2 – Gac fruit pigment	34.59±0.11[c]	30.19±0.07[c]	23.96±0.25[c]	18.70±0.16[c]	31.67±0.17[c]	27.03±0.28[c]	22.36±0.14[c]
T_3 – Marigold pigment	55.14±0.08[b]	50.62±0.14[b]	44.74±0.09[b]	39.44±0.12[b]	52.40±0.13[b]	47.81±0.14[b]	42.77±0.24[b]
S.Em±	0.15	0.14	0.16	0.15	0.13	0.21	0.18
C.D (0.05)	0.48	0.44	0.49	0.47	0.42	0.64	0.56

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

4.6.1.6.3 FRAP assay (µl/ml)

FRAP value of guava squash increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 90). The increase was rapid in the guava squash stored under ambient condition as compared to refrigerated condition. Before storage, FRAP value in the guava squash without pigment, added with gac fruit and marigold pigment was 0.744±0.005, 0.693±0.004 and 0.608±0.003 µl/ml, respectively. After three months of storage, the highest ferric reducing antioxidant power (1.326±0.031 µl/ml) was observed in guava squash added with marigold pigment stored under refrigerated condition, whereas the lowest ferric reducing antioxidant power (6.586±0.082 µl/ml) was noticed in guava squash without pigment stored under ambient condition.

4.6.1.7 Sensory evaluation of guava squash (9-point hedonic scale)

Data on mean sensory scores of guava squash without pigment, added with gac fruit and marigold pigment during initial, first, second and three months after storage under ambient and refrigerated conditions are presented in Tables 91, 92, 93, respectively. Sensory scores of guava squash declined during storage period, irrespective of treatments and storage conditions. The rate of decrease was faster in the guava squash stored under ambient condition as compared to refrigerated condition. After three months of storage, guava squash added with gac fruit pigment stored under refrigerated condition recorded highest sensory score (45.60), while the lowest (37.44) was noticed in the guava squash added with gac fruit pigment stored under ambient condition.

Note: Non-enzymatic browning in guava squash was not observed.

Table 90: Changes in DPPH, ABTS and FRAP antioxidant activity of guava squash incorporated with carotenoid pigment during storage

Treatments	Initial	DPPH activity (µl/ml)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	0.710±0.004^{a}	0.743±0.005^{a}	1.409±0.027^{a}	8.118±0.123^{a}	0.720±0.002^{a}	1.052±0.010^{a}	4.943±0.232^{a}
T_2 – Gac fruit pigment	0.582±0.005^{b}	0.680±0.006^{b}	0.814±0.006^{b}	1.286±0.028^{b}	0.618±0.004^{b}	0.712±0.004^{b}	0.931±0.012^{b}
T_3 – Marigold pigment	0.541±0.001^{c}	0.615±0.004^{c}	0.777±0.005^{b}	1.098±0.024^{b}	0.586±0.004^{c}	0.675±0.005^{c}	0.832±0.011^{b}
S.Em±	0.003	0.005	0.016	0.074	0.003	0.007	0.134
C.D (0.05)	0.011	0.016	0.050	0.231	0.011	0.022	0.418
	ABTS activity (µl/ml)						
T_1 – No pigment	0.687±0.003^{a}	0.716±0.004^{a}	1.250±0.027^{a}	4.771±0.170^{a}	0.696±0.002^{a}	0.969±0.008^{a}	2.930±0.076^{a}
T_2 – Gac fruit pigment	0.543±0.004^{b}	0.686±0.006^{b}	0.865±0.013^{b}	1.134±0.017^{b}	0.589±0.007^{b}	0.723±0.009^{b}	0.944±0.011^{b}
T_3 – Marigold pigment	0.515±0.005^{c}	0.6410.004^{c}	0.809±0.008^{c}	1.013±0.019^{b}	0.550±0.004^{c}	0.684±0.006^{c}	0.937±0.011^{b}
S.Em±	0.004	0.005	0.018	0.099	0.005	0.008	0.045
C.D (0.05)	0.012	0.015	0.055	0.309	0.015	0.024	0.140
	FRAP activity (µl/ml)						
T_1 – No pigment	0.744±0.005^{a}	1.028±0.012^{a}	3.185±0.032^{a}	6.586±0.082^{a}	0.758±0.004^{a}	2.073±0.039^{a}	5.387±0.277^{a}
T_2 – Gac fruit pigment	0.693±0.004^{b}	0.987±0.017^{b}	1.518±0.028^{b}	2.249±0.022^{b}	0.713±0.007^{b}	1.195±0.024^{b}	1.804±0.023^{b}
T_3 – Marigold pigment	0.608±0.003^{c}	0.823±0.003^{c}	1.054±0.008^{c}	1.523±0.007^{c}	0.644±0.007^{c}	0.880±0.015^{c}	1.326±0.031^{b}
S.Em±	0.004	0.012	0.025	0.049	0.006	0.028	0.161
C.D (0.05)	0.013	0.037	0.078	0.153	0.018	0.087	0.503

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 91: Sensory quality of guava squash incorporated with carotenoid pigment (Initial)

Treatments	Initial						
	Appearance	**Colour**	**Flavour**	**Body and consistency**	**After-taste**	**Overall acceptability**	**Total score**
T_1 – No pigment	8.00	8.00	9.00	9.00	9.00	8.60	51.60
T_2 – Gac fruit pigment	8.20	8.20	8.80	9.00	8.80	8.60	51.60
T_3 – Marigold pigment	8.20	8.20	8.80	8.80	8.80	8.56	51.36
Kendall's W test value	0.20	0.20	0.20	0.20	0.20	0.03	

Kendall's W value: Kendall's coefficient of concordance

Table 92: Changes in sensory quality of guava squash incorporated with carotenoid pigment stored under ambient condition

Treatments	One MAS under ambient condition						
	Appearance	Colour	Flavour	Body and consistency	After-taste	Overall acceptability	Total score
T_1 – No pigment	7.20	6.80	8.00	8.00	8.20	7.64	45.84
T_2 – Gac fruit pigment	7.40	7.60	8.00	8.00	8.20	7.84	47.04
T_3 – Marigold pigment	7.20	7.00	7.80	7.80	8.00	7.56	45.36
Kendall's W test value	0.10	0.65	0.20	0.20	0.20	0.84	
	Two MAS under ambient condition						
T_1 – No pigment	6.20	6.20	7.00	7.80	7.80	7.00	42.00
T_2 – Gac fruit pigment	6.20	6.40	7.20	7.80	7.60	7.04	42.24
T_3 – Marigold pigment	6.00	6.00	7.00	7.60	7.60	6.84	41.04
Kendall's W test value	0.07	0.15	0.07	0.20	0.10	0.30	
	Three MAS under ambient condition						
T_1 – No pigment	5.40	5.40	6.40	6.80	7.40	6.28	37.68
T_2 – Gac fruit pigment	5.60	5.60	6.20	7.00	6.80	6.24	37.44
T_3 – Marigold pigment	5.60	5.60	6.20	6.80	7.00	6.28	37.48
Kendall's W test value	0.02	0.04	0.07	0.20	0.47	0.01	

Kendall's W value: Kendall's coefficient of concordance

Table 93: Changes in sensory quality of guava squash incorporated with carotenoid pigment stored under refrigerated condition

Treatments	One MAS under refrigerated condition						
	Appearance	Colour	Flavour	Body and consistency	After-taste	Overall acceptability	Total score
T_1 – No pigment	8.00	8.00	8.20	8.40	8.40	8.20	49.20
T_2 – Gac fruit pigment	8.20	8.20	8.40	8.40	8.40	8.32	49.92
T_3 – Marigold pigment	8.00	8.20	8.20	8.20	8.20	8.16	48.96
Kendall's W test value	0.20	0.20	0.20	0.10	0.20	0.20	
	Two MAS under refrigerated condition						
T_1 – No pigment	7.80	7.40	8.00	8.20	8.20	7.92	47.52
T_2 – Gac fruit pigment	8.00	7.60	8.20	8.40	8.00	8.04	48.24
T_3 – Marigold pigment	8.00	7.40	8.00	8.20	8.00	7.92	47.52
Kendall's W test value	0.20	0.10	0.10	0.20	0.20	0.19	
	Three MAS under refrigerated condition						
T_1 – No pigment	7.00	6.80	7.40	7.60	7.80	7.32	43.92
T_2 – Gac fruit pigment	7.40	7.20	7.80	7.80	7.80	7.60	45.60
T_3 – Marigold pigment	7.40	6.80	7.60	7.60	7.60	7.40	44.40
Kendall's W test value	0.07	0.20	0.30	0.20	0.20	0.30	

Kendall's W value: Kendall's coefficient of concordance

4.6.2 Guava jelly coloured with carotenoid pigment

4.6.2.1 Lycopene content (µg/100 g)

Lycopene content of guava jelly decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 94). The decrease was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, lycopene content in the guava jelly added with gac fruit and marigold pigment was 124.85±0.10 µg/100 g and 22.85±0.13 µg/100 g, respectively. After three months of storage, the highest retention of lycopene content (108.26±0.16 µg/100 g) was noticed in guava jelly added with gac fruit pigment stored under refrigerated condition, whereas the lowest retention (7.93±0.17 µg/100 g) was observed in guava jelly added with marigold pigment stored under ambient condition.

4.6.2.2 β-carotene content (µg/100 g)

β-carotene content of guava jelly decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 95). The decrease was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, β-carotene content in the guava jelly added with gac fruit and marigold pigment was 75.20±0.32 µg/100 g and 52.34±0.22 µg/100 g, respectively. After three months of storage, the highest retention of β-carotene content (54.22±0.28 µg/100 g) was noticed in guava jelly added with gac fruit pigment stored under refrigerated condition, whereas the minimum retention (30.10±0.12 µg/100 g) was observed in guava jelly added with marigold pigment stored under ambient condition.

4.6.2.3 Lutein content (µg/100 g)

Lutein content of guava jelly decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 96). The decrease was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, lutein content in the guava jelly added with gac fruit and marigold pigment was 18.92±0.07 µg/100 g and 151.61±0.21 µg/100 g,

respectively. After three months of storage, the highest retention of lutein content (132.02±0.19 µg/100 g) was noticed in guava jelly added with marigold pigment stored under refrigerated condition, whereas the minimum retention (1.69±0.13 µg/100 g) was observed in guava jelly added with gac fruit pigment stored under ambient condition.

4.6.2.4 Total carotenoid content (µg/100 g)

Total carotenoid content of guava jelly decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 97). The decrease was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, total carotenoid content in the guava jelly added with gac fruit and marigold pigment was 231.85±1.03 µg/100 g and 239.69±0.37 µg/100 g, respectively. After three months of storage, the highest retention of total carotenoid content (195.77±0.49 µg/100 g) was noticed in guava jelly added with marigold pigment stored under refrigerated condition, whereas the lowest retention (169.29±0.36 µg/100 g) was observed in guava jelly added with gac fruit pigment stored under ambient condition.

4.6.2.5 Colour properties

4.6.2.5.1 Instrumental colour values

Instrumental colour values for L^* in guava jelly decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 98). The decrease was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for L^* in guava jelly without pigment, added with gac fruit and marigold pigment was 99.51±0.07, 87.55±0.16 and 92.47±0.16, respectively. After three months of storage, the highest L^* value (87.49±0.20) was noticed in guava jelly without pigment stored under refrigerated condition, whereas the lowest L^* value (71.35±0.09) was observed in guava jelly added with gac fruit pigment stored under ambient condition.

Table 94: Changes in lycopene content of guava jelly incorporated with carotenoid pigment during storage

Treatments	Initial	Lycopene content (µg/100 g)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	NA	NA	NA	NA	NA	NA	NA
T_2 – Gac fruit pigment	124.85±0.10[a]	117.65±0.13[a]	112.70±0.51[a]	103.68±0.39[a]	119.86±0.22[a]	113.47±0.20[a]	108.26±0.16[a]
T_3 – Marigold pigment	22.85±0.13[b]	18.37±0.10[b]	11.27±0.15[b]	7.93±0.17[b]	20.94±0.15[b]	13.07±0.46[b]	9.83±0.46[b]
S.Em±	0.09	0.09	0.31	0.24	0.15	0.29	0.28
C.D (0.05)	0.29	0.29	0.96	0.76	0.48	0.91	0.88

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)
NA = not-available

Table 95: Changes in β-carotene content of guava jelly incorporated with carotenoid pigment during storage

Treatments	Initial	β-carotene content (µg/100 g)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	NA	NA	NA	NA	NA	NA	NA
T_2 – Gac fruit pigment	75.20±0.32[a]	68.79±0.28[a]	59.10±0.24[a]	48.96±0.27[a]	71.65±0.14[a]	63.29±0.22[a]	54.22±0.28[a]
T_3 – Marigold pigment	52.34±0.22[b]	47.24±0.09[b]	40.86±0.28[b]	30.10±0.12[b]	49.95±0.12[b]	45.06±0.18[b]	37.87±0.20[b]
S.Em±	0.22	0.17	0.22	0.12	0.11	0.16	0.20
C.D (0.05)	0.69	0.52	0.67	0.54	0.33	0.50	0.61

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)
NA = not-available

Table 96: Changes in lutein content of guava jelly incorporated with carotenoid pigment during storage

Treatments	Initial	Lutein content (µg/100 g)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	NA	NA	NA	NA	NA	NA	NA
T_2 – Gac fruit pigment	18.92±0.07^{b}	14.64±0.11^{b}	7.83±0.26^{b}	1.69±0.13^{b}	15.80±0.15^{b}	13.50±0.14^{b}	8.01±0.10^{b}
T_3 – Marigold pigment	151.61±0.21^{a}	140.96±0.21^{a}	136.12±0.08^{a}	119.82±0.31^{a}	143.87±0.13^{a}	138.88±0.20^{a}	132.02±0.19^{a}
S.Em±	0.13	0.14	0.16	0.19	0.12	0.14	0.13
C.D (0.05)	0.41	0.43	0.49	0.6	0.36	0.44	0.39

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)
NA = not-available

Table 97: Changes in total carotenoid content of guava jelly incorporated with carotenoid pigment during storage

Treatments	Initial	Total carotenoid content (µg/100 g)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	NA	NA	NA	NA	NA	NA	NA
T_2 – Gac fruit pigment	231.85±1.03^{b}	210.79±0.73^{b}	187.38±0.56^{b}	169.29±0.36^{b}	219.21±0.55^{b}	203.79±0.60^{b}	186.74±0.48^{b}
T_3 – Marigold pigment	239.69±0.37^{a}	218.22±1.35^{a}	199.65±0.46^{a}	179.36±0.17^{a}	232.26±1.17^{a}	208.76±0.41^{a}	195.77±0.49^{a}
S.Em±	0.63	0.89	0.42	0.23	0.75	0.42	0.40
C.D (0.05)	1.97	2.76	1.29	0.72	2.32	0.6	1.23

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)
NA = not-available

Instrumental colour values for *a** in guava jelly decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 99). The decrease was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *a** in guava jelly without pigment, added with gac fruit and marigold pigment was 29.30±0.14, 47.44±0.19 and 30.59±0.19, respectively. After three months of storage, the lowest *a** value (11.76±0.41) was noticed in guava jelly added with marigold pigment stored under ambient condition, whereas the highest *a** value (38.34±0.09) was observed in guava jelly added with gac fruit pigment stored under refrigerated condition.

Instrumental colour values for *b** in guava jelly decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 100). The decrease was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *b** in guava jelly without pigment, added with gac fruit and marigold pigment was 40.45±0.14, 25.58±0.13 and 90.34±0.10, respectively. After three months of storage, the lowest *b** value (14.50±0.12) was noticed in guava jelly added with gac fruit pigment stored under ambient condition, whereas the highest *b** value (79.01±0.27) was observed in guava jelly added with marigold pigment stored under refrigerated condition.

Instrumental colour values for *hue angle* (°) in guava jelly increased significantly throughout the storage period, irrespective of storage conditions except in guava jelly without pigment (Table 101). The increase was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *hue angle* in guava jelly without pigment, added with gac fruit and marigold pigment was 84.46±0.15°, 40.47±0.13° and 81.13±0.16°, respectively. After three months of storage, the highest *hue angle* value (91.32±0.16°) was noticed in guava jelly added with marigold pigment stored under ambient condition, whereas the lowest *hue angle* value (54.47±0.15°) was observed in guava jelly added with gac fruit pigment stored under refrigerated condition.

Instrumental colour values for *chroma* in guava jelly decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 102). The decrease was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *chroma* in guava jelly without pigment, added with gac fruit and marigold pigment was 50.36±0.08, 48.97±0.15 and 67.55±0.11, respectively. After three months of storage, the highest *chroma* value (47.78±0.13) was noticed in guava jelly added with marigold pigment stored under refrigerated condition, whereas the lowest *chroma* value (35.50±0.11) was observed in guava jelly added with gac fruit pigment stored under ambient condition.

4.6.2.6 Antioxidant properties

4.6.2.6.1 DPPH assay (µl/ml)

DPPH value of guava jelly increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 103). The increase was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, DPPH value in the guava jelly without pigment, added with gac fruit and marigold pigment was 5.117±0.048, 0.835±0.001 and 0.608±0.002 µl/ml, respectively. After three months of storage, the highest DPPH radical scavenging activity (1.238±0.007 µl/ml) was observed in guava jelly added with marigold pigment stored under refrigerated condition, whereas the lowest activity (28.665±0.250 µl/ml) was noticed in guava jelly without pigment stored under ambient condition.

4.6.2.6.2 ABTS assay (µl/ml)

ABTS value of guava jelly increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 103). The increase was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, ABTS value in the guava jelly without pigment, added with gac fruit and marigold pigment was 4.138±0.113, 0.815±0.005 and 0.598±0.001 µl/ml, respectively.

Table 98: Changes in *L value of guava jelly incorporated with carotenoid pigment during storage**

Treatments	Initial	*L** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	99.51±0.07[a]	90.50±0.10[a]	86.49±0.14[a]	81.43±0.17[a]	98.30±0.10[a]	92.49±0.14[a]	87.49±0.20[a]
T_2 – Gac fruit pigment	87.55±0.16[c]	83.68±0.07[c]	81.14±0.12[c]	71.35±0.09[c]	85.74±0.22[c]	82.64±0.14[c]	75.54±0.14[c]
T_3 – Marigold pigment	92.47±0.16[b]	89.49±0.09[b]	85.70±0.16[b]	79.58±0.16[b]	90.76±0.13[b]	86.82±0.27[b]	81.71±0.20[b]
S.Em±	0.14	0.09	0.14	0.14	0.16	0.19	0.18
C.D (0.05)	0.42	0.27	0.44	0.45	0.5	0.6	0.57

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 99: Changes in *a value of guava jelly incorporated with carotenoid pigment during storage**

Treatments	Initial	*a** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	29.30±0.14^{c}	26.40±0.10^{b}	22.52±0.06^{b}	17.45±0.07^{b}	27.42±0.14^{b}	24.41±0.12^{c}	20.48±0.09^{c}
T_2 – Gac fruit pigment	47.44±0.19^{a}	44.11±0.04^{a}	39.80±0.05^{a}	33.99±0.06^{a}	45.42±0.27^{a}	42.46±0.12^{a}	38.34±0.09^{a}
T_3 – Marigold pigment	30.59±0.19^{b}	26.29±0.15^{b}	21.29±0.21^{c}	11.76±0.41^{c}	27.95±0.11^{b}	25.11±0.11^{b}	21.27±0.18^{b}
S.Em±	0.17	0.11	0.13	0.24	0.19	0.12	0.13
C.D (0.05)	0.54	0.34	0.40	0.75	0.58	0.36	0.40

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 100: Changes in *b value of guava jelly incorporated with carotenoid pigment during storage**

Treatments	Initial	*b** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	40.45±0.14^{b}	36.74±0.35^{b}	32.50±0.08^{b}	28.14±0.32^{b}	38.48±0.16^{b}	35.43±0.13^{b}	32.19±0.20^{b}
T_2 – Gac fruit pigment	25.58±0.13^{c}	22.63±0.14^{c}	19.36±0.10^{c}	14.50±0.12^{c}	23.54±0.18^{c}	20.69±0.15^{c}	17.66±0.13^{c}
T_3 – Marigold pigment	90.34±0.10^{a}	84.39±0.16^{a}	77.19±0.09^{a}	66.44±0.16^{a}	87.42±0.14^{a}	83.45±0.12^{a}	79.01±0.27^{a}
S.Em±	0.13	0.24	0.09	0.22	0.16	0.14	0.21
C.D (0.05)	0.39	0.74	0.28	0.68	0.50	0.42	0.65

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 101: Changes in *hue angle* (º) value of guava jelly incorporated with carotenoid pigment during storage

Treatments	Initial	*Hue angle* (º) value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	84.46±0.15^{a}	80.39±0.14^{b}	75.31±0.08^{b}	69.36±0.13^{b}	82.51±0.09^{a}	79.24±0.12^{b}	74.17±0.05^{b}
T_2 – Gac fruit pigment	40.47±0.13^{c}	44.37±0.20^{c}	51.32±0.14^{c}	60.39±0.08^{c}	41.45±0.13^{b}	47.45±0.13^{c}	54.47±0.15^{c}
T_3 – Marigold pigment	81.13±0.16^{b}	34.04±0.16^{a}	88.41±0.16^{a}	91.32±0.16^{a}	82.58±0.16^{a}	84.39±0.13^{a}	89.87±0.16^{a}
S.Em±	0.15	0.17	0.13	0.13	0.13	0.12	0.13
C.D (0.05)	0.45	0.53	0.40	0.40	0.40	0.39	0.41

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 102: Changes in *chroma* value of guava jelly incorporated with carotenoid pigment during storage

Treatments	Initial	*Chroma* value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	50.36±0.08^{b}	47.70±0.10^{b}	43.46±0.10^{b}	39.44±0.10^{b}	49.37±0.12^{b}	45.68±0.15^{b}	41.20±0.10^{b}
T_2 – Gac fruit pigment	48.97±0.15^{c}	44.48±0.16^{c}	39.51±0.16^{c}	35.50±0.11^{c}	47.24±0.25^{c}	41.63±0.22^{c}	37.44±0.14^{c}
T_3 – Marigold pigment	67.55±0.11^{a}	63.19±0.74^{a}	57.57±0.16^{a}	41.56±0.13^{a}	65.57±0.17^{a}	61.54±0.11^{a}	47.78±0.13^{a}
S.Em±	0.12	0.44	0.14	0.12	0.19	0.17	0.12
C.D (0.05)	0.36	1.37	0.45	0.36	0.59	0.52	0.39

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 103: Changes in DPPH, ABTS and FRAP antioxidant activity of guava jelly incorporated with carotenoid pigment during storage

Treatments	Initial	DPPH activity (µl/ml)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	5.117±0.048^{a}	9.012±0.064^{a}	18.618±0.213^{a}	28.665±0.250^{a}	6.448±0.017^{a}	13.177±0.058^{a}	21.373±0.086^{a}
T_2 – Gac fruit pigme	0.835±0.001^{b}	1.057±0.001^{b}	1.337±0.002^{b}	1.889±0.004^{b}	0.950±0.004^{b}	1.033±0.001^{b}	1.406±0.003^{b}
T_3 – Marigold pigme	0.608±0.002^{c}	0.786±0.003^{c}	0.984±0.001^{c}	1.368±0.004^{c}	0.728±0.001^{c}	0.869±0.002^{c}	1.238±0.007^{c}
S.Em±	0.028	0.037	0.123	0.144	0.010	0.033	0.050
C.D (0.05)	0.087	0.116	0.383	0.450	0.031	0.104	0.155
	ABTS activity (µl/ml)						
T_1 – No pigment	4.138±0.113^{a}	9.273±0.092^{a}	15.741±0.108^{a}	23.805±0.183^{a}	4.288±0.043^{a}	8.257±0.104^{a}	15.413±0.207^{a}
T_2 – Gac fruit pigme	0.815±0.005^{b}	1.009±0.001^{b}	1.299±0.002^{b}	1.777±0.006^{b}	0.927±0.003^{b}	1.007±0.003^{b}	1.358±0.005^{b}
T_3 – Marigold pigme	0.598±0.001^{c}	0.753±0.002^{c}	0.961±0.002^{c}	1.311±0.007^{c}	0.715±0.002^{c}	0.837±0.002^{b}	1.205±0.006^{b}
S.Em±	0.065	0.053	0.062	0.106	0.025	0.060	0.120
C.D (0.05)	0.204	0.166	0.194	0.330	0.077	0.188	0.373
	FRAP activity (µl/ml)						
T_1 – No pigment	2.576±0.012^{a}	5.168±0.025^{a}	9.307±0.070^{a}	16.157±0.200^{a}	4.757±0.013^{a}	7.120±0.043^{a}	12.152±0.118^{a}
T_2 – Gac fruit pigme	0.890±0.002^{b}	1.139±0.001^{b}	1.766±0.003^{b}	3.308±0.011^{b}	1.080±0.009^{b}	1.318±0.009^{b}	2.223±0.018^{b}
T_3 – Marigold pigme	0.633±0.002^{c}	0.831±0.003^{c}	1.342±0.005^{c}	2.138±0.017^{c}	0.805±0.005^{c}	0.999±0.005^{c}	1.793±0.014^{c}
S.Em±	0.007	0.015	0.041	0.116	0.009	0.026	0.069
C.D (0.05)	0.023	0.046	0.127	0.362	0.029	0.080	0.215

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

After three months of storage, the highest ABTS radical scavenging activity (1.205±0.006 µl/ml) was observed in guava jelly added with marigold pigment stored under refrigerated condition, whereas the lowest activity (23.805±0.183 µl/ml) was noticed in guava jelly without pigment stored under ambient condition.

4.6.2.6.3 FRAP assay (µl/ml)

FRAP value of guava jelly increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 103). The increase was rapid in the guava jelly stored under ambient condition as compared to refrigerated condition. Before storage, FRAP value in the guava jelly without pigment, added with gac fruit and marigold pigment was 2.576±0.012, 0.890±0.002 and 0.633±0.002 µl/ml, respectively. After three months of storage, the highest ferric reducing antioxidant power (1.793±0.014 µl/ml) was observed in guava jelly added with marigold pigment stored under refrigerated condition, whereas the lowest ferric reducing antioxidant power (16.157±0.200 µl/ml) was noticed in guava jelly without pigment stored under ambient condition.

4.6.2.7 Sensory evaluation of guava jelly (9-point hedonic scale)

Data on mean sensory scores of guava jelly without pigment, added with gac fruit and marigold pigment during initial, first, second and three months after storage under ambient and refrigerated conditions are presented in Tables 104, 105, 106, respectively. Sensory scores of guava jelly declined during storage period, irrespective of treatments and storage conditions. The rate of decrease was faster in the guava jelly stored under ambient condition as compared to refrigerated condition. After three months of storage, guava jelly added with marigold pigment stored under refrigerated condition recorded highest sensory score (40.25), while the lowest (29.40) was noticed in the guava jelly without pigment stored under ambient condition.

Table 104: Sensory quality of guava jelly incorporated with carotenoid pigment (Initial)

Treatments	Initial					
	Visual appearance and transparency	**Colour**	**Flavour**	**After-taste**	**Overall acceptability**	**Total score**
T_1 – No pigment	8.20	8.00	8.00	8.00	8.05	40.25
T_2 – Gac fruit pigment	8.60	8.60	8.20	8.20	8.40	42.00
T_3 – Marigold pigment	8.80	9.00	8.40	8.20	8.60	43.00
Kendall's W test value	0.47	0.76	0.30	0.20	0.86	

Kendall's W value: Kendall's coefficient of concordance

Table 105: Changes in sensory quality of guava jelly incorporated with carotenoid pigment stored under ambient condition

Treatments	One MAS under ambient condition					
	Visual appearance and transparency	Colour	Flavour	After-taste	Overall acceptability	Total score
T_1 – No pigment	8.00	7.80	7.80	7.80	7.85	39.25
T_2 – Gac fruit pigment	8.20	8.40	8.00	8.00	8.15	40.75
T_3 – Marigold pigment	9.00	9.00	8.20	8.00	8.55	42.75
Kendall's W test value	0.75	0.80	0.30	0.20	0.93	
	Two MAS under ambient condition					
T_1 – No pigment	7.20	7.60	7.60	7.60	7.50	37.50
T_2 – Gac fruit pigment	7.60	8.20	7.80	7.80	7.85	39.25
T_3 – Marigold pigment	8.20	8.40	7.80	7.80	8.05	40.25
Kendall's W test value	0.76	0.65	0.20	0.20	0.96	
	Three MAS under ambient condition					
T_1 – No pigment	6.60	7.00	7.60	7.60	7.20	29.40
T_2 – Gac fruit pigment	7.40	7.60	7.80	7.80	7.65	30.85
T_3 – Marigold pigment	7.60	7.80	7.80	7.80	7.75	31.15
Kendall's W test value	0.75	0.65	0.20	0.20	0.74	

Kendall's W value: Kendall's coefficient of concordance

Table 106: Changes in sensory quality of guava jelly incorporated with carotenoid pigment stored under refrigerated condition

Treatments	One MAS under refrigerated condition					
	Visual appearance and transparency	Colour	Flavour	After-taste	Overall acceptability	Total score
T_1 – No pigment	8.20	8.20	7.80	7.80	8.00	40.00
T_2 – Gac fruit pigment	8.40	8.60	8.00	8.00	8.25	41.25
T_3 – Marigold pigment	9.00	9.00	8.40	8.00	8.60	43.00
Kendall's W test value	0.65	0.60	0.47	0.20	0.93	
	Two MAS under refrigerated condition					
T_1 – No pigment	7.40	7.80	7.80	7.80	7.70	38.50
T_2 – Gac fruit pigment	8.40	8.00	8.00	8.00	8.10	40.50
T_3 – Marigold pigment	8.60	8.60	8.00	8.00	8.30	41.50
Kendall's W test value	0.95	0.65	0.20	0.20	0.93	
	Three MAS under refrigerated condition					
T_1 – No pigment	7.00	7.60	7.60	7.60	7.45	37.25
T_2 – Gac fruit pigment	7.60	7.80	7.80	7.80	7.75	38.75
T_3 – Marigold pigment	8.20	8.20	8.00	7.80	8.05	40.25
Kendall's W test value	0.80	0.47	0.30	0.20	0.86	

Kendall's W value: Kendall's coefficient of concordance

Table 107: Changes in non-enzymatic browning of guava jelly incorporated with carotenoid pigment during storage

Treatments	Initial	Non-enzymatic browning (OD value)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	0.167±0.013	0.275±0.003	0.374±0.029	0.379±0.052	0.241±0.007	0.341±0.035	0.364±0.030
T_2 – Gac fruit pigment	0.160±0.015	0.266±0.003	0.354±0.021	0.362±0.010	0.233±0.012	0.330±0.032	0.358±0.005
T_3 – Marigold pigment	0.161±0.021	0.261±0.006	0.340±0.010	0.351±0.015	0.228±0.014	0.325±0.038	0.347±0.013
S.Em±	0.017	0.004	0.022	0.032	0.011	0.035	0.019
C.D (0.05)	NS	NS	NS	NS	NS	NS	NS

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

4.6.2.8 Non-enzymatic browning

The data pertaining to non-enzymatic browning is presented in the Table 27. Non-significant variation was recorded with respect to non-enzymatic browning throughout the storage period, irrespective of treatments and storage conditions. Before storage, non-enzymatic browning in guava jelly without pigment, added with gac fruit and marigold pigment was 0.167±0.013, 0.160±0.015 and 0.161±0.021, respectively. After three months of storage, numerically highest value (0.379±0.052) was noticed in guava jelly without pigment stored under ambient condition, whereas numerically lowest value (0.347±0.013) was observed in guava jelly added with marigold pigment stored under refrigerated condition.

4.6.3 Ash gourd candy coloured with carotenoid pigment

4.6.3.1 Lycopene content (µg/100 g)

Lycopene content of ash gourd candy decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 108). The decrease was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, lycopene content in the ash gourd candy added with gac fruit and marigold pigment was 129.99±0.06 µg/100 g and 28.36±0.09 µg/100 g, respectively. After three months of storage, the highest retention of lycopene content (112.34±0.17 µg/100 g) was noticed in ash gourd candy added with gac fruit pigment stored under refrigerated condition, whereas the lowest retention (12.74±0.17 µg/100 g) was observed in ash gourd candy added with marigold pigment stored under ambient condition.

4.6.3.2 β-carotene content (µg/100 g)

β-carotene content of ash gourd candy decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 109). The decrease was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, β-carotene content in the ash gourd candy added with gac fruit and marigold pigment was 78.68±0.40 µg/100 g and 56.21±0.22 µg/100 g, respectively. After three months of storage, the highest

retention of β-carotene content (61.64±0.27 µg/100 g) was noticed in ash gourd candy added with gac fruit pigment stored under refrigerated condition, whereas the lowest retention (31.81±0.11 µg/100 g) was observed in ash gourd candy added with marigold pigment stored under ambient condition.

4.6.3.3 Lutein content (µg/100 g)

Lutein content of ash gourd candy decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 110). The decrease was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, lutein content in the ash gourd candy added with gac fruit and marigold pigment was 22.20±0.07 µg/100 g and 154.89±0.21 µg/100 g, respectively. After three months of storage, the highest retention of lutein content (135.29±0.19 µg/100 g) was noticed in ash gourd candy added with marigold pigment stored under refrigerated condition, whereas the lowest retention (3.43±0.10 µg/100 g) was observed in ash gourd candy added with gac fruit pigment stored under ambient condition.

4.6.3.4 Total carotenoid content (µg/100 g)

Total carotenoid content of ash gourd candy decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 111). The decrease was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, total carotenoid content in the ash gourd candy added with gac fruit and marigold pigment was 236.43±1.03 µg/100 g and 244.27±0.37 µg/100 g, respectively. After three months of storage, the highest retention of total carotenoid content (200.35±0.49 µg/100 g) was noticed in ash gourd candy added with marigold pigment stored under refrigerated condition, whereas the minimum retention (173.87±0.36 µg/100 g) was observed in ash gourd candy added with gac fruit pigment stored under ambient condition.

Table 108: Changes in lycopene content of ash gourd candy incorporated with carotenoid pigment during storage

Treatments	Initial	Lycopene content (µg/100 g)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	NA	NA	NA	NA	NA	NA	NA
T_2 – Gac fruit pigment	129.99±0.06^{a}	124.04±0.13^{a}	115.88±0.38^{a}	105.02±0.34^{a}	125.91±0.22^{a}	120.12±0.29^{a}	112.34±0.17^{a}
T_3 – Marigold pigment	28.36±0.09^{b}	23.76±0.10^{b}	18.72±0.15^{b}	12.74±0.17^{b}	24.55±0.15^{b}	20.73±0.13^{b}	14.09±0.07^{b}
S.Em±	0.06	0.09	0.24	0.22	0.15	0.18	0.11
C.D (0.05)	0.19	0.29	0.74	0.69	0.47	0.57	0.34

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)
NA = not-available

Table 109: Changes in β-carotene content of ash gourd candy incorporated with carotenoid pigment during storage

Treatments	Initial	β-carotene content (µg/100 g)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	NA	NA	NA	NA	NA	NA	NA
T_2 – Gac fruit pigment	78.68±0.40[a]	71.94±0.28[a]	63.46±0.21[a]	54.84±0.28[a]	74.00±0.26[a]	68.33±0.21[a]	61.64±0.27[a]
T_3 – Marigold pigment	56.21±0.22[b]	50.89±0.10[b]	42.59±0.08[b]	31.81±0.11[b]	55.74±0.10[b]	49.09±0.18[b]	39.89±0.20[b]
S.Em±	0.26	0.17	0.13	0.17	0.16	0.16	0.19
C.D (0.05)	0.81	0.53	0.40	0.54	0.51	0.50	0.60

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)
NA = not-available

Table 110: Changes in lutein content of ash gourd candy incorporated with carotenoid pigment during storage

Treatments	Initial	Lutein content (µg/100 g)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	NA	NA	NA	NA	NA	NA	NA
T_2 – Gac fruit pigment	22.20±0.07[a]	17.92±0.11[a]	11.10±0.26[a]	3.43±0.10[a]	19.07±0.15[a]	16.77±0.14[a]	11.28±0.10[a]
T_3 – Marigold pigment	154.89±0.21[b]	144.24±0.21[b]	139.39±0.08[b]	123.09±0.31[b]	147.14±0.13[b]	142.15±0.20[b]	135.29±0.19[b]
S.Em±	0.13	0.14	0.16	0.19	0.12	0.14	0.13
C.D (0.05)	0.40	0.43	0.49	0.58	0.36	0.44	0.39

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)
NA = not-available

Table 111: Changes in total carotenoid content of ash gourd candy incorporated with carotenoid pigment during storage

Treatments	Initial	Total carotenoid content (µg/100 g)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	NA	NA	NA	NA	NA	NA	NA
T_2 – Gac fruit pigment	236.43±1.03^{b}	215.36±0.73^{b}	191.95±0.56^{c}	173.87±0.36^{b}	223.79±0.55^{b}	208.36±0.60^{b}	191.32±0.48^{b}
T_3 – Marigold pigment	244.27±0.37^{a}	222.80±1.35^{a}	204.23±0.46^{a}	183.94±0.17^{c}	236.84±1.17^{a}	213.34±0.42^{a}	200.35±0.49^{a}
S.Em±	0.63	0.89	0.42	0.23	0.75	0.42	0.39
C.D (0.05)	1.97	2.76	1.29	0.71	2.32	1.32	1.23

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)
NA = not-available

4.6.3.5 Colour properties

4.6.3.5.1 Instrumental colour values

Instrumental colour values for *L** in ash gourd candy decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 112). The decrease was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *L** in ash gourd candy without pigment, added with gac fruit and marigold pigment was 98.88±0.10, 97.68±0.13 and 93.54±0.13, respectively. After three months of storage, the highest *L** value (92.24±0.24) was noticed in ash gourd candy added with gac fruit pigment stored under refrigerated condition, whereas the lowest *L** value (76.71±0.11) was observed in ash gourd candy without pigment stored under ambient condition.

Instrumental colour values for *a** in ash gourd candy decreased significantly throughout the storage period, irrespective of storage conditions except in ash gourd candy without pigment (Table 113). The decrease was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *a** in ash gourd candy without pigment, added with gac fruit and marigold pigment was 0.06±0.004, 24.41±0.19 and 28.84±0.20, respectively. After three months of storage, the lowest *a** value (10.01±0.21) was noticed in ash gourd candy added with marigold pigment stored under ambient condition, whereas the highest *a** value (20.19±0.18) was observed in ash gourd candy added with marigold pigment stored under refrigerated condition.

Instrumental colour values for *b** in ash gourd candy decreased significantly throughout the storage period, irrespective of storage conditions except in ash gourd candy without pigment (Table 114). The decrease was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *b** in ash gourd candy without pigment, added with gac fruit and marigold pigment was 15.51±0.12, 23.47±0.13 and 88.41±0.11, respectively. After three months of storage, the lowest *b** value (12.39±0.12) was noticed in ash gourd candy added with gac fruit pigment stored under ambient

condition, whereas the highest *b** value (77.06±0.27) was observed in ash gourd candy added with marigold pigment stored under refrigerated condition.

Instrumental colour values for *hue angle* (°) in ash gourd candy decreased significantly throughout the storage period, irrespective of storage conditions except in ash gourd candy added with marigold pigment (Table 115). The decrease was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *hue angle* in ash gourd candy without pigment, added with gac fruit and marigold pigment was 106.41±0.14°, 104.07±0.18° and 79.67±0.16°, respectively. After three months of storage, the highest *hue angle* value (98.07±0.07°) was noticed in ash gourd candy without pigment stored under refrigerated condition, whereas the lowest *hue angle* value (86.10±1.12°) was observed in ash gourd candy added with marigold pigment stored under refrigerated condition.

Instrumental colour values for *chroma* in ash gourd candy decreased significantly throughout the storage period, irrespective of storage conditions except in ash gourd candy without pigment (Table 116). The decrease was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, instrumental colour value for *chroma* in ash gourd candy without pigment, added with gac fruit and marigold pigment was 38.38±0.04, 21.33±0.11 and 77.50±0.13, respectively. After three months of storage, the highest *chroma* value (68.21±0.07) was noticed in ash gourd candy added with marigold pigment stored under refrigerated condition, whereas the lowest *chroma* value (13.57±0.14) was observed in ash gourd candy added with gac fruit pigment stored under ambient condition.

Table 112: Changes in *L value of ash gourd candy incorporated with carotenoid pigment during storage**

Treatments	Initial	*L** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	98.88±0.10^{a}	95.54±0.13^{a}	87.55±0.08^{b}	76.71±0.11^{c}	96.64±0.05^{a}	88.48±0.10^{c}	80.58±0.07^{c}
T_2 – Gac fruit pigment	97.68±0.13^{b}	91.70±0.09^{b}	89.37±0.09^{a}	86.70±0.27^{a}	95.44±0.10^{b}	94.47±0.16^{a}	92.24±0.24^{a}
T_3 – Marigold pigment	93.54±0.13^{c}	88.86±0.17^{c}	85.58±0.16^{c}	80.99±0.11^{b}	92.10±0.20^{c}	89.50±0.11^{b}	85.82±0.33^{b}
S.Em±	0.12	0.14	0.12	0.18	0.13	0.13	0.24
C.D (0.05)	0.37	0.42	0.36	0.55	0.42	0.39	0.74

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 113: Changes in *a value of ash gourd candy incorporated with carotenoid pigment during storage**

Treatments	Initial	*a** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	-0.06±0.004^{c}	-1.24±0.01^{c}	-2.98±0.01^{c}	-5.56±0.12^{c}	-0.95±0.01^{c}	-1.89±0.03^{c}	-3.07±0.12^{c}
T_2 – Gac fruit pigment	24.41±0.19^{b}	22.34±0.15^{b}	20.47±0.14^{a}	16.47±0.18^{a}	23.71±0.08^{b}	21.51±0.15^{b}	18.34±0.12^{b}
T_3 – Marigold pigment	28.84±0.20^{a}	24.54±0.15^{a}	19.54±0.21^{b}	10.01±0.41^{b}	26.20±0.11^{a}	23.36±0.11^{a}	20.19±0.18^{a}
S.Em±	0.16	0.13	0.14	0.27	0.08	0.11	0.14
C.D (0.05)	0.49	0.39	0.45	0.83	0.24	0.34	0.45

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 114: Changes in *b value of ash gourd candy incorporated with carotenoid pigment during storage**

Treatments	Initial	*b** value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	15.51±0.12^{c}	17.29±0.15^{c}	20.50±0.07^{b}	24.56±0.14^{b}	16.54±0.14^{c}	18.79±0.21^{b}	20.59±0.21^{b}
T_2 – Gac fruit pigment	23.47±0.13^{b}	20.52±0.14^{b}	17.25±0.10^{c}	12.39±0.12^{c}	21.43±0.18^{b}	18.58±0.15^{b}	15.55±0.13^{c}
T_3 – Marigold pigment	88.41±0.11^{a}	82.44±0.16^{a}	75.24±0.09^{a}	64.49±0.16^{a}	85.47±0.14^{a}	81.50±0.12^{a}	77.06±0.27^{a}
S.Em±	0.12	0.15	0.09	0.14	0.15	0.17	0.21
C.D (0.05)	0.37	0.47	0.28	0.44	0.48	0.52	0.66

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 115: Changes in *hue angle* (º) value of ash gourd candy incorporated with carotenoid pigment during storage

Treatments	Initial	*Hue angle* (º) value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	106.41±0.14^{a}	103.48±0.15^{a}	99.91±0.03^{a}	93.22±0.14^{b}	104.40±0.09^{a}	101.68±0.16^{a}	98.07±0.07^{a}
T_2 – Gac fruit pigment	104.07±0.18^{b}	101.59±0.13^{b}	99.05±0.06^{b}	95.53±0.15^{a}	102.37±0.20^{b}	99.46±0.15^{b}	96.47±0.17^{b}
T_3 – Marigold pigment	79.67±0.16^{c}	82.37±0.11^{c}	86.40±0.11^{c}	91.42±0.07^{c}	81.42±0.07^{c}	84.39±0.13^{c}	86.10±1.12^{c}
S.Em±	0.16	0.13	0.08	0.13	0.13	0.15	0.66
C.D (0.05)	0.50	0.39	0.24	0.39	0.41	0.45	2.05

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 116: Changes in *chroma* value of ash gourd candy incorporated with carotenoid pigment during storage

Treatments	Initial	*Chroma* value					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	38.38±0.04^{b}	40.71±0.15^{b}	43.45±0.12^{b}	46.35±0.13^{b}	39.24±0.06^{b}	41.69±0.12^{b}	43.00±0.16^{b}
T_2 – Gac fruit pigment	21.33±0.11^{c}	19.27±0.36^{c}	16.75±0.20^{c}	13.57±0.14^{c}	20.53±0.14^{c}	17.71±0.10^{c}	15.51±0.14^{c}
T_3 – Marigold pigment	77.50±0.13^{a}	72.36±0.16^{a}	67.27±0.14^{a}	61.45±0.11^{a}	74.51±0.10^{a}	71.45±0.13^{a}	68.21±0.07^{a}
S.Em±	0.10	0.24	0.16	0.13	0.10	0.12	0.13
C.D (0.05)	0.31	0.75	0.48	0.40	0.32	0.37	0.40

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

4.6.3.6 Antioxidant properties

4.6.3.6.1 DPPH assay (µl/ml)

DPPH value of ash gourd candy increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 117). The increase was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, DPPH value in the ash gourd candy without pigment, added with gac fruit and marigold pigment was 5.026±0.040, 0.632±0.001 and 0.554±0.001 µl/ml, respectively. After three months of storage, the highest DPPH radical scavenging activity (0.778±0.003 µl/ml) was observed in ash gourd candy added with marigold pigment stored under refrigerated condition, whereas the lowest activity (22.509±0.123 µl/ml) was noticed in ash gourd candy without pigment stored under ambient condition.

4.6.3.6.2 ABTS assay (µl/ml)

ABTS value of ash gourd candy increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 117). The increase was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, ABTS value in the ash gourd candy without pigment, added with gac fruit and marigold pigment was 3.446±0.038, 0.541±0.001 and 0.514±0.001 µl/ml, respectively. After three months of storage, the highest ABTS radical scavenging activity (0.813±0.001 µl/ml) was observed in ash gourd candy added with marigold pigment stored under refrigerated condition, whereas the lowest activity (18.222±0.332 µl/ml) was noticed in ash gourd candy without pigment stored under ambient condition.

4.6.3.6.3 FRAP assay (µl/ml)

FRAP value of ash gourd candy increased significantly throughout the storage period, irrespective of treatments and storage conditions (Table 117). The increase was rapid in the ash gourd candy stored under ambient condition as compared to refrigerated condition. Before storage, FRAP value in the ash gourd candy without

pigment, added with gac fruit and marigold pigment was 2.433±0.011, 0.813±0.007 and 0.644±0.007 µl/ml, respectively. After three months of storage, the highest ferric reducing antioxidant power (1.326±0.031 µl/ml) was observed in ash gourd candy added with marigold pigment stored at refrigerated condition, whereas the lowest ferric reducing antioxidant power (13.081±0.059 µl/ml) was noticed in ash gourd candy without pigment stored under ambient condition.

4.6.3.7 Sensory evaluation of ash gourd candy (9-point hedonic scale)

Data on mean sensory scores of ash gourd candy without pigment, added with gac fruit and marigold pigment during initial, first, second and three months after storage under ambient and refrigerated conditions are presented in Tables 118, 119, 120, respectively. Sensory scores of ash gourd candy declined during storage period, irrespective of treatments and storage conditions. The rate of decrease was faster in the ash gourd candy stored under ambient condition as compared to refrigerated condition. After three months of storage, ash gourd candy added with marigold pigment stored under refrigerated condition recorded highest sensory score (48.00), while the lower score (41.28) was noticed in the ash gourd candy without pigment and added with marigold pigment stored under ambient condition.

Note: Non-enzymatic browning in ash gourd candy was not observed.

Table 117: Changes in DPPH, ABTS and FRAP antioxidant activity of ash gourd candy incorporated with carotenoid pigment during storage

Treatments	Initial	DPPH activity (µl/ml)					
		Ambient condition			Refrigerated condition		
		One MAS	Two MAS	Three MAS	One MAS	Two MAS	Three MAS
T_1 – No pigment	5.026±0.040^{a}	7.481±0.044^{a}	13.048±0.098^{a}	22.509±0.123^{a}	5.625±0.013^{a}	8.912±0.026^{a}	17.139±0.045^{a}
T_2 – Gac fruit pigment	0.632±0.001^{b}	0.764±0.001^{b}	1.199±0.002^{b}	3.574±0.007^{b}	0.653±0.001^{b}	0.777±0.001^{b}	0.993±0.001^{b}
T_3 – Marigold pigment	0.554±0.001^{c}	0.665±0.001^{c}	0.980±0.001^{c}	1.322±0.002^{c}	0.626±0.001^{c}	0.670±0.001^{c}	0.778±0.003^{c}
S.Em±	0.023	0.026	0.054	0.071	0.007	0.015	0.026
C.D (0.05)	0.072	0.080	0.170	0.222	0.023	0.047	0.081
	ABTS activity (µl/ml)						
T_1 – No pigment	3.446±0.038^{a}	6.093±0.179^{a}	10.508±0.245^{a}	18.222±0.332^{a}	4.479±0.005^{a}	7.533±0.027^{a}	12.164±0.266^{a}
T_2 – Gac fruit pigment	0.541±0.001^{b}	0.626±0.001^{b}	0.771±0.002^{b}	1.002±0.003^{b}	0.575±0.003^{b}	0.687±0.002^{b}	0.962±0.002^{b}
T_3 – Marigold pigment	0.514±0.001^{b}	0.695±0.003^{b}	0.844±0.003^{b}	1.471±0.003^{b}	0.548±0.001^{c}	0.638±0.001^{b}	0.813±0.001^{b}
S.Em±	0.022	0.103	0.141	0.191	0.004	0.016	0.154
C.D (0.05)	0.068	0.322	0.441	0.596	0.011	0.05	0.478
	FRAP activity (µl/ml)						
T_1 – No pigment	2.433±0.011^{a}	4.621±0.020^{a}	7.672±0.047^{a}	13.081±0.059^{a}	3.906±0.009^{a}	6.746±0.009^{a}	10.762±0.188^{a}
T_2 – Gac fruit pigment	0.813±0.007^{b}	0.987±0.017^{b}	2.040±0.032^{b}	5.046±0.075^{b}	0.866±0.006^{b}	1.195±0.024^{b}	1.804±0.023^{b}
T_3 – Marigold pigment	0.644±0.007^{c}	1.321±0.008^{c}	1.518±0.028^{c}	2.249±0.022^{c}	0.707±0.004^{c}	0.880±0.015^{c}	1.326±0.031^{c}
S.Em±	0.009	0.016	0.037	0.056	0.006	0.017	0.111
C.D (0.05)	0.027	0.05	0.114	0.176	0.02	0.053	0.345

Note: Similar alphabets within the column represent non-significant differences at ($p<0.05$)

Table 118: Sensory quality of ash gourd candy incorporated with carotenoid pigment (Initial)

Treatments	Initial						
	Visual appearance	**Colour**	**Flavour**	**Texture**	**After-taste**	**Overall acceptability**	**Total score**
T_1 – No pigment	8.40	8.20	8.40	8.60	8.00	8.32	49.92
T_2 – Gac fruit pigment	8.60	8.40	8.80	8.80	8.40	8.60	51.60
T_3 – Marigold pigment	8.80	8.80	8.60	8.80	8.20	8.64	51.84
Kendall's W test value	0.30	0.47	0.30	0.20	0.30	0.75	

Kendall's W value: Kendall's coefficient of concordance

Table 119: Changes in sensory quality of ash gourd candy incorporated with carotenoid pigment stored under ambient condition

Treatments	One MAS under ambient condition						
	Visual appearance	Colour	Flavour	Texture	After-taste	Overall acceptability	Total score
T_1 – No pigment	8.40	8.20	8.20	8.20	7.80	8.16	48.96
T_2 – Gac fruit pigment	8.40	8.20	8.20	8.40	8.00	8.24	49.44
T_3 – Marigold pigment	8.20	7.60	7.40	8.20	7.80	7.84	47.04
Kendall's W test value	0.20	0.60	0.80	0.20	0.20	0.93	
	Two MAS under ambient condition						
T_1 – No pigment	7.80	7.80	7.40	7.40	7.60	7.60	45.60
T_2 – Gac fruit pigment	8.00	8.00	7.60	7.80	7.80	7.84	47.04
T_3 – Marigold pigment	7.80	7.40	7.20	7.40	7.60	7.48	44.88
Kendall's W test value	0.20	0.47	0.30	0.40	0.20	0.70	
	Three MAS under ambient condition						
T_1 – No pigment	6.60	6.20	7.00	7.00	7.60	6.88	41.28
T_2 – Gac fruit pigment	7.40	7.00	7.00	7.40	7.80	7.32	43.92
T_3 – Marigold pigment	6.60	6.40	6.60	7.20	7.60	6.88	41.28
Kendall's W test value	0.80	0.65	0.40	0.30	0.20	0.88	

Kendall's W value: Kendall's coefficient of concordance

Table 120: Changes in sensory quality of ash gourd candy incorporated with carotenoid pigment stored under refrigerated condition

Treatments	One MAS under refrigerated condition						
	Visual appearance	Colour	Flavour	Texture	After-taste	Overall acceptability	Total score
T_1 – No pigment	8.40	8.20	8.40	8.40	7.80	8.24	49.44
T_2 – Gac fruit pigment	8.40	8.20	8.40	8.60	8.00	8.32	49.92
T_3 – Marigold pigment	8.60	8.80	8.20	8.60	8.00	8.44	50.64
Kendall's W test value	0.20	0.60	0.20	0.20	0.20	0.50	
	Two MAS under refrigerated condition						
T_1 – No pigment	8.20	8.00	8.20	8.20	7.80	8.08	48.48
T_2 – Gac fruit pigment	8.00	8.20	8.20	8.40	8.00	8.16	48.96
T_3 – Marigold pigment	8.40	8.60	8.00	8.40	8.00	8.28	49.68
Kendall's W test value	0.30	0.47	0.20	0.20	0.20	0.20	
	Three MAS under refrigerated condition						
T_1 – No pigment	7.00	7.40	7.60	7.60	7.60	7.44	44.64
T_2 – Gac fruit pigment	7.60	7.60	8.00	7.80	7.80	7.76	46.56
T_3 – Marigold pigment	8.40	8.60	7.60	7.80	7.60	8.00	48.00
Kendall's W test value	0.86	0.95	0.40	0.20	0.20	0.96	

Kendall's W value: Kendall's coefficient of concordance

Discussion

5. DISCUSSION

The discussion pertaining to the study is presented under the following heads.

5.1 Standardization of extraction method for anthocyanin from butterfly pea flowers and mangosteen fruit rind

5.1.1 Butterfly pea flowers

5.1.1.1 Recovery percentage of anthocyanin pigment concentrate and TMAC

Depending on the quantity of bioactive compounds obtained in the extract, final recovery varies upon vacuum concentration. Significantly higher recovery percentages were seen in acidified aqueous (74.03±0.84 %), MAE (73.17±1.76 %), and aqueous (72.03±1.24 %) extraction methods. It was noted that a large amount of the pigment extract itself was produced during the extraction process, which might have been caused by the petals' low dry matter content. Hence, regardless of the solvent and procedures used, the pigment concentrate recovery percentage after vacuum concentration was higher in all treatments. The plant material and the chemistry of the bioactive substances it contains determine the solubility of the various components, the extraction yield, and the antioxidant activity.

Five different extraction methods were used in the current investigation, among them aqueous (water) extraction technique turned out to be the most effective, producing a higher TMAC of 7925.29±36.07 mg/L. High dielectric constant (ε) (ε = 78.30) (Chemat *et al*., 2019), water at higher temperatures (<100 °C) has more energy and provides a high diffusion rate, enhancing the interaction between the sample and solvent (water), and maximizing the extraction of anthocyanins in the medium, as it is highly soluble in water (Kang *et al*., 2021).

Although the effect varies depending on the type of acid used, several studies have shown that the interaction of acidified solvents increases the release of pigments, such as anthocyanins, from the cell wall membrane. In our study, the anthocyanin yield was less (3772.28±116.74mg/L) in the solvent and acidified solvent method (5771.13±54.19 mg/L). The steps involved in pigment extraction could be the cause

of variances in a given solvent/acid combination's efficacy. This is particularly true if a concentration step is required, as the concentration of acid and anthocyanins in a solution may both increase as the extraction solvent is removed, potentially causing anthocyanin hydrolysis to occur or be prolonged and affecting the final pigment recovery (Garcia-Viguera *et al*., 1998).

Toxicities arise when anthocyanin pigments are extracted using organic solvents like methanol and ethanol. Although ethanol is typically regarded as a safe extraction medium, isolating anthocyanins via water-based extraction is seen to be a more eco-friendly approach (Khoo *et al*., 2017). The MAE method has good reproducibility and enables for a quicker extraction process with less solvent. On the other hand, while boosting chemical diffusion from matrix to solvent may increase extraction yield, it also makes it simpler to extract compounds that are not intended for extraction. Low to moderate powers are used with prolonged MAE extraction times to reduce overheating; however, this does not always prevent anthocyanin degradation as it is very sensitive to temperature (Garofulic *et al*., 2013). This might be the reason for getting a lower (6146.86±22.98 mg/L) anthocyanin yield compared to aqueous extraction method.

Butterfly pea flower is one of the most significant sources of polyacylated blue-coloured anthocyanins, which are highly soluble in water with higher stability, especially at low-acid and neutral conditions. Thus, it is considered the most potential food colourant (Coelho *et al*., 2021; Gamage *et al*., 2021; Marpaung *et al*., 2017; Thuy *et al*., 2021). The ideal extraction temperature and time for anthocyanins were 45 °C and 60 min, respectively. If the duration is too short or too long, high total anthocyanin content won't be obtained because if the time is too short, the anthocyanins might not fully dissolve in the extraction solvent. Yet, because they are thermo-labile pigments, they deteriorate when extracted over an extended period of time at high temperatures (Thuy *et al*., 2021).

There were no abnormalities or symptoms of mortality when albino Wistar rats were given an oral dose of the butterfly pea flower's aqueous ethanol extract (2000 mg/kg body weight), and also, there was no discernible change in the

haematological data (Srichaikul, 2018). Butterfly pea flowers have the potential to be employed as functional foods incorporated into various food products or even as pharmaceuticals or supplements combined with conventional treatments to improve patient outcomes (Jeyaraj *et al*., 2021).

5.1.1.2 Colour properties

The colour values in the pigment concentrate from butterfly pea flower were significantly different. *L*, a**, *b**, *chroma* and *hue* values ranged from 18.09±3.95 to 49.24±1.13; 36.78±0.92 to 46.90±0.77; -63.25±0.13 to -38.02±0.41; 52.90±0.92 to 78.74±0.38; and 306.56±0.50 to 319.44±1.56, respectively. In terms of lightness or luminosity (*L**), the diluted pigment concentrate samples were darker (18.09±3.95) in all treatments except for the acidified aqueous extraction method (49.24±1.13). Lower *L** values indicate darker colour. Due to the samples' positive values, the red/green colour component, *a**, showed the red colour tone. Negative values in the blue/yellow colour component, *b**, were used to denote blue hues.

Among different extraction methods, the pigment concentrate produced by MAE methods displayed a bright blue colour (-63.25±0.13), which was followed by the aqueous method (-54.79±0.88). In contrast, the acidified aqueous extraction method produced a light blue colour (-38.02±0.41). As the chroma, or colour intensity, approached 100, it showed pure colour (depth of the colour). The MAE method had the highest colour intensity (78.74±0.38), followed by the aqueous extraction method (69.30±0.13), while the acidified aqueous extraction method had the lowest colour intensity (52.90±0.92). In MAE and the acidified solvent extraction method, the *hue angle* indicated the chromatic colour values in the range of bright blue-violet (306.56±0.50) to blue-violet (319.44±1.56), respectively. The colour of pigment concentrates is depicted in Plate 8.

5.1.1.3 Antioxidant properties

Plant-derived anthocyanins have antioxidant properties that greatly impact health and therapeutic outcomes. The high antioxidant activity of anthocyanin is a result of its glycosylated B-ring structure, and orthohydroxylation and methoxylation

considerably boost antioxidant activity (Bors *et al*., 1990). Anthocyanin chalcones and quinoidal bases with a double bond conjugated to the keto group are examples of antioxidants that scavenge free radicals (Wang *et al*., 1997).

In the present study, higher antioxidant activity with respect to DPPH (3.49±0.59 µl/ml), FRAP (3.99±1.10 µl/ml), and ABTS (2.42±0.01 µl/ml) was observed in the pigment concentrate extracted using water as the solvent by the MAE method. According to Kamkaen and Wilkinson (2009), after 15 minutes of extraction, the antioxidant capability of extracts produced with various solvents (DPPH assay) showed that the water extract was more potent than the 100 per cent ethanol extract. Anthocyanins extracted in water were more powerful and had more antioxidant activity than extracts made with 100% and 50% methanol (Prado *et al*., 2019). Further, a random crossover experiment by Chusak *et al*. (2018) found that drinking or consuming *C. ternatea* floral extract in a short time frame boosted plasma antioxidant capacity. Putra *et al*. (2021) opined that the amount of total phenolics and flavonoids in the anthocyanin pigment extract from *C. ternatea* was directly correlated with its capacity to scavenge free radicals, and that the higher antioxidant activity of the phenolic compound was due to the presence of a high number of hydroxyl groups (Do *et al*., 2014). In addition, Jeyaraj *et al*. (2021) observed that the antioxidant activity of anthocyanin extracts from butterfly pea flower obtained by water and 50% ethanol extraction using DPPH and FRAP assays did not differ significantly. This implied that anthocyanins from butterfly pea flower might be extracted using distilled water and used as a natural blue colouring agent with high antioxidant activity (Gamage *et al*., 2021).

5.1.1.4 Total phenolics and total flavonoid content

In our study, higher total phenolics (29.78±1.79 mg GAE/100 g) and flavonoid (20.13±0.40 mg QE/100 g) contents were observed in MAE with water, which might be due to the greater effectiveness of the microwave power in rupturing cell membranes, which effectively introduces these substances into the pigment extract.

T_1 T_2 T_3 T_4 T_5

Plate 8: Anthocyanin pigment concentrates of butterfly pea flower petals as influenced by different extraction methods

Treatments details:

T_1 Aqueous (distilled water) extraction

T_2 Acidified aqueous extraction (1 % citric acid)

T_3 Solvent extraction (50% ethanol)

T_4 Acidified solvent extraction (50 % ethanol with 1 % citric acid)

T_5 Microwave assisted extraction with aqueous medium

Using water as the extraction solvent, a comparable value (26.70 mg GAE/g) for total phenolics was obtained from *C. ternatea* flowers (Lakshan *et al*., 2019). Due to the nature of phenolic compounds, which are thermo-labile, key factors in the process include the extraction temperature, time, and solvent. Effective mass transfer from the plant material into the extract requires increasing the diffusion rate and solubility of the compounds, ideal temperature (80 °C), time (45 min), and adequate solvent. Protocatechuic acid, gallic acid, and chlorogenic acids made up the majority of the phenolic acids in *C. ternatea* (Azima *et al*., 2017).

Similarly, flavonoids are phenolic substances that are also obtained from vascular plants and are well-known for having antioxidant properties. The ability of the sweet cherry extract to act as an antioxidant was shown to be correlated with its flavonoid content (González-Gómez *et al*., 2010). In *C. ternatea* flowers, myricetin was the most prevalent flavonoid, followed by delphinidin, epicatechin, rutin, kaempferol, and quercetin (Azima *et al*., 2017).

In general, plant phenolics have significant antioxidant activity and are well-known scavengers of free radicals (Rabeta and An Nabil, 2013), by a number of processes, such as metal chelation, hydrogen donation, and singlet oxygen quenching capabilities, they can neutralize free radicals (Venkatesan *et al*., 2019). After molecular deprotonation and stabilisation in an alkaline media, greater electron donation capacity may be the cause of the rise in antioxidant activity of phenolic compounds with an increase in pH (Altunkaya *et al*., 2016).

5.1.2 Mangosteen fruit rind

5.1.2.1 Recovery percentage of anthocyanin pigment and TMAC

The recovery percentage ranged (from 30.23±1.37 to 55.03±2.98 %). Since the chemistry of various bioactive substances in plants varies, different extraction solvents may have varying effects on the solubility, extraction yield and antioxidant activity of the phytochemicals (Azabou *et al*., 2020). Microwave assisted extraction with acidified solvent yielded significantly higher recovery of the pigment extract compared to other treatments. This could be because when microwave energy comes

into contact with the moisture in the matrix, it causes evaporation, which puts pressure on cell walls, causing them to rupture and release the bioactive components (Ren *et al*., 2012). Compared to a typical extraction processes, it is believed that this methodology could extract the target compounds more efficiently and allow for higher compound recovery. In addition, the results showed that ethanol, a polar protic solvent provided higher extraction yields, which may indicate that the fruit rind is also rich in polar secondary metabolites. This demonstrated that the extraction solvents as well as the extraction technique have an impact on the extraction yield because some chemical components may be soluble in both water and/or organic solvents, utilizing hydro-alcoholic as an extraction solvent may make it easier to use those components (Truong *et al*., 2019) as they have at least one hydrogen atom directly bonded to an electronegative atom, polar protic liquids can form hydrogen bonds (such as O-H or N-H).

In different treatments, TMAC ranged from 8721.95±382.80 to 17652.54±139.47 mg/L. Acidified solvent extraction method resulted in the highest TMAC of 17652.54±139.47 mg/L. A temperature of 45 °C softens plant tissue during the extraction process, accelerating molecular movements and solvent penetration into the plant material which subsequently improves the pigment solubility and diffusivities in the solvent leading to increased extraction yield (Yong *et al*., 2006). The highest recovery of anthocyanin content was noticed at 50 % ethanol strength since it minimized the pigment decomposition and allowed the extraction of anthocyanins in their native form (Oancea *et al*., 2012). Additionally, the acidified solvent denatures the membranes of the cell tissue in which most of the anthocyanin pigments are located (Giusti and Wrolstad, 2005) and also stabilizes anthocyanins in the form of flavylium cation maintaining a low acidic pH (Joshi and Devi, 2014) resulting in higher pigment in the extract. All these conditions during the extraction process might have resulted in the high anthocyanin yield in the extract. The primary method for obtaining bioactive chemicals from biomass materials is extraction, which maximizes the number of target chemicals and aims to obtain the extracts with the highest levels of biological activity. Both the extraction process and the extraction solvent impact on the extraction yield and biological activity of the resultant extract

(Azabou *et al*., 2020). The extraction of bioactive compounds from plant material has been carried out using a variety of solvents *i.e.,* methanol, ethanol, acetone and water. The best solvent for extraction relies on the specific plant materials and the compounds to be extracted due to the range of bioactive chemicals found in plant materials and their varying solubility qualities in different solvents (Truong *et al*., 2019).

5.1.2.2 Colour properties

The colour values in the treatments of mangosteen fruit rind pigment concentrate were significantly different, and *L*, a*, b*, chroma,* and *hue* values ranged from 3.17±0.38 to 61.65±0.94, 19.24±1.08 to 59.53±0.85, 5.09±0.52 to 23.75±2.04, 25.33±1.28 to 60.80±1.00 and 28.06±0.33 to 46.42±2.43, respectively. The pigment concentrates showed red to orange hues differing in their colour intensities as influenced by the different extraction methods, wherein acidified solvent and microwave assisted extraction methods showed darker shade towards the direction of redness. The colour of pigment concentrates is depicted in Plate 9.

5.1.2.3 Antioxidant properties

The DPPH and ABTS free radical scavenging activity in mangosteen fruit rind pigment concentrate ranged from 2.29±0.24 to 9.57±1.28 and 2.02±0.03 to 8.80±0.29 µl/ml, respectively. The DPPH is a proton-free radical-containing molecule with a maximum absorption wavelength of 517 nm. The purple colour of DPPH fades quickly when it comes into contact with proton radical scavengers. The lowest value reflects the pigment concentrate's strongest potential to act as DPPH scavenger. Significantly higher free radical scavenging activity was found in all the treatments except the aqueous extraction method, which might be due to higher phenol content. Similar results were observed in a study where the amount of phenolic compounds in organic extracts was higher than in aqueous extracts (Othman *et al*., 2007). According to Pearson correlation at $P \leq 0.05$ significance value, both DPPH scavenging value (IC_{50}) and total phenolics were negatively correlated (r = - 0.524*), which indicated that increase in the total phenolics content (32.25±0.30 mg GAE/100 g), there was an

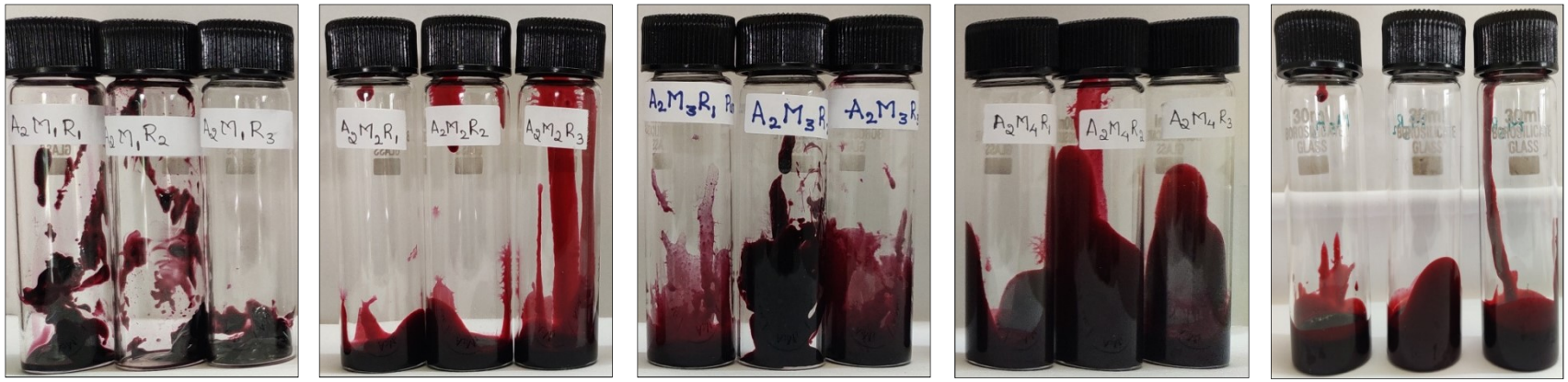
increase in the DPPH scavenging activity (2.29±0.24 µg/ml) indicating higher antioxidant activity by the pigment concentrate.

Similarly, higher antioxidant potential among the pigment concentrates based on the FRAP assay was also noticed in all the treatments compared to the aqueous extraction method. This could be due to the antioxidant mechanisms of anthocyanins towards free radicals along with phenolic compounds. In addition, there was a highly negative correlation (r = - 0.675**) between reducing power by FRAP assay and phenolic content according to Pearson correlation at P≤0.01. The present findings are in agreement with the study, wherein a strong correlation between TPC and FRAP assay was observed (Benzie and Stezo, 1999).

With respect to ABTS assay results, significantly higher free radical scavenging activity was found in the microwave assisted extraction with the acidified solvent method. Higher radical scavenging activity in the mangosteen fruit peel was noticed as compared to the extracts of *Clitoria ternatea* flowers, *Syzygium cumini* fruits and *Ardisia colourata* var. *elliptica* fruits, which might be due to the presence of high concentration of gallic acid in the fruit as compared to other parts (Azima *et al*., 2017). When considering the food application, the antioxidant activity must be maintained together with the pigment yield in terms of enhancing extra benefits and our results clearly showed that the antioxidant activity was successfully preserved.

5.1.2.4 Total phenolics and Total flavonoid content

Total phenolics and flavonoid content of mangosteen rind pigment concentrate ranged from 12.30±1.53 to 32.25±0.30 mg GAE/100 g and 15.47±0.02 to 40.02±3.52 mg QE/100 g, respectively. Higher total phenolics and flavonoid content was observed in mangosteen peel as compared to the extracts of *Clitoria ternatea* flowers, *Syzygium cumini* fruits and *Ardisia colourata* var. *elliptica* fruits (Azima *et al*., 2017). Protocatechuic acid was the most abundant chemical in mangosteen peel followed by vanillic, caffeic, and ferulic acids (Zadernowski *et al*., 2009). Epicatechin was shown to be the most abundant flavonoid in mangosteen peel, followed by catechin, cyanidin 3-sophoroside, cyanidin 3-glucoside, myricetin, rutin, kaempferol, and quercetin. In our study, higher total phenolic and flavonoid compounds were observed in the



T_1 **T_2** **T_3** **T_4** **T_5**

Plate 9: Anthocyanin pigment concentrates of mangosteen fruit rind as influenced by different extraction methods

Treatments details:

T_1 Aqueous (distilled water) extraction

T_2 Acidified aqueous extraction (1 % citric acid)

T_3 Solvent extraction (50 % ethanol)

T_4 Acidified solvent extraction (50 % ethanol with 1 % citric acid)

T_5 Microwave assisted extraction with acidified solvent (50 % ethanol with 1 % citric acid)

microwave assisted extraction with an acidified aqueous solvent which might be due to the lower temperature prevalent during the extraction process, thereby lowering the degradation of these compounds. Also, the higher efficiency of the microwave power in rupturing the cell membranes and getting these components efficiently into the pigment extract.

5.2 Evaluation of guava squash, guava jelly and ash gourd candy coloured with anthocyanin pigment

5.2.1 Total monomeric anthocyanin content

Anthocyanins are a class of bioactive, water soluble flavonoids that are found throughout the plant kingdom and give plant tissues colours like pink, red, purple, and blue (Grotewold, 2006). Anthocyanins are a safe and extremely nutritious food colouring agent with a plethora of potential. However, anthocyanins are highly unstable in nature and are easily degraded by external conditions like temperature, pH, light, oxygen, enzymes, metal ions *etc* (Escher *et al*., 2020). Extreme pH and temperatures are frequently encountered during food processing, and anthocyanin breakdown and discolouration typically occur during storage and processing (Zang *et al*., 2021). The aforementioned stability-related issue presents significant challenges for the preservation of anthocyanins (Fu *et al*., 2021).

In our study, the TMAC in all the products *i.e.,* guava squash, guava jelly and ash candy decreased significantly throughout the storage period, irrespective of treatments and storage conditions (Plate 10, 11 and 12). After three months of storage, the maximum retention of TMAC was noticed in the products with butterfly pea pigment stored under refrigerated condition, whereas the minimum retention was in the products added with mangosteen pigment stored at ambient condition. These results are in accordance with a study conducted on blueberry juice (Skrede *et al*., 2000), wherein higher temperatures caused a faster degradation of delphinidin-3-*O*-glucoside (D_3G) and cyanidin-3-*O*-glucoside (C_3G). A significant decrease in anthocyanin content of squash was recorded during the storage and more retention of anthocyanins was observed under refrigerated storage than ambient condition and the

loss of anthocyanins in squash might be due to their high susceptibility to auto-oxidative degradation during storage.

The oxidation process may be the cause of anthocyanin degradation in all products. The accelerated degradation of anthocyanins at the higher temperature also might be ascribed to the hydrolysis of the glycosidic bonds, which connect aglycones with glycosyl moieties (Mazza and Miniati, 1993). Aglycones (anthocyanidins), the basic structural skeleton (C_6-C_3-C_6) of anthocyanins, are considerably less stable than their glycosylated counterparts, and as a result, anthocyanins will degrade more quickly as sugars are lost by hydrolysis of the glycosidic linkages (Mazza and Miniati, 1993). Also, ascorbic acid radicals can be regenerated to ascorbic acid by oxidizing one molecule of anthocyanin into its well established radical form; so degradation of anthocyanins occurs. Our results indicate that the anthocyanin pigment from butterfly pea pigment is more stable than mangosteen anthocyanin pigment under ambient as well as refrigerated storage conditions in all the products. This present results are in alignment to the report that ternatins *i.e.,* blue coloured anthocyanins from butterfly pea flowers are amongst the most stable anthocyanins (Terahara *et al*., 1990). The high stability of ternatins might be due to the intra-molecular copigmentation configured by a hydrophobic interaction between the aromatic acids and anthocyanin chromophore (Yoshida *et al*., 2009). Recent research, however, has demonstrated that anthocyanin stability is impacted by both the intrinsic qualities of the product and the processing parameters, in addition to the processing temperature (Garzón and Wrolstad, 2002).

5.2.2 Instrumental colour values

The equipment required to meet the demands of today's consumers is provided by colour measuring instruments. The main purpose of colour measuring equipment is to assess the quality of coloured products. There are many different ways to describe colour contrasts verbally. To express colour contrasts, words like "stronger," "duller," "slightly blue," or "too grey" are employed. Unfortunately one person's slightly may be another person's strongly. When standardized visual evaluation techniques are followed and the standard and trial have the same gloss and texture, instrument

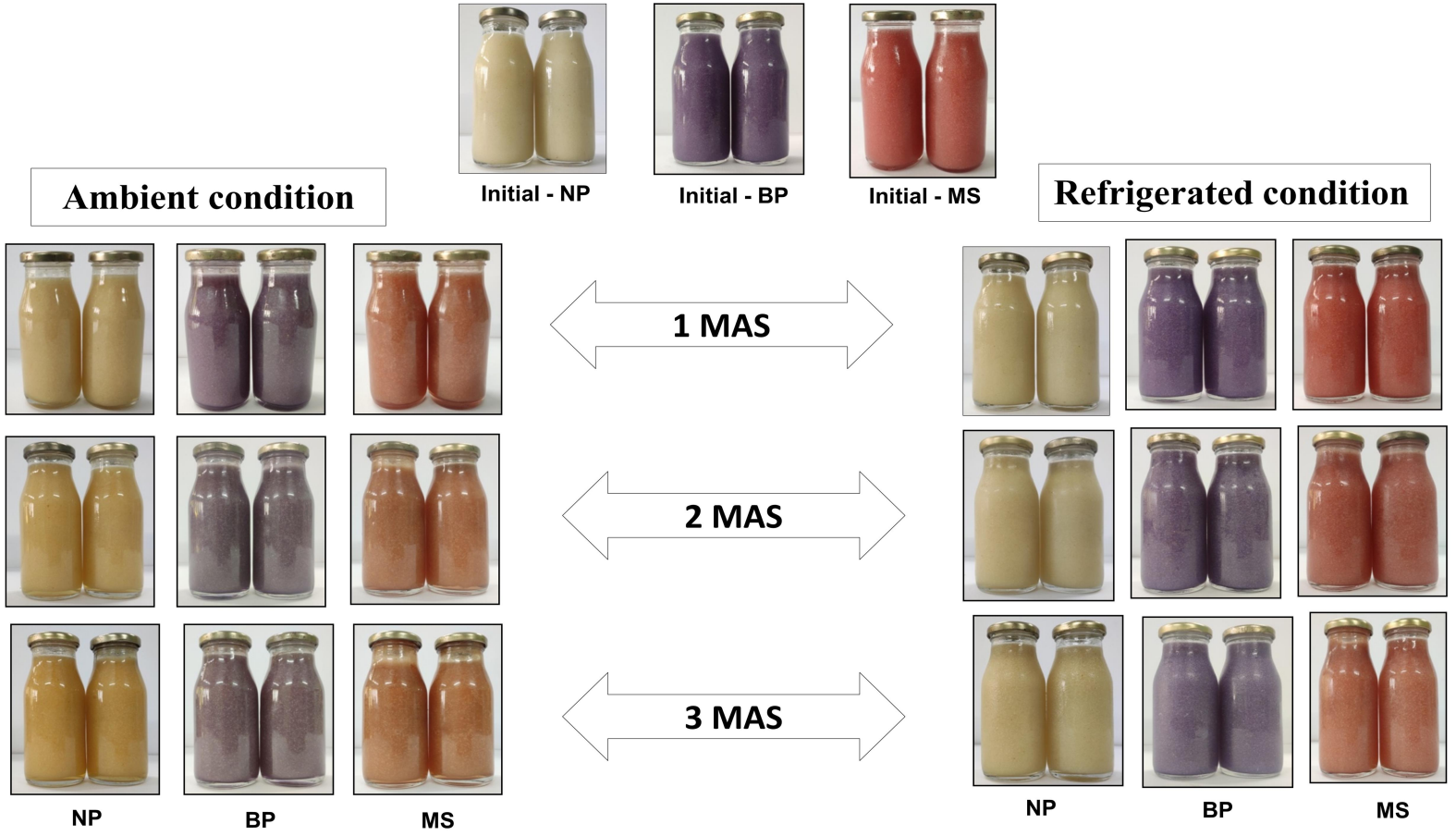


Plate 10: Guava squash with and without anthocyanin pigment at different storage periods under ambient and refrigerated storage conditions

NP – No pigment; BP – Butterfly pea pigment; MS – Mangosteen pigment

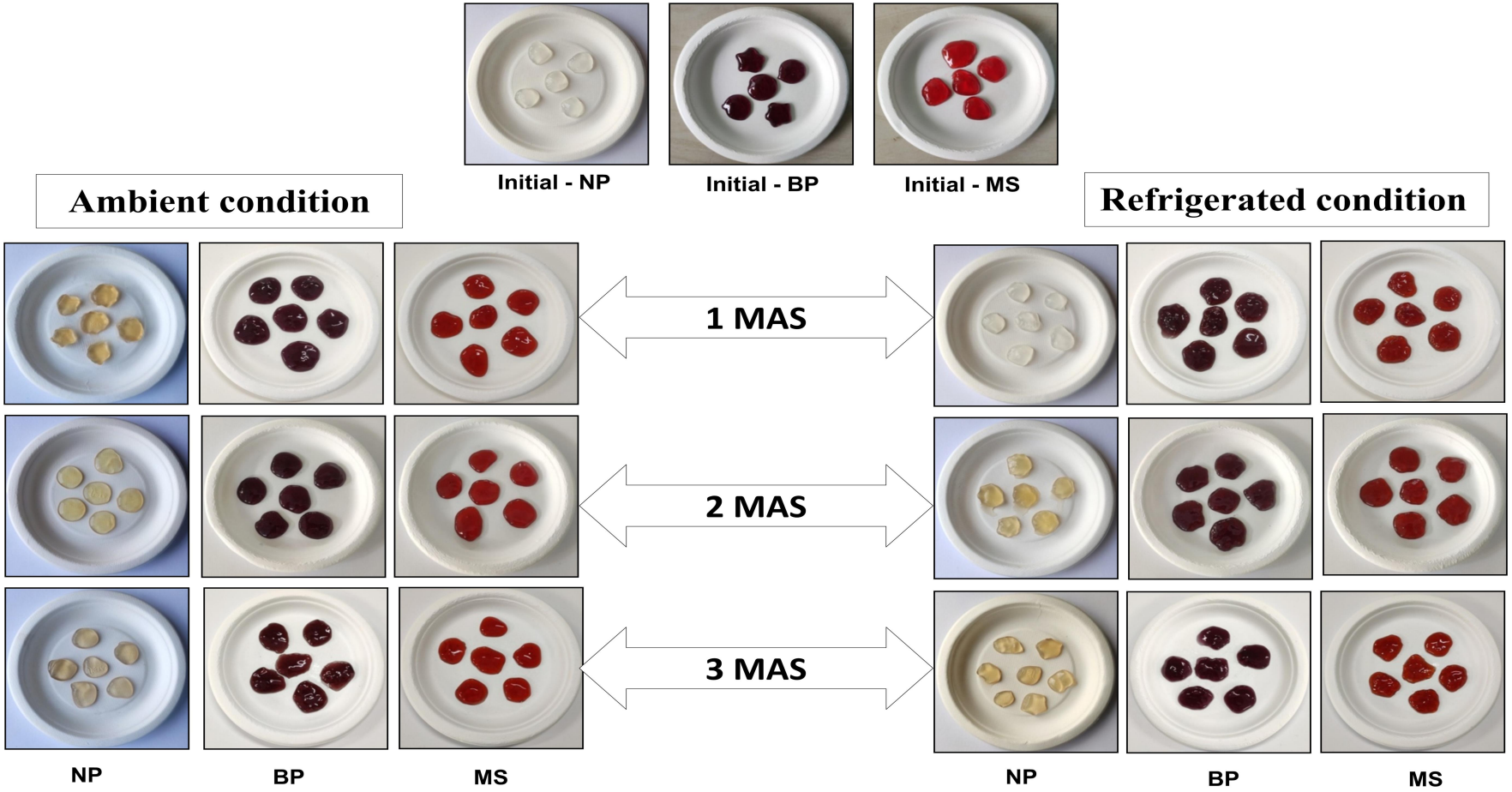


Plate 11: Guava jelly with and without anthocyanin pigment at different storage periods under ambient and refrigerated storage conditions

NP – No pigment; BP – Butterfly pea pigment; MS – Mangosteen pigment

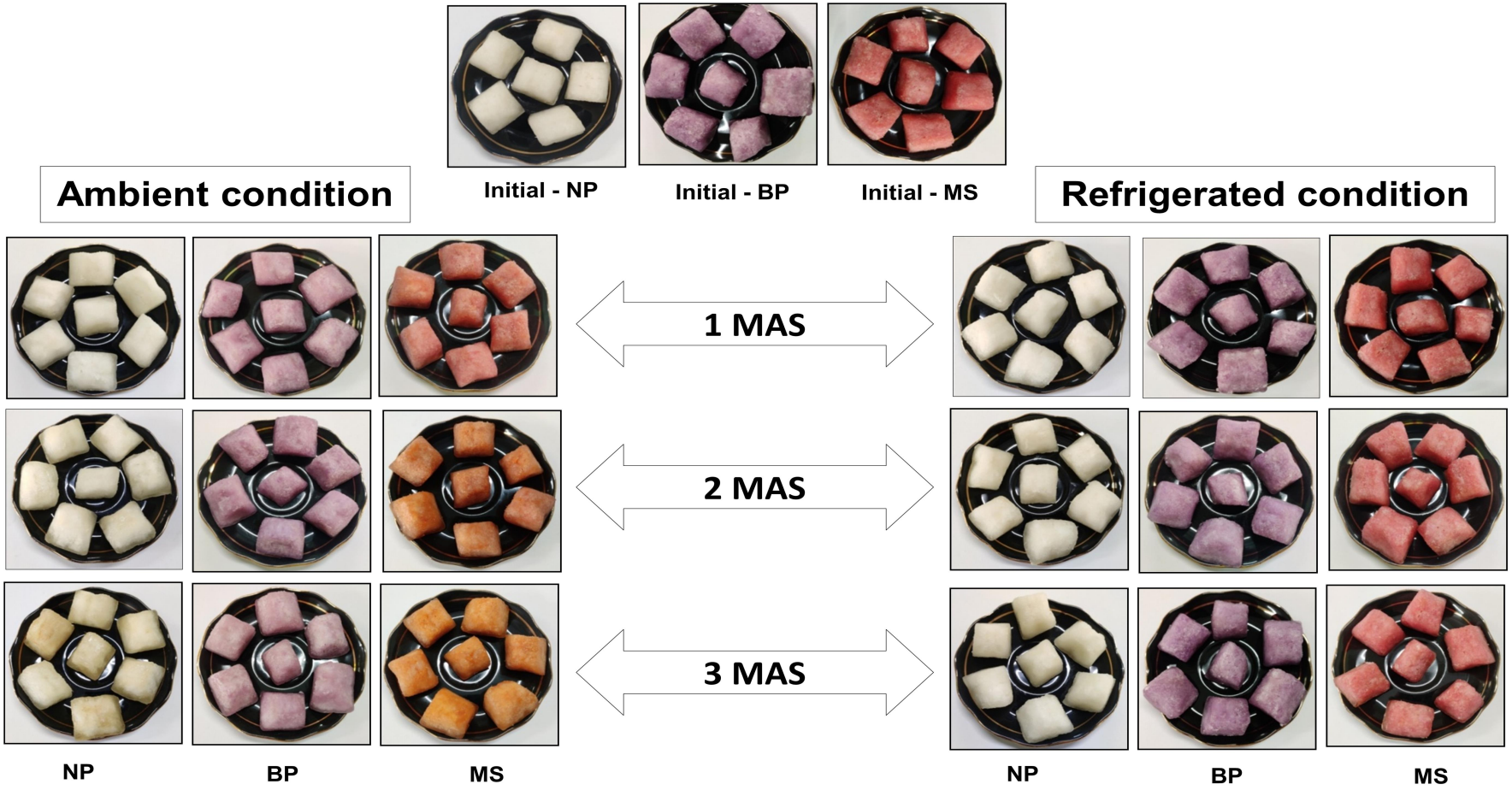


Plate 12: Ash gourd candy with and without anthocyanin pigment at different storage periods under ambient and refrigerated storage conditions

NP – No pigment; BP – Butterfly pea pigment; MS – Mangosteen pigment

measurements will correlate with visual evaluation the best. Instrumental measurements are enhanced and given more validity by visual inspection.

The increasing temperature and time resulted in anthocyanin pigments of all the products becoming darker in all irrespective of the storage condition and period corresponding to significant decreases ($P < 0.05$) in L^* and C^* values as indicators for browning. Lozano and Ibarz (1997), who stated that the change in L^* could be used to evaluate the browning of a heat-treated concentrated fruit pulp, provided evidence in favour of this finding. L^*, C^*, and $h°$ values were positively linked with anthocyanin content degradation. This could be the result of a reduction in visual colour, particularly one that is primarily brought on by the breakdown of anthocyanin pigments during storage and storage conditions. Mozetic *et al*. (2004) reported a similar finding, stating that variations in C^* were substantially correlated with variations in anthocyanin concentration. According to Duangmal *et al*. (2008), changes in L^* and C^* were substantially associated with the degradation of a beverage that contained powdered roselle anthocyanin. A similar result of the changes in a^*, b^*, and L^* values was also reported by Kara and Ercelebi (2013) in Urmu mulberry concentrate. Our results are also in line with the study conducted by Loypimai *et al*. (2016).

5.2.3 Antioxidant properties

The ability to contribute hydrogen atoms or electrons to free radicals and displace them is what is known as the antioxidant characteristic. This prevents the damage that the free radicals would otherwise inflict (Tan and Lim, 2015). In the present study, the antioxidant activities assessed by DPPH, ABTS and FRAP assays showed significant decrease in all the products irrespective of the storage condition and period. In our investigation, all of the products *viz.,* guava squash, guava jelly, and ash gourd candy saw a significant decline in antioxidant activity during the course of storage, irrespective of source of anthocyanin pigment and storage conditions. Our results also indicate that under both ambient and refrigerated storage conditions, the antioxidant activity of products added with butterfly pea pigment was greater than that of products added with mangosteen anthocyanin pigment, after three months of

storage. This is likely due to the bioactive chemicals degrading and absorbing water during the preparation of the products which dilutes the active substances. During preparation and processing of the products, the polyphenols that are present in the products may be eliminated or changed into other phytochemicals. Also, the loss in the antioxidant activity in all the products may possibly be due to structural changes, oxidation of phenolic compounds during processing procedures, and interactions of the phenolic antioxidants with other dietary components (Isabelle *et al*., 2010). A considerable decline in antioxidant activity is always linked to fall in phenolic total polyphenol (Tsai *et al*., 2002). The decrease in the total phenol content during storage might be due to involvement of phenols in the formation of polymeric compounds by complexing with protein and their subsequent precipitation as observed by Abers and Wrolstad (1979). The addition of 10 % butterfly pea flower extracts showed that an increase in antioxidant activity in fermented beverages from every kind of plant-based milk (almond, soy and combination of almond and soy milk), since butterfly pea flower extract has high antioxidant activity and contains bioactive compounds which can also act as antioxidants (Lakshmi *et al*., 2014). Similarly, a study was carried out to develop a functional beverage using blue pea flower extract, stevia extract and lime, and the antioxidant property of the functional beverage measured with DPPH, ABTS, FRAP and oxygen radical absorbance capacity (ORAC) assays were IC_{50} 247.6 µl/ml, 35.8 µl/ml, 14.9 mg Trolox equivalent/L and 122.2 mg Trolox equivalent/L, respectively.

5.2.4 Sensory evaluation (9-point hedonic scale)

Colour is essential to the acceptance of foods because it is one of the main senses used by consumers to swiftly identify and eventually accept foods (Giusti and Wrolstad, 2003). In the present study, 9-point hedonic scale was used to assess the sensory qualities of the products. With respect to guava squash the attributes like appearance, colour, flavour, body and consistency, after-taste and overall acceptability were considered, for guava jelly, visual appearance and transparency, colour, flavour, after-taste and overall acceptability and in case of ash gourd candy, appearance, colour, flavour, texture, after-taste and overall acceptability were evaluated. Highest scores with respect to the sensory attributes for all the products

evaluated on 9-point hedonic scale were noted in coloured products and the least values were scored by the control (without any colour) in the present study.

The difference and reduction in all the sensory attributes may be probably due to storage condition and duration. Indicating that storage period and condition had a substantial impact on how consumers perceived the products, a slight reduction in the visual appearance, colour, flavour, body and consistency, texture, after-taste and overall-acceptability of the products was seen as the storage period increased. A related investigation on the storage stability of guava squash by Rashid *et al*. (2018) noted the appearance loss. A decrease in the products' colour ratings over storage could be caused by the anthocyanin pigment's deterioration, which changed the product's colour. The retention of higher colour ratings under refrigerated storage conditions may be attributed to anthocyanins' slower rate of deterioration than in ambient circumstances. The loss of aroma scores during storage might be due to the possible loss of volatile aromatic compounds, whereas the decrease in taste scores of product during storage might be due to loss of sugar-acid blend. Retention of higher taste scores in refrigerated conditions might be due to the better retention of original sugar-acid blend as a result of slower reaction rate at low temperature. The aftertaste is a very important parameter in a way to know the taste remaining in the mouth after eating or drinking. Decrease in overall acceptability scores during storage might be due to cumulative loss in all other sensory attributes of the products during storage, whereas retention of higher overall acceptability scores in refrigerated conditions might be due to the better retention of all other sensory attributes of the product as a result of slower reaction rate at low temperature.

5.3 Standardization of extraction method for betalain from basella berries and amaranthus leaves

5.3.1 Basella berries

5.3.1.1 Recovery percentage of betalain pigment concentrate and content of betalain

The final recovery of the pigment concentrate varies depending on the quantity of bioactive components extracted during the extraction process and subsequently during vacuum concentration. A significantly higher recovery percentage of pigment concentrate (53.13±0.41 %) was observed in acidified aqueous extraction method (1 % citric acid). The hydroxyl groups (-OH) on the structures of betalain give rise to charge polarisation and the formation of hydrogen bonding results in high hydrophilicity (Fathordoobady *et al*., 2016). Because they serve as neutralising agents for the electrophilic core of betalains, acidifying substances like citric acid have been widely used to increase the pigments' stability (Delgado-Vargas *et al.,* 2000; Prakash-Maran *et al*., 2013). Hence, the hydrophilic nature combined with slight acidification of the extracting solution might have resulted in the higher recovery of the betalain pigment and other bioactive compounds in the extract and upon vacuum concentration of the pigment extract.

The phytochemical extraction from plant materials relies upon the extraction strategy being influenced by the extraction parameters used. In the present study, MAE (50% ethanol) method recorded significantly higher betacyanin (605.83±4.10 mg/g), betaxanthin content (86.35±1.67 mg/g) and total betalain content (692.18±2.52 mg/g). Microwave assisted extraction is a promising alternative to conventional extraction (Eskilsson and Bjorklund, 2000) methods. During microwave irradiation, the cells become thermally stressed; as a result, the temperature and pressure inside the cell increase to levels that result in the rupture of cell walls releasing intracellular compounds. It was reported that using a combination of ethanol and water will increase betalain extraction yields (Fu *et al*., 2020), and the extracts can be easily used in biological systems. It should be noted that the applicability of the final extracted product must be taken into account when choosing the suitable solvent for extracting any phytochemical, as some alternatives (such as methanol and ethyl acetate) are not recommended if the product is intended to be a food additive. In light of this, it is advised to use solvents like water, ethanol, or a combination of these solvents (Calva-Estrada *et al*., 2022).

5.3.1.2 Colour properties

The retention of colour properties is crucial in developing of a novel extraction protocol for the recovery of colourants from plant-based matrices. The colour parameters *L** indicating lightness, *a** indicating greenness and redness, *b** vector indicating blueness and yellowness, chroma (*C**, showing the dullness/vividness of the product), and hue angle (*h*°, the color perception by the human eye), considered to be the best descriptors of colour, were used to characterize the different betalain pigment concentrates recovered by the different extraction methods.

The results of the present study revealed the instrumental colour values of *a** (52.27±0.24), *b** (23.24±0.16), *chroma* (58.75±0.19) and *hue angle* (27.40±0.17) indicating bright purple-red colour of the pigment concentrate in the MAE method with ethanol (50%) as solvent. The colour of the pigment concentrates is depicted in Plate 13. It indicates that the *L*, a**, *b**, *chroma* and *hue angle* values may be linked to a higher betalain extraction yield in the above said extraction method. The colour visible to our naked eyes is not because of the individual vector of the instrumental colour values but is the combination of all the colour vectors in group. A similar thought was put-forth by Paciulli *et al*. (2016), who opined that combinations of *L*, a*, b** parameters correlated better with pigments than each single parameter alone. The results obtained in the present work are in line with the studies of Lombardelli *et al*. (2021) and Cejudo-Bastante *et al*. (2016), who concluded that there is a strong correlation between the total betalain content and the instrumental colour values with respect to *L*, a*, b*, hue* and *chroma* values.

5.3.1.3 Antioxidant properties, total phenolics, and flavonoid content

The study revealed higher antioxidant activity *viz.,* DPPH (3.40±0.04 µl/ml), FRAP (3.69±0.19 µl/ml), and ABTS (2.99±0.04 µl/ml), total phenolics (211.37±0.29 mg GAE/100 g) and total flavonoids (124.07±2.53 mg QE/100 g) observed in the pigment concentrate extracted using 50% ethanol as the solvent by MAE method.

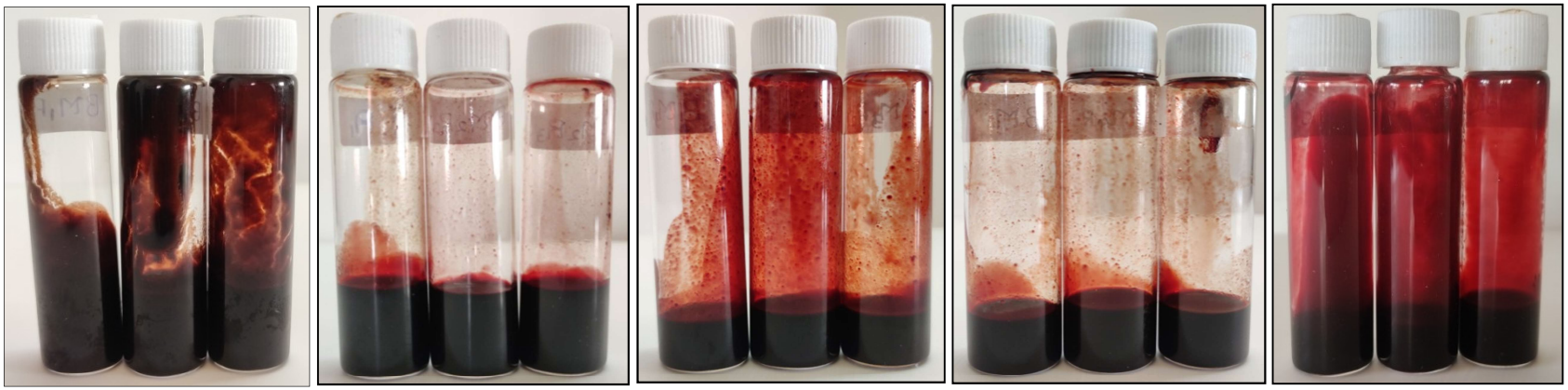
Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid peroxidation, and reducing power of a compound may serve as an indicator of its potential antioxidant activity. The reducing properties are

generally associated reductones, which have been shown to exert antioxidant action by breaking the free radical chain by denoting a hydrogen atom. The reason for the high antioxidant activity of the pigment concentrate may be due to the presence of high total betalain content, phenolic compounds, and flavonoid compounds in the pigment concentrate owing to the better extractability of these bioactive compounds by the extraction method used, since during the process of extraction the thermal treatment involved release the bound form of antioxidant compounds due to disruption of cell membranes and cell walls (Pandey *et al*., 2018). Structurally, betalains are immonium derivatives of betalamic acid that contain an aromatic amino compound that is able to stabilize radicals. This stabilization is tightly linked to betalain's electron donation ability (Slimen *et al*., 2017). Several studies strongly confirmed the high radical scavenging activity of betalains (Gandía-Herrero *et al*., 2016; Tesoriere *et al*., 2009) which are considered as a class of dietary cationized antioxidants (Kanner *et al*., 2001). Also, the radical scavenging capacity might be mostly related to their concentration of phenolic and hydroxyl group. The antiradical activity of phenolic compound depends on their molecular structure the availability of phenolic hydrogen, and on the possibility for stabilization of the resulting phenoxyl radicals formed by hydrogen donation. A highly significant correlation was observed between the total antioxidant capacity and phenolics content.

5.3.2 Red amaranthus leaves

5.3.2.1 Recovery percentage of betalain pigment concentrate and content of betalain

A significantly higher recovery percentage of red amaranthus betalain pigment concentrate (58.73±0.43 %) was observed in acidified aqueous extraction method (1% citric acid). Betalain is a water soluble compound that can be easily extracted and dissolved in water to obtain high betalain pigments. Additionally, the presence of citric acid in the extraction mixture will exhibit stabilizing effects on the maintenance of betalain pigment (Sigwela *et al*., 2021). For the extraction of betalains from *Bougainvillea spectabilis*, *Celosia argentea* inflorescence, and *Amaranthus*



T_1 **T_2** **T_3** **T_4** **T_5**

Plate 13: Betalain pigment concentrates of basella berries as influenced by different extraction methods

Treatments details:

T_1 Aqueous (distilled water) extraction

T_2 Acidified aqueous extraction (1 % citric acid)

T_3 Solvent extraction (50 % ethanol)

T_4 Acidified solvent extraction (50 % ethanol with 1 % citric acid)

T_5 Microwave assisted extraction with solvent medium (50% ethanol)

gangeticus, water proved to be the most effective solvent (Chong *et al*., 2014; Lavanya *et al*., 2019).

Natural pigments are often extracted *via* a solid-liquid process. Cell wall materials and other cellular components are released along with the mechanical breakdown of the tissues, necessitating additional purification of the extracts. Therefore, it is highly desired to have technologies and procedures that make it possible to extract from matrices as intact as possible (Stintzing and Carle, 2007). In the present study, MAE (50% ethanol) method recorded significantly higher betacyanin (601.15±2.25 mg/g), betaxanthin content (75.63±0.55 mg/g) and total betalain content (676.78±2.79 mg/g). Since MAE causes frustule permeabilization but prevents frustule explosion, it is viewed as a clean procedure. It is an effective method that uses higher temperatures without destroying the pigment (Pasqueta *et al*., 2011). Ethanol has been recognized as a suitable solvent for bioactive and polyphenol extraction and is not harmful to human consumption. This indicates the combination of MAE with aqueous ethanol as a solvent together is more efficient for recovering higher pigment yields. The highest total betalain concentration was found in the ethanol extract of *Amaranthus blitum* (Chong *et al*., 2014; Lavanya *et al*., 2019). The results are in line with the results observed by Sanchez-Gonzalez *et al*. (2013), wherein 20% aqueous-ethanolic extract from Opuntia joconostle had the highest total betalain concentration. The type and amount of chemicals extracted from the material depend on the ratio of ethanol to water. Higher ethanol concentrations resulted in higher phenolic and betaxanthin contents, whereas extracts with ethanol concentrations of 40 and 60 per cent had the highest betacyanin concentrations (Thiyajai and Koyama, 2022).

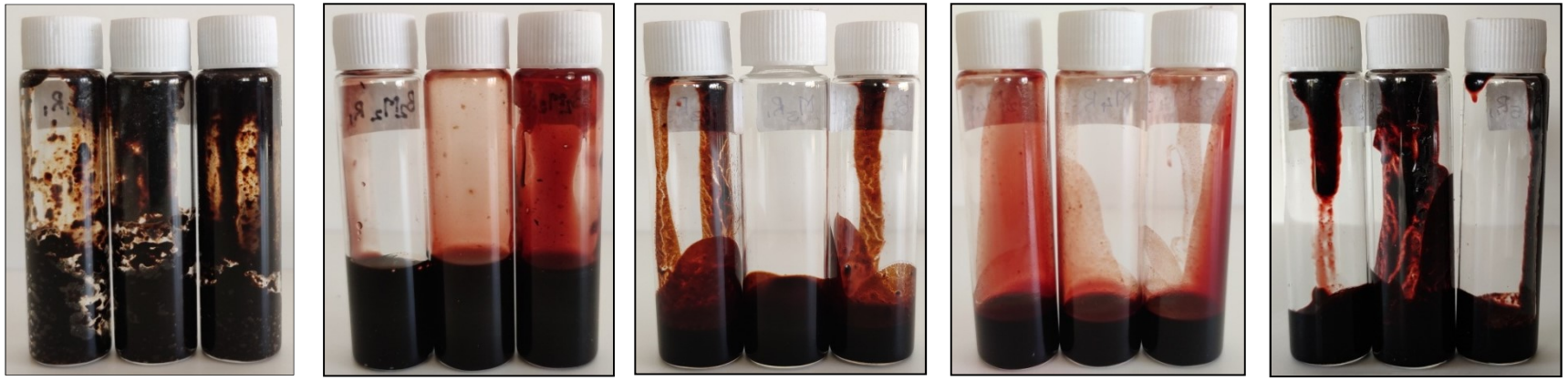
5.3.2.2 Colour properties

The red amaranthus pigment concentrate presented the instrumental colour values *viz.., L** (41.94±0.06), *a** (33.37±0.22), *b**(56.76±0.02) *chroma* (63.37±0.17) and *hue angle* (58.41±0.02) showing a darker and reddish colour shade of the pigment concentrate in the MAE method with ethanol (50%) as solvent. The colour of pigment concentrates is depicted in Plate 14.The obtained results in the present study

are in line with observations made by Thiyajai and Koyama (2022), wherein 40% aqueous-ethanolic extract had the most intense colour, while the 80% aqueous-ethanolic extract had the least intense colour as it was brighter (high *L**), less red (less positive *a**), and less blue (less negative *b**) than the others. Our results indicate that the *L**, *a**, *b**, hue angle and chroma values may be linked to a higher betalain extraction yield in the above said extraction method. Darker extracts (lower values of *L**) corresponded to those with higher contents of betalain (Tabio-García *et al*., 2021). The colour visible to our naked eyes is not because of the individual vector of the instrumental colour values but is the combination of all the colour vectors in group. A similar thought was put-forth by Paciulli *et al*. (2016), who opined that combinations of *L*, a*, b** parameters correlated better with pigments than every single parameter alone. The results obtained in the present work are in line with the studies of Lombardelli *et al*. (2021) and Cejudo-Bastante *et al*. (2016), who concluded that there is a strong correlation between the total betalain content and the instrumental colour values with respect to *L, a*, b*, hue* and *chroma* values.

5.3.2.3 Antioxidant properties, total phenolics, and flavonoids content

The study revealed higher antioxidant activity *viz.,* DPPH (1.34±0.01 µl/ml), FRAP (3.69±0.19 µl/ml), and ABTS (0.81±0.01 µl/ml), total phenolics (190.03±0.22 mg GAE/100 g) and total flavonoids (179.07±0.49 mg QE/100 g) in MAE method with ethanol (50%) as solvent. Betalains and phenolics are located in vacuoles in the cell wall (Grotewold, 2006), whereas phenolics are mostly in primary cell walls (Wang *et al*., 2020). The higher antioxidant activity of the red amaranthus pigment extract in the present study can be attributed to the presence of higher pigment components, *i.e*., betaxanthin and betacyanin, accounting for the total betalain content and also higher phenolics owing to the efficient extraction of the betalains and increased release of the bound phenolics into the extract by the MAE with ethanol as solvent. The aromatic ring in the structure of phenols and betalains have at least one 1-OH group. The H-atom from phenols is then given to the free radical, resulting in the formation of phenoxyl radicals through resonance (Thiyajai and Koyama, 2022). The electron is distributed throughout the aromatic ring and is delocalized. The imino and tetrahydropyridine groups can also support the electronic resonance system in the



T_1 T_2 T_3 T_4 T_5

Plate 14: Betalain pigment concentrates of red amaranthus as influenced by different extraction methods

Treatments details:

T_1 Aqueous (distilled water) extraction

T_2 Acidified aqueous extraction (1 % citric acid)

T_3 Solvent extraction (50 % ethanol)

T_4 Acidified solvent extraction (50 % ethanol with 1 % citric acid)

T_5 Microwave assisted extraction with solvent medium (50% ethanol)

case of betalains (Craft *et al*., 2012; Slimen *et al*., 2017; Thiyajai and Koyama, 2022). A strong correlation was observed both between antioxidant activities and total phenolic content, in addition to total betalain content (Nour *et al*., 2013; Waszkowiak and Gliszczyńska-Świgło, 2016). Phenolic compounds were the major contributors to antioxidant activities and in addition, moderate to strong correlations were observed between betaxanthin (BX) and antioxidant activities (Thiyajai and Koyama, 2022). Lavanya *et al*. (2019) also reported promising antioxidant activities from water extract of *Celosia argentea* inflorescence measured by ABTS, DPPH, FRAP, cupric ion reducing antioxidant capacity, and chelating potential assays.

5.4 Evaluation of guava squash, guava jelly and ash gourd candy coloured with betalain pigment

In recent years, there has been an increase in interest in using natural additives in the food industry, such as colourants and bioactive compounds, prioritizing the focus on the nutritional value and sensory qualities of the products as well as enhancing food safety (Kanatt, 2020; Zin *et al*., 2020a). Due to their pigmentation, antioxidant, antibacterial, and other bioactivities linked to possible human health benefits, betalains represent a group of phytochemicals with tremendous promise for the enrichment and supplementing of foods (Prieto-Santiago *et al*., 2020; Zin *et al*., 2020b). However, there are difficulties with maintaining betalains' chemical stability and, consequently, their bioactivities and usefulness as pigments when using them as antioxidants and/or natural colourants in industrially produced goods.

5.4.1 Total betalain content

In our study, the total betalain content in all the products *i.e.,* guava squash, guava jelly and ash gourd candy decreased significantly throughout the storage period, irrespective of the pigment source and storage conditions (Plate 15-17). After three months of storage, the maximum retention of betalain was noticed in the products with basella berries pigment stored under refrigerated condition, whereas the minimum retention was in the products added with red amaranthus pigment stored at ambient condition. One of the factors that most affects the structure of betalains is temperature. The breakdown of betalains is accelerated by rising

storage temperatures (Kayin *et al*. 2019; Prieto-Santiago *et al*., 2020) whose structural changes are brought about by the processes of hydrolysis, isomerization, dehydrogenation, deglycosylation, and decarboxylation (Herbach *et al*., 2006) and the degree of heating during processing, the presence of oxygen, the amount of pigments present *etc* all influence how rapidly betalains degrade (Dos Santos *et al*., 2018; Laqui-Vilca *et al*., 2018). Another important aspect in the breakdown of betalains is oxygen, especially considering how it interacts with other factors like light and temperature to cause a joint breakdown (Barba-Espin *et al*., 2018). According to Chhikara *et al*. (2019), betalains are easily broken down by the presence of light and the severity of the effect is dependent on the amount of light, the presence of oxygen, the concentration of betalains, and their reactivity (Kayin *et al*., 2019). The presence of all these factors limits the application of betalains in food, which is why several techniques have been used for their preservation. All these factors have an impact on the structure of betalains in some way, which is reflected in a change in the colour parameters (Prieto-Santiago *et al*., 2020). When compared to guava squash and ash gourd candy added with betalain pigment, the retention of total betalain content in betalain pigmented guava jelly was higher under refrigerated condition which might be due to lower water activity of the jelly product. The current findings are in line with those of Rodrguez-Sánchez *et al*. (2017), who used betaxanthins from yellow pitaya (*Stenocereus pruinosus*) fruit as a colouring for jelly gummies. They found that betaxanthin stability was highest when the product was stored at low temperatures and in the dark, and they also found that these pigments were more stable in the gummies because of the food matrix (a protective effect was provided by their interactions with proteins) and their low water activity. Low water activity enhances betalain stability, with 0.63 or less being the most beneficial (Kearsley and Katsaboxakis, 1980).

5.4.2 Instrument colour values

In the present study, instrumental colour evaluation was done using the colour coordinates *L**, *a**, *b**, *chroma* and *hue angle* initially and for a period of three months at monthly interval under refrigerated and ambient conditions in the products *viz.,* guava squash, guava jelly and ash gourd candy with and without addition of betalain pigment from basella berries and red amaranthus. The combination of all the

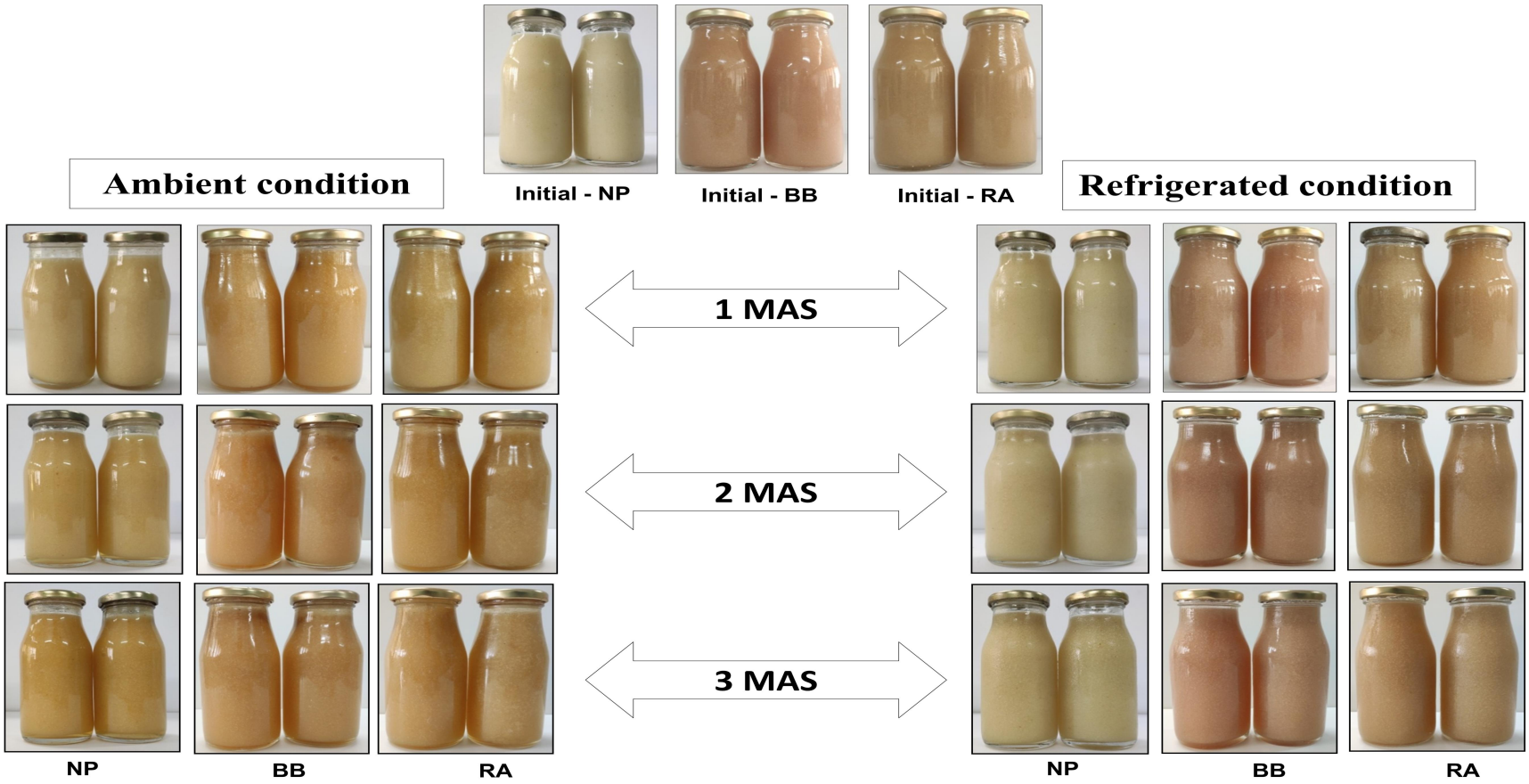


Plate 15: Guava squash with and without betalain pigment at different storage periods under ambient and refrigerated storage conditions

NP – No pigment; BB – Basella berries pigment; RA – Red amaranthus pigment

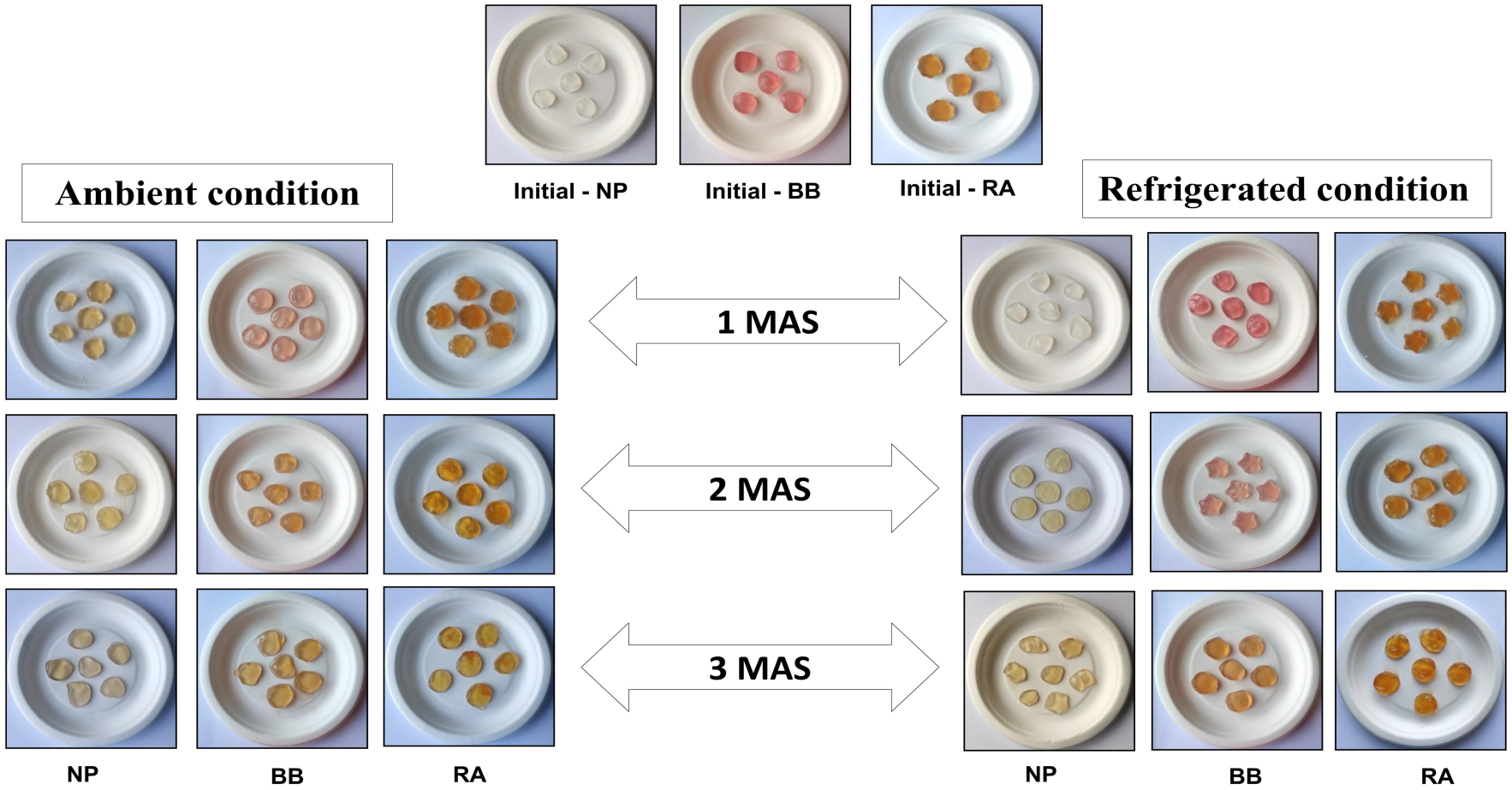


Plate 16: Guava jelly with and without betalain pigment at different storage periods under ambient and refrigerated storage conditions

NP – No pigment; BB – Basella berries pigment; RA – Red amaranthus pigment

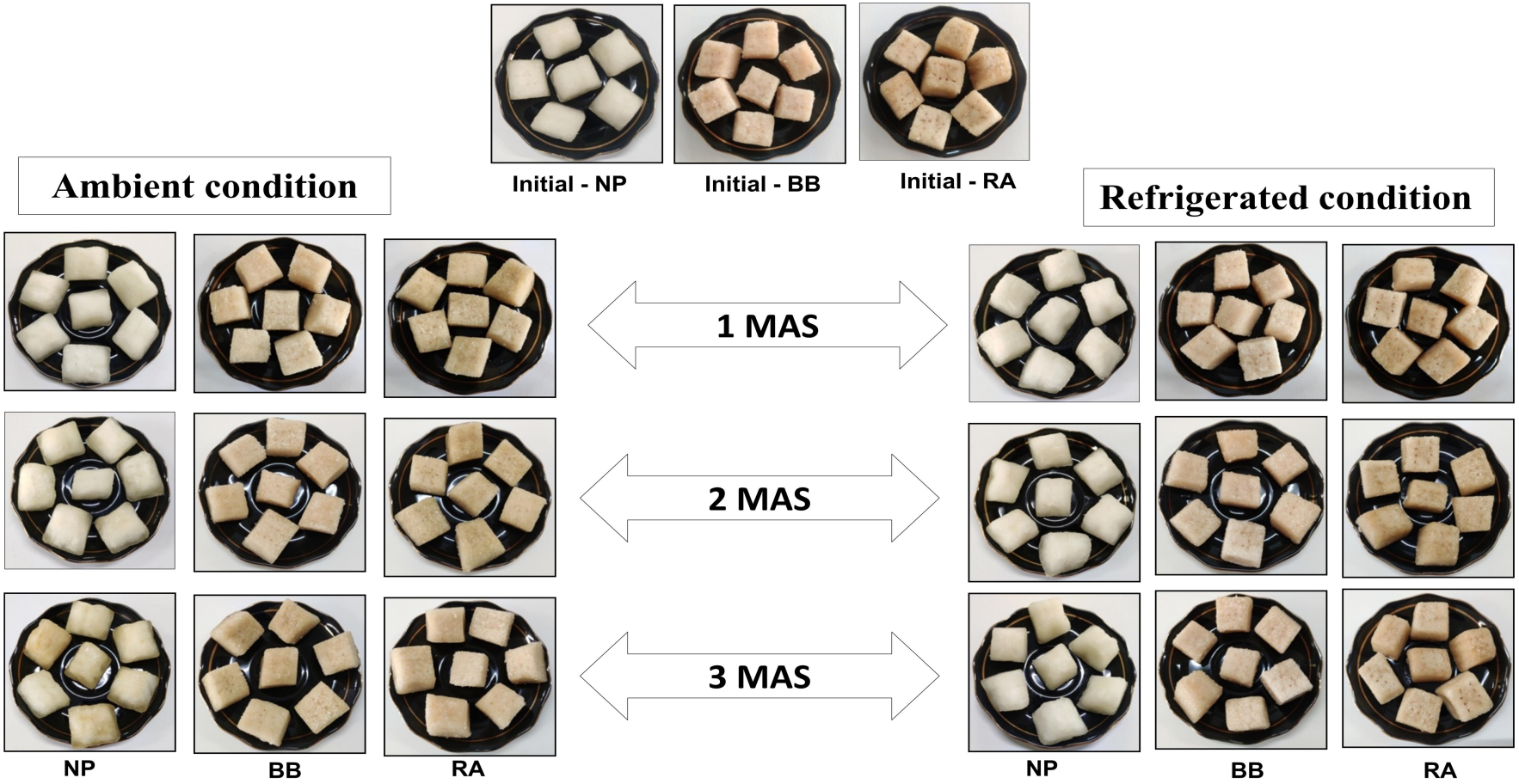


Plate 17: Ash gourd candy with and without betalain pigment at different storage periods under ambient and refrigerated storage conditions

NP – No pigment; BB – Basella berries pigment; RA – Red amaranthus pigment

colour coordinates indicated bright pink colour of the products added with basella pigment than the red amaranthus pigment. The products added with red amaranthus pigment also had light tinge of green colour since the pigment obtained by solvent (ethanol) extraction method was used in the products. Among the three products, guava jelly with basella pigment gave very attractive pink colour to the product. It was noticed that there was significant reduction in the colour of the products irrespective of the source of the pigment added throughout the storage period and both ambient and refrigerated conditions. However, the products stored under refrigerated condition and added with basella pigment has retained the pink colour to a higher extent than the products stored under ambient condition and added with red amaranthus pigment. These results conclude that the retention of colour was higher under refrigerated condition which can be correlated with the total betalain content of the products and also slower rate of degradation of the betalain pigment and overall quality of the products.

5.4.3 Antioxidant properties

Betalains exhibit antioxidant capabilities because of their two structural constituents, a phenolic and a cyclic amine, are effective electron donors (Kanner *et al*., 2001). The electron donor capacity, bond dissociation energy, and ionization potential of betanin have all been examined and used to assess and explain its antioxidant and antiradical properties (Gliszczyńska-Swigło *et al*., 2006). In the present study, the antioxidant activities assessed by DPPH, ABTS and FRAP assays showed significant decrease in all the products *viz.,* guava squash, guava jelly and ash gourd candy added with betalain pigment irrespective of the pigment source, storage condition and period. The antioxidant activity declined over the course of storage and was temperature-dependent, suggesting that a lower storage temperature could slow the decline of the antioxidant activity. Our results also indicate that under both ambient and refrigerated storage conditions, the loss of antioxidant activity of products added with basella pigment was more prominent than that of products added with red amaranthus pigment, after three months of storage which indicates that the basella betalain pigment stability is higher than that of red amaranthus betalain pigment in all the products studied.

According to Coria-Cayupán and Nazareno (2015), the assessment of the protective effect of betalain pigments, oxidative damage was inhibited by more than 80 per cent in yoghurt and 50 per cent in cream during the oxidation of the systems without the pigment additions. The current finding is in line with that found by Myojin *et al*. (2008), who found that higher storage temperatures led to a greater decline in the antioxidant activity measured by DPPH scavenging activity. Yang *et al*. (2007) reported that storing the noni juice for 30 days at the temperature of 4 and 24 °C, free radical scavenging capacity was found to decrease by 36 and 83 per cent, respectively depicting the decrease of antioxidant activity with increasing storage temperature. Within three months of storage at 25 °C ambient temperature, Jiménez-Zamora *et al*. (2016) showed a substantial decline in the overall antioxidant activity of 26 different tea varietals. The findings of the current investigation are in line with those of Zorić *et al*. (2017) who found that Marasca sour cherry powder's antioxidant activity significantly decreased at 37 °C in comparison to samples maintained at 4 °C. The antioxidant capacity of the samples at 4 °C after 12 months was nearly identical to that at 37 °C after just three months, according to the authors, demonstrating that phenolic chemicals degrade more quickly at higher temperatures. The phenolic compounds may have oxidised as a result of being stored at high temperatures with exposure to light (Guimarães *et al*., 2011; Jiménez-Zamora *et al*., 2016), which may have resulted in the loss of antioxidant activity.

5.4.4 Sensory evaluation

Sensory evaluation also referred to as organoleptic evaluation, has a major role to play in the product development and used to determine consumer acceptability of a food. In the present study, 9-point hedonic scale was used to assess the sensory qualities of the products. With respect to guava squash the attributes like appearance, colour, flavour, body and consistency, after-taste and overall acceptability were considered, for guava jelly, visual appearance and transparency, colour, flavour, after-taste and overall acceptability and in case of ash gourd candy, appearance, colour, flavour, texture, after-taste and overall acceptability were evaluated. In all the products, the first treatment was the product without pigment followed by the products with pigment. The sensory evaluation was done initially and for a period of

three months at monthly interval under refrigerated and ambient conditions in the products. Refrigerated storage temperature was found to be superior over other methods for storage of banana RTS beverage (Yadav *et al*., 2013). The extensive application of betalains in food is restricted by the instability of the pigment to light, alkaline, oxygen, and metal ions (Khan and Giridhar, 2014).

5.5 Standardization of extraction method for carotenoid from gac fruit seed aril and marigold petals

5.5.1 Gac fruit seed aril

5.5.1.1 Recovery percentage of carotenoid pigment concentrate and content of carotenoid

The final recovery of the pigment concentrate varies depending on the quantity of bioactive components extracted during the extraction process and subsequently during vacuum concentration. A significantly higher recovery percentage of pigment concentrate (89.53±0.13 %) was observed in MAE with the ethanol method. The obtained results are in line with the study of Jaeschke *et al*. (2017), who opined that a concentration higher than 75% ethanol, there was a possible disruption of the cell structure due to interactions between the solvent and the cell phospholipid membrane that are associated with ethanol favorably interacting with the carotenoids. Due to the availability of technology, the ease of operation, and the high extraction efficiency, MAE and UAE have been identified as two of the most feasible approaches on the industrial scale (Wani *et al*., 2016). The MAE relies on the help of electromagnetic radiation with frequencies ranging from 0.3 to 300 GHz, which heats up the material by causing the molecules to rotate dipolarly and conduct ions (Camel, 2001). The heat produced by this process and the activation of these molecules may weaken or shatter the cell walls, making it easier for the bioactive compounds to be liberated from the material matrix and discharged into the extraction solvents (Kaufmann and Christen, 2002). In a microwave-assisted extraction, the extraction mixture's temperature rises as a result of absorbing microwave energy. When the temperature rises, the solvent becomes less viscous; this speeds up the diffusion of the target chemicals into the extraction media (Eskilsson and Björklund, 2000).

Microwave heating also causes material cell walls to break down, allowing solvent to enter the solid matrix, dissolve it, and release the chemicals inside the cells into the liquid phase (Zhou and Liu, 2006).

The phytochemical extraction from plant materials relies upon the extraction strategy being influenced by the extraction parameters used. In the present study, the ethyl lactate extraction method had given significantly higher total carotenoid content (2069.83±4.38 μg/g), β-carotene (689.43±1.04 μg/g), lycopene (1052.31±0.87 μg/g) and lutein content (216.96±0.22 μg/g). One of the most important considerations in developing new processes and technology for extraction is the safety issue of the final products when used as food. A critical and demanding step is the selection of proper safe, food-grade solvents. Recently, ethyl lactate has been examined as an extraction solvent with promising results (Ishida and Chapman, 2009). Ethyl lactate is an environmentally friendly solvent produced from the fermentation of carbohydrate feedstocks available from the corn and soybean industries.

Ethyl lactate is a potent solvent that can dissolve in both aqueous (polar) and hydrocarbon (non-polar) environments, according to Kua *et al*. (2014). Hence, it has the ability to recover chemicals from crude palm oil with a wide range of polarity without the use of a co-solvent. In addition, it was demonstrated the use of ethyl lactate and ethanol as green and safe solvents to extract phytonutrients such as carotenes and tocols from crude palm olein (CPO) before they are lost during the oil refining process (Kua *et al*., 2018).

Strati and Oreopoulou (2011) found that ethyl lactate, is the most efficient solvent to extract carotenoids (lycopene) from tomato waste at 70 °C, rather than being an environmental-friendly solvent as compared to acetone, ethyl acetate, hexane and ethanol. Even at ambient temperature, ethyl lactate extracted more lycopene than other solvents at a higher temperature. Thirty minutes was found to be adequate to extract lycopene from tomato waste. Prolonged extraction was undesirable due to isomerisation and oxidation of carotenoids at high operating temperature.

Szabo *et al*. (2022) reported that the highest values for lycopene (1324.89 μg/g dw) were obtained when ethyl lactate was applied as a solvent, followed by ethyl

acetate with slightly smaller differences (1313.54 µg/g dw) in tomato processing by-products. The achieved yields of total carotenoid content, β-carotene, lycopene and lutein content in the present study agree with previous studies, which reported that ethyl lactate as an excellent solvent for extracting bioactive compounds.

5.5.1.2 Colour properties

The instrumental colour values *viz*., the luminosity (L*) represent how light and dark the sample is, varying between 0 (black) and 100 (white). Higher luminosity values indicate whiter colours. The chrome a* values (x-axis) vary from green (-) to red (+); and chrome b* values (y-axis) from blue (-) to yellow (+).

The results of the present study revealed the instrumental colour values of *L** (60.44±0.12), *a** (61.35±0.20), *b** (43.24±0.10), *chroma* (65.62±0.10) and *hue angle* (36.64±0.18) indicating red colour of the pigment concentrate in the ethyl lactate extraction method. The colour of gac pigment concentrates is depicted in Plate 18.

Chrome, *a** and *b** from the tomatoes presented positive values, that is to say, reddish and yellowish tones, due to the presence of pigmentation, lycopene and β-carotene, respectively. However, it was determined that the intensity of the red component (chrome *a**) was higher than the yellow component (chrome *b**), because the fruits were in their ripe and red stage (Vieira *et al*., 2020). As well as for the luminosity, the tendency of chrome b* is to decrease with the ripening, which can be related to the fact that lycopene can reach its highest concentration.

It indicates that the *L*, a**, *b**, *chroma,* and *hue angle* values may be linked to a higher carotenoid extraction yield in the above said extraction method. The colour that is visible to our naked eyes is not because of the individual vector of the instrumental colour values but is the combination of all the colour vectors in group. Similar thought was put-forth by Paciulli *et al*. (2016), who have opined that combinations of *L*, a*, b** parameters correlated better with pigments than every single parameter alone. The results obtained in the present work are in line with the studies of Vieira *et al*. (2020), who concluded that there is a strong correlation

between the total carotenoid content and the instrumental colour values with respect to *L*, a*, b*, hue* and *chroma* values.

5.5.1.3 Antioxidant properties and total phenolics

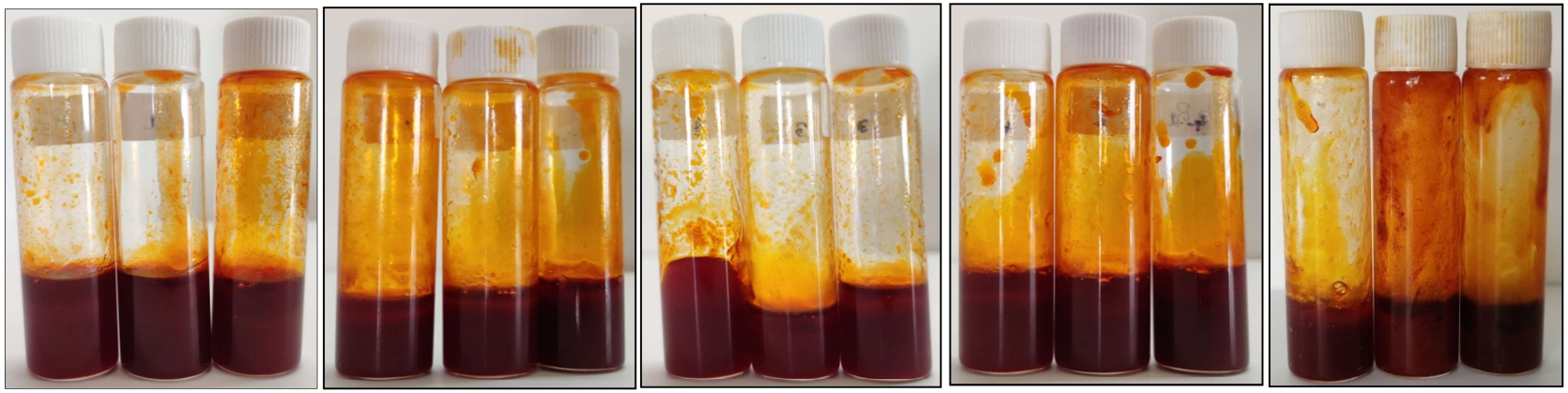
The study revealed higher antioxidant activity *viz.,* DPPH (0.59±0.005 µl/ml), FRAP (0.63±0.004 µl/ml), ABTS (0.58±0.005 µl/ml), and total phenolics (211.57±0.13 mg GAE/100 g) observed in ethyl lactate extraction method.

DPPH, ABTS, and FRAP tests provide a convenient, cost effective, and evident observation regarding the potential antioxidant capacities of plant extracts based on colour changes. The results of DPPH and FRAP exhibited strong antioxidant activity in the aril and peel parts of the fruit (Abdulqader *et al*., 2019). Recently several studies have also demonstrated that gac fruit parts possessed antioxidant capacities which were consistent with these findings (Le *et al*., 2018; Tran *et al*., 2016). In this study, both the aril and peel appeared to possess higher contents of carotenoids, phenolics, and flavonoids. The total contents of these phytochemicals are positively linked with the antioxidant capacities (Kubola and Siriamornpun, 2011; Ranneh *et al*., 2018).

Jang and Kim (2014) reported that gac fruit the seed aril extract showed stronger activity than the pulp extract. Lycopene, was more efficient than β-carotene, was active similar to α-tocopherol. Owing to the gac fruits having powerful antioxidants from DPPH and ABTS methods, which suggest that gac fruits in the diet or in functional food products might provide greater health beneficial effects. It was opined that the use of ethyl lactate and ethanol as green and safe solvents to extract phytonutrients such as carotenoids and α-tocopherol antioxidant from crude palm oil (Kua *et al*., 2018).

5.5.2 Marigold petals

5.5.2.1 Recovery percentage of carotenoid pigment concentrate and content of carotenoid



T_1 T_2 T_3 T_4 T_5

Plate 18: Carotenoid pigment concentrates of gac fruit seed aril as influenced by different extraction methods

Treatments details:

T_1 Ethanol

T_2 Ethyl lactate

T_3 Ethanol and ethyl lactate (1:1 ratio)

T_4 Microwave assisted extraction with ethanol

T_5 Microwave assisted extraction with ethanol and ethyl lactate (1:1 ratio)

Among the different extraction methods used, ethyl lactate extraction method recorded significantly higher recovery percentage (93.33±0.27 %) of pigment concentrate in the marigold pigment concentrates. Globally, there is an increasing demand for organic compounds that are non-toxic and safe for human consumption. It is projected that increased demand for green solvents in a variety of industries, particularly food and beverage, will have a beneficial impact on market growth in the near future. The fermentation of a carbohydrate feedstock results in the production of ethyl lactate, which is completely biodegradable in CO_2 and water. It is suitable to extract a wide variety of metabolites due to its miscibility with both hydrophilic and hydrophobic substances and it also has the benefit of extracting cis and trans-lycopene isomers. This might be the reason for obtaining highest recovery of carotenoid pigment concentrate in the present study. The results of our study are in line with the study conducted by Nie *et al*. (2021) who opined that in the extraction of fucoxanthin from *Sargassum fusiforme*, an edible brown macroalga rich in carotenoid content, ethyl lactate performed similarly to ethanol/acetone in terms of efficiency, suggesting that it might be utilised as a substitute for conventional solvents.

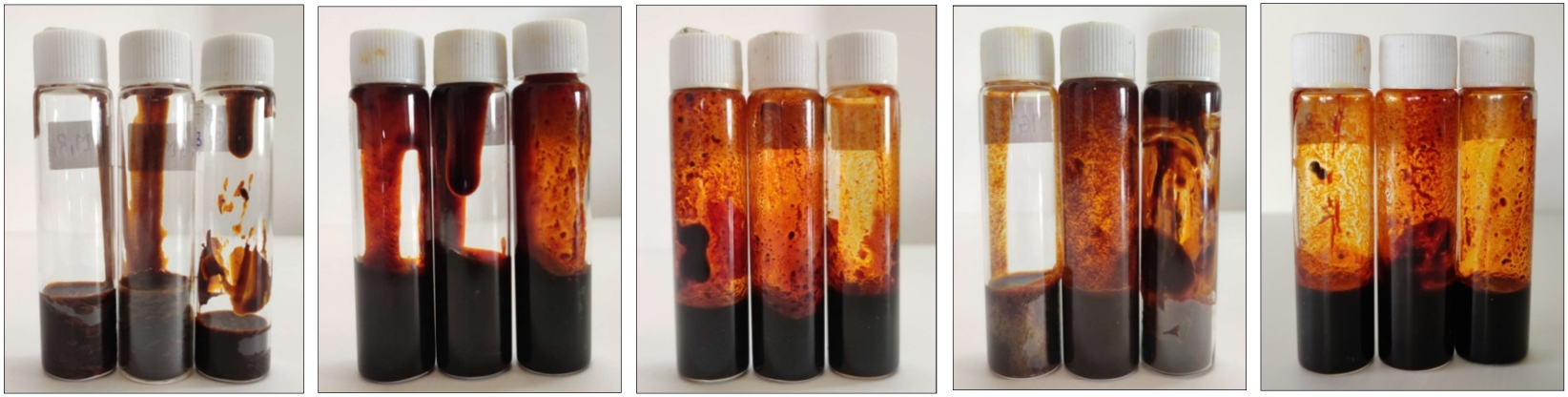
There is a growing corpus of scientific research on the extraction of carotenoids from sources that are based on plants (Cante *et al*., 2022). The traditional organic solvent extraction, either alone or in conjunction with other techniques, is one of the most often used methods for carotenoid recovery extraction. However, the petrochemical industry provides the great majority of the solvents (which are highly flammable, corrosive, carcinogenic, and harmful substances) required in the process (Saini and Keum, 2018). The removal of the remaining solvents to produce a secure extract that can be included into food products is another key drawback of the traditional solvent extraction process (Calvo *et al*., 2007). In this regard, ethyl lactate, a biodegradable environmentally friendly solvent, produced from the fermentation of carbohydrate feedstock can offer advantages for the extraction of carotenoids. A technique for extracting carotenoids from dry plant material using ethyl lactate as a green solvent was patented by Ishida and Chapman (2009). In our study, the highest total carotenoid content (2276.93±3.61 µg/g), β-carotene (491.03±0.85 µg/g), lycopene (236.96±0.10 µg/g) and lutein content (1310.02±0.27 µg/g) was found in the

extraction method with ethyl lactate solvent. This might be related to the fact that ethyl lactate is capable of dissolving in both aqueous (polar) and hydrocarbon (non-polar) mediums and so it has the potential to recover compounds with a wide range of polarity without the presence of co-solvent.

When ethyl lactate was used as a solvent, Szabo *et al*. (2022) reported the highest results for lycopene (1324.89 g/g DW), followed by ethyl acetate with slightly lower variations (1313.54 g/g DW). Strati and Oreopoulou (2011) studied the extraction of carotenoids from tomato waste with ethyl lactate and several other conventional organic solvents and found that the extraction rate of ethyl lactate was 243.00 mg/kg which is highest carotenoid yield from tomato waste compared to acetone or ethyl acetate and the maximum yield was achieved by operating with three successive extractions using a dry tomato waste/ solvent ratio of 1:10, for 30 min each, at 70 ºC, whereas the extraction rate by acetone, ethyl acetate, n-hexane, and ethanol were below 60 mg/kg, indicating that the high extraction efficiency of ethyl lactate is attributed to the fact that ethyl lactate could be dissolved in both water (polar) and hydrocarbon solvents (non-polar). The main finding of the study made by Szabo *et al*. (2022), ethyl lactate exhibits a high level of performance in the recovery of lycopene from wet samples (1324.89 g/g DW), followed by ethyl acetate with barely changed values (1313.54 g/g DW). . Similarly, According to Wu *et al*. (2011), ethyl lactate outperformed acetone and ethanol in terms of its ability to extract astaxanthin from red yeast *Xanthophyllomyces dendrorhous*.

5.5.2.2 Colour properties

In our work, highest instrumental colour values *viz., L** (70.20±0.12), *a** (10.48±0.14), *b** (76.49±0.15) and *hue angle* (82.44±0.19) were recorded in ethanol extraction method. The combination of the values of the instrumental colour vectors indicated bright yellow colour of the pigment concentrate obtained in extraction with ethanol as solvent, whereas, orange-yellow with much higher values for *chroma* in the pigment concentrate obtained in the extraction done by ethyl lactate was observed. The colour of marigold pigment concentrates is depicted in Plate 19. This difference in the hues of the pigment concentrate might be due the dominance in the extraction



T_1 T_2 T_3 T_4 T_5

Plate 19: Carotenoid pigment concentrates of marigold flower petals as influenced by different extraction methods

Treatments details:

T_1 Ethanol

T_2 Ethyl lactate

T_3 Ethanol and ethyl lactate (1:1 ratio)

T_4 Microwave assisted extraction with ethanol

T_5 Microwave assisted extraction with ethanol and ethyl lactate (1:1 ratio)

of higher amount of lutein by ethanol and much higher quantity of the combination of β-carotene, lutein and lycopene content by ethyl lactate is evident from our study. The colour of the pigment concentrate can be correlated with the concentration of β-carotene, lutein and lycopene content. Hence, it is proved from our study that the extraction efficiency of ethyl lactate alone with respect to extraction of carotenoids namely lutein, lycopene and β-carotene is highest as compared to ethanol, combination of ethanol and ethyl lactate as well as microwave assisted extraction using ethanol and ethyl lactate in marigold.

The marigold spent obtained by the of extraction process indicating the respective hues by ethanol and ethyl lactate as solvents can be noticed in the Plate 20.

Plate 20: Image indicating the colour of the marigold spent obtained after the extraction process by using ethanol and ethyl lactate as extraction solvents. Right side of the picture indicates the spent obtained from ethyl lactate and left side of the spent was from ethanol as solvent.

5.5.2.3 Antioxidant properties and total phenolics

Carotenoids are organic antioxidants that protect the body against a number of degenerative conditions, such as cancer and cardiovascular disease (Lores *et al*., 2015). They have anti-inflammatory properties and reduce the oxidation of low density lipoprotein (Rafi *et al*., 2007). In the present study highest antioxidant activity *viz.,* DPPH (0.355±0.03 µl/ml), FRAP (0.557±0.003 µl/ml), ABTS (0.201±0.002 µl/ml) and total phenolics (337.61±0.23 mg GAE/100 g) were obtained when ethyl lactate was applied as a solvent. The highest antioxidant activity of the pigment concentrate might be due to the presence of highest lutein, β-carotene, lycopene, total carotenoid content as well as highest phenolic content which was found in the

extraction method with ethyl lactate solvent. During the process of carotenoid pigment extraction, a temperature of 45 ºC for a period of 45 min was maintained, so this thermal processing might have released more bound phenolic acids due to the breakdown of cellular constituents since phenolic acids occur in plants as metabolic intermediates, and they also accumulate in the vacuoles (Chism and Haard, 1996). Ethyl lactate has resulted in efficient extraction solvent for polyphenols from a wild plant *Cytisus scoparius*, yielding extracts with high levels of plant phenolics and antioxidant activity. In the study conducted by Lores *et al*. (2015), ethyl lactate was found to exert higher efficiency (total phenolic content and antiradical activity) than methanol at temperatures higher than 120 °C, whereas El-Malah *et al*. (2015) concluded ethyl lactate as extraction solvent giving high value in both total phenolics and lycopene content since it act as hydrophilic and lipophilic solvent which is used newly and widely as eco-friendly solvent. The FRAP values of differently dried materials of marigold indicated that combination of far-infrared radiation with hot air convection (FIR-HA) drying had the greatest reducing power (972.7 µmol FeSO4/g DW), followed by fresh (821.0 µmol FeSO4/g DW), freeze drying (FD) (811.0 µmol FeSO4/g), and then hot-air (HA) (730.7 µmol FeSO4/g DW). The FIR-HA dried gave high FRAP values that may be due to their higher levels of total phenol content (TPC), total flavonoid content (TFC), lycopene, β-carotene and lutein. The increase in antioxidant activity of the thermally processed marigold could be explained by the increased amount of lycopene, β-carotene and lutein, a major phytochemical in marigold, and other bound phytochemicals released from the matrix with thermal processing (Siriamornpuna *et al*., 2012). Synergistic effects of other phytochemicals could be another explanation for increased antioxidant activity (Eberhardt *et al*., 2000).

5.6 Evaluation of guava squash, guava jelly and ash gourd candy coloured with carotenoid pigment

The pigment concentrate obtained from the best extraction method for gac fruit seed aril and marigold flower petal was incorporated in the processed products like guava squash, guava jelly and ash gourd candy at 0.3 g/200 ml concentration. With respect to the gac fruit pigment concentrate added to guava squash and ash

gourd candy, the bright red colour was not prominently visible which indicated that the concentration used (0.3 g/200 ml) was not sufficient to give the products bright red hue, whereas marigold petal pigment concentrate incorporated in guava squash, the yellow hue was not prominently visible indicated that the pigment concentration (0.3 g/200 ml) was not sufficient to give the visible bright yellow hue to the squash. Upon incorporation, the products were stored for a period of three months under ambient and refrigerated condition.

5.6.1 Lycopene, β-carotene, lutein and total carotenoid content

In all the products, the pigment (lycopene, β-carotene, lutein and total carotenoid content) content significantly reduced throughout the storage period irrespective of the product, source of pigment (gac and marigold pigment) and storage conditions. However, maximum retention of the pigment concentration was noticed in the products stored under refrigerated condition, and the concentration being high in the products added with marigold pigment. This implies that the marigold pigment has higher stability than the gac fruit pigment in the processed products which might be due to the difference in the compounds present in the pigment concentrate specific to the crop and also their interaction with the product contents. The food industry uses carotenoids as natural pigments to colour a variety of foods and beverages. However, they are extremely unstable and readily damaged when exposed to oxygen or light during storage or food production because of their highly conjugated structure (Zakynthinos and Varzakas, 2016). Additionally, the incorporation of carotenoids into ingredient systems may cause them to degrade quickly (Ribeiro *et al*., 2003). Carotenoids may undergo reactions that result in the loss of double bonds or the molecule's scission (Zakynthinos and Varzakas, 2016). In addition, isomerization to the cis-configuration of the double bonds in carotenoids is possible (Xianquan *et al*., 2005). Since cis-isomers of carotenoids like lycopene are expected to be more accessible and bioactive, isomerization reactions may even be advantageous (Schieber and Carle, 2005).

Our results are in accordance with Rodriguez-Amaya (1997), who mentioned that polyene chain of carotenoid compounds' vulnerability to oxidation and geometric

isomerization is what causes their instability and subsequent breakdown. According to Sanchez-Moreno *et al*. (2003), the main reason for carotenoid loss is oxidation, which is a spontaneous free-radical chain reaction in the presence of oxygen, light, metals, enzymes, and peroxides. Odriozola-Serrano *et al*. (2009) assessed the carotenoid stability of orange juices stored in the refrigerator and revealed that the total carotenoid content fell considerably during the exposure time, which is consistent with the effects seen in this study.

5.6.2 Instrumental colour values

In all the products evaluated, the combination of instrumental colour values with respect to *L**, *a**, *b**, *chroma* and *hue angle* showed decrease in the brightness of the red and yellow colour of the products throughout the storage period irrespective of the product, source of pigment and storage conditions (Plate 21, 22 and 23). However, maximum retention of the colour brightness was noticed in the products stored under refrigerated condition, and it was high in the products added with marigold pigment. The reduction in the brightness of the colour may be correlated with the reduction in the lycopene, β-carotene, lutein and total carotenoid content in the products. During storage, when the products are exposed to the light, oxygen, varied temperatures etc the products' internal as well as external quality deteriorates. And this process of deterioration and degradation is much higher under ambient condition than the refrigerated conditions.

5.6.3 Antioxidant properties

Fruit and vegetables have a very diverse range of bioactive compounds present in them, and this diversity affects the quantity and kind of antioxidants present in them. However, other authors contend that vitamin C, a potent antioxidant found in fruits and vegetables can increase the antioxidant capacity of juices. Several studies have found a strong association between phenolic content and antioxidant activity (Reddy *et al*., 2010). Because of this, the antioxidant activities in the investigated products cannot be only attributed to their pigment/phenolic content but also to the actions of other antioxidant chemicals and their interactions.

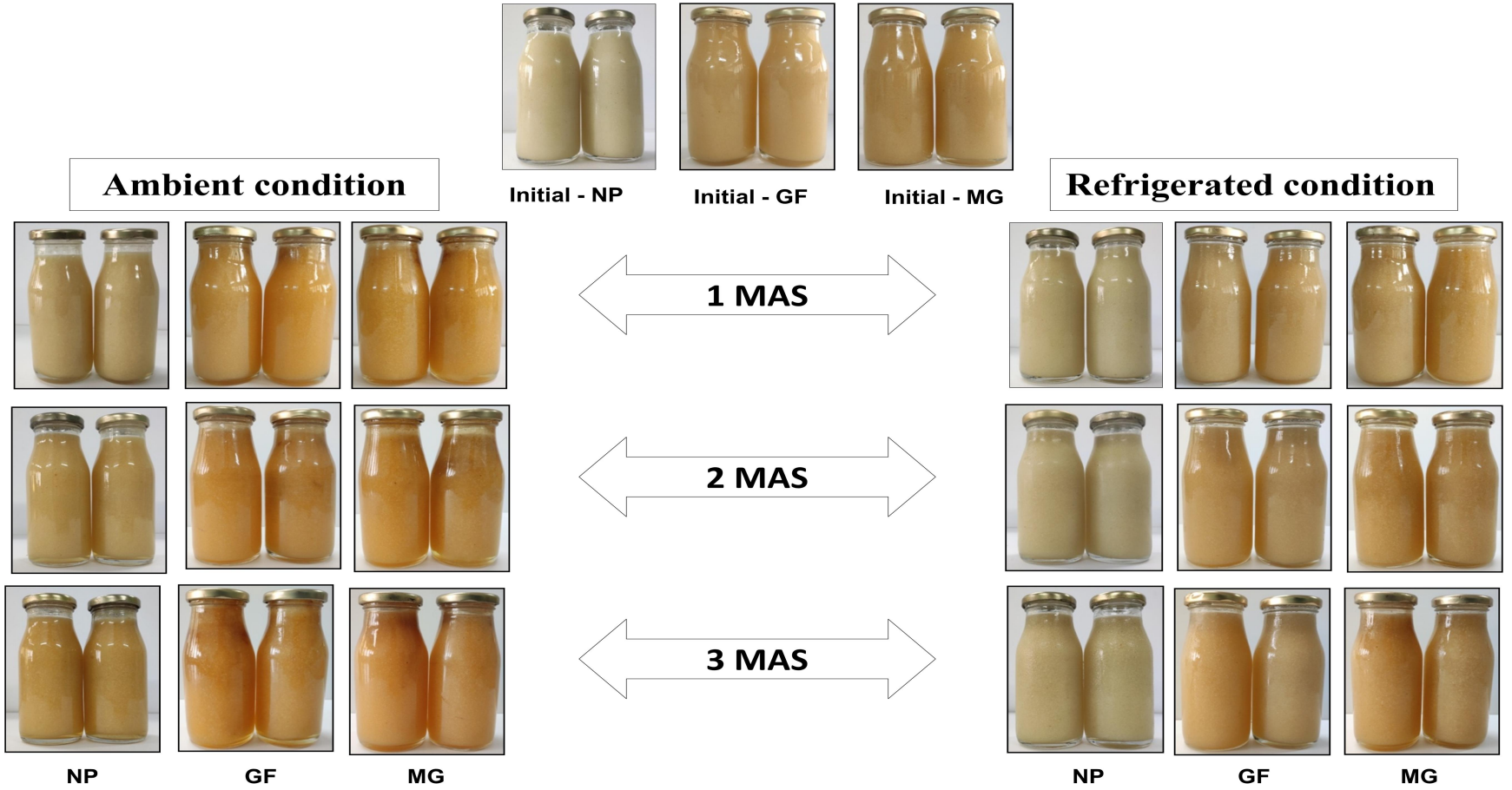


Plate 21: Guava squash with and without carotenoid pigment at different storage periods under ambient and refrigerated storage conditions

NP – No pigment; GF – Gac fruit pigment; MG – Marigold pigment

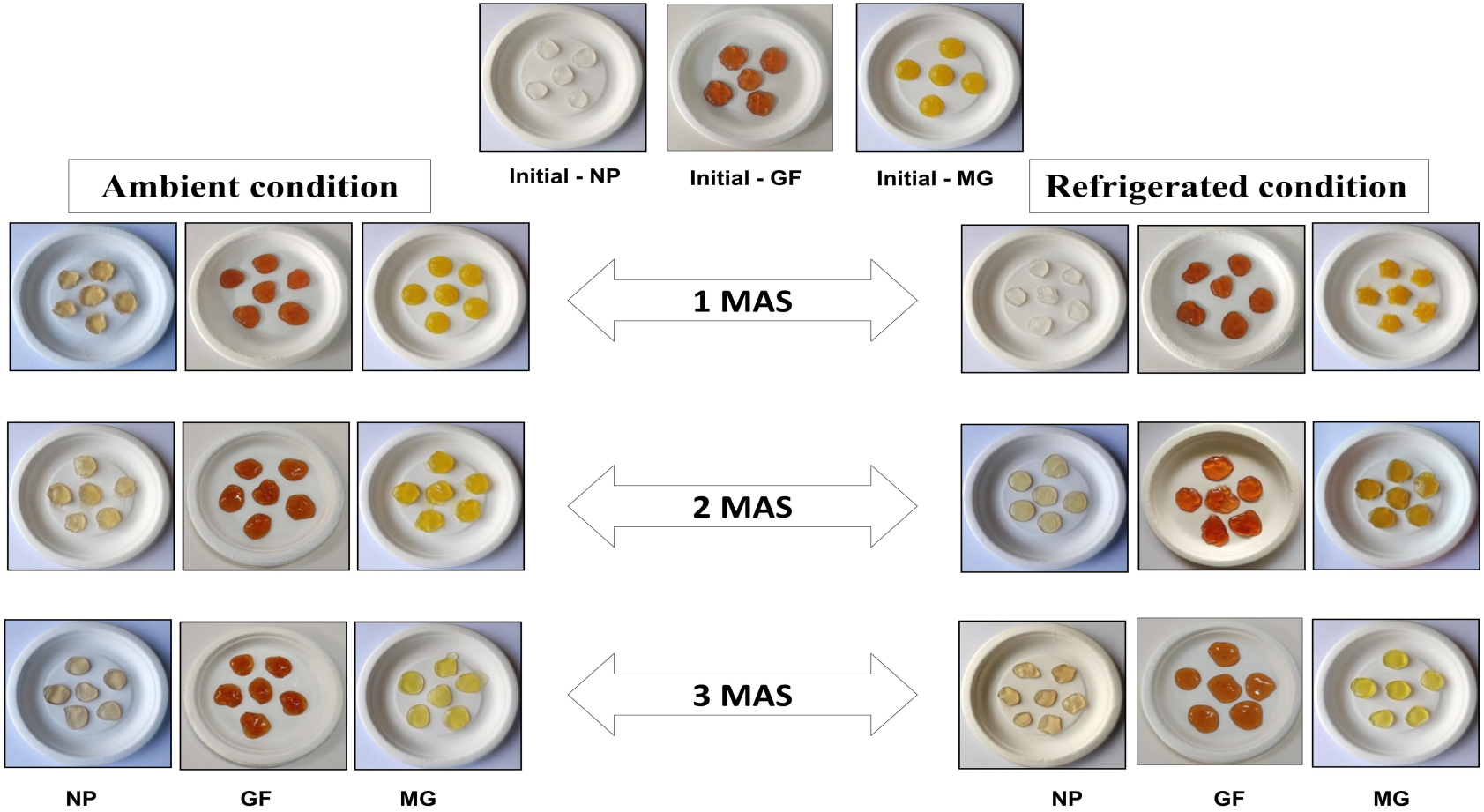


Plate 22: Guava jelly with and without carotenoid pigment at different storage periods under ambient and refrigerated storage conditions

NP – No pigment; GF – Gac fruit pigment; MG – Marigold pigment

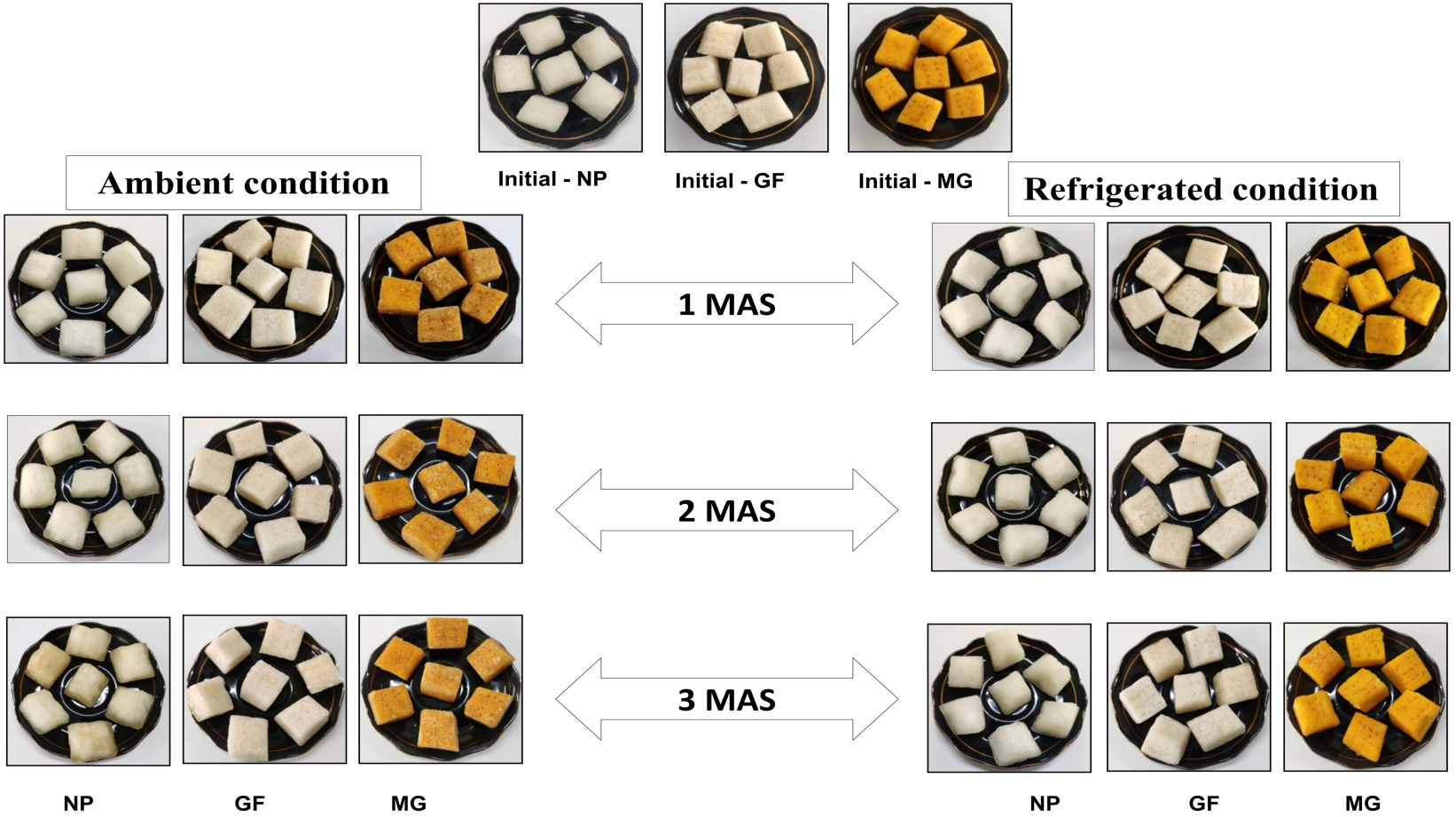


Plate 23: Ash gourd candy with and without carotenoid pigment at different storage periods under ambient and refrigerated storage conditions

NP – No pigment; GF – Gac fruit pigment; MG – Marigold pigment

In all the products, the antioxidant activity analyzed by DPPH, ABTS and FRAP significantly reduced throughout the storage period irrespective of the product, source of pigment (gac and marigold pigment) and storage conditions. However, maximum retention of the antioxidant activity was noticed in the products stored under refrigerated condition, and the antioxidant activity being high in the products added with marigold pigment. Even though the assays described above initially showed good levels of antioxidant activity, some authors claimed that the observed decrease during product storage may have been caused by antioxidant antagonism, which is connected to the presence of various bioactive compounds and their interactions, resulting in a decrease in antioxidant activity (Zielinski *et al*., 2014).

5.6.4 Sensory evaluation

Since different reactions during storage, such as isomerization and oxidation of carotenoids, result in the loss of many organoleptic characteristics, including colour and flavour, among others, which are the most significant characteristics related to product quality influencing choice of purchase, the stability of carotenoid content during storage affects the quality of the product (Baker and Günter, 2004).

The sensory scores for the respective attributes selected for the products (guava squash, guava jelly and ash gourd candy) decreased as the storage period increased under ambient as well as refrigerated storage conditions. However, the products stored under refrigerated condition were able to score higher as compared to the products stored under ambient condition. Lower temperature during storage will reduce the rate of deterioration process of the product and the product quality is well maintained. The most effective way to preserve the quality of products can be thought of as storage at low temperatures without oxygen or light. Our results showed that the loss of total carotenoids along with lycopene, β-carotene and lutein was significantly decreased at low storage temperatures so the products retained their appeal and other sensorial qualities obtained higher scores.

Summary

6. SUMMARY

The main objectives of the study were to standardize the process for extraction of plant pigments from selected fruits (mangosteen), vegetables (red amaranthus, basella, gac fruit), and flowers (butterfly pea, marigold) and to assess application of extracted pigments in food products (guava squash, guava jelly and ash gourd candy).

The present study was focused on the extraction of anthocyanin, betalain and carotenoid pigments. Two crops for each pigment group were taken *viz.*, butterfly pea flowers and mangosteen fruit rind for anthocyanin pigment; basella berries and red amaranthus leaves for betalain pigment; gac fruit seed aril and marigold flower petals for carotenoid pigment.

Depending on the quantity of bioactive compounds obtained in the extract its final recovery varies upon vacuum concentration. Significantly higher recovery percentage of anthocyanin pigment concentrate from butterfly pea flower was seen in acidified aqueous (74.03±0.84 %) extraction method (1 % citric acid), whereas aqueous (distilled water) extraction method has proved to be the most efficient, yielding a higher total monomeric anthocyanin content (TMAC) of 7925.29±36.07 mg/L. The colour values in the different treatments of anthocyanin pigment concentrate from butterfly pea flower were significantly different. Instrumental colour values indicating brilliant-blue colour *viz., a** (46.90±0.77), *b** (-63.25±0.13), *chroma* (78.74±0.38) and *hue angle* (306.56±0.50°) were observed in the microwave assisted extraction (MAE) method with aqueous medium. Anthocyanins extracted from plants have antioxidant properties that contribute significantly to health and therapeutic effects. Higher antioxidant activity *viz.,* DPPH (3.49±0.59 µl/ml), FRAP (3.99±1.10 µl/ml) and ABTS ((2.42±0.01 µl/ml), total phenolics (29.78±1.79 mg GAE/100 g), total flavonoids (20.13±0.40 mg QE/100 g) were recorded in MAE method with aqueous medium.

Since the chemistry of various bioactive substances in plants varies, different extraction solvents may have varying effects on the solubility, extraction yield and antioxidant activity of the phytochemicals. Microwave assisted extraction method with acidified solvent recorded significantly higher recovery percentage of

anthocyanin pigment concentrate from mangosteen fruit rind (55.03±2.98 %), whereas the acidified solvent extraction method resulted in highest TMAC from mangosteen fruit rind (17652.54±139.47 mg/L). Instrumental colour values indicating purple-red colour *viz.., L** (3.17±0.38), *a** (59.53±0.85), *b** (5.09±0.52), *chroma* (60.80±1.00) and *hue angle* (28.06±0.33°) were observed in the MAE method with acidified solvent. Higher antioxidant activity *viz.,* DPPH (2.29±0.24 µl/ml), FRAP (2.83±0.21 µl/ml) and ABTS (2.02±0.03 µl/ml), total phenolics (32.25±0.30 mg GAE/100 g) and total flavonoids (40.02±3.52 mg QE/100 g) were recorded in the MAE method with acidified solvent.

The TMAC, instrumental colour properties, radical scavenging antioxidant activities assessed by DPPH, ABTS and FRAP assays and sensory scores of processed products *i.e.,* guava squash, guava jelly and ash gourd candy decreased significantly throughout the storage period, irrespective of treatments and storage conditions. After three months of storage, the highest retention of TMAC, highest instrumental colour properties, radical scavenging antioxidant activities and sensory scores were noticed in the products with butterfly pea pigment stored under refrigerated condition, whereas the lowest retention of TMAC, lowest instrumental colour properties, radical scavenging antioxidant activities and sensory scores were recorded in the products added with mangosteen pigment stored at ambient condition, which indicates that the butterfly pea anthocyanin pigment's stability is higher than that of mangosteen.

The phytochemical extraction from plant materials relies upon the extraction strategy being influenced by the extraction parameters used. A significantly higher recovery percentage of betalain pigment concentrate from basella berries (53.13±0.41 %) was observed in acidified aqueous extraction method (1 % citric acid), whereas MAE (50 % ethanol) method recorded significantly higher betacyanin (605.83±4.10 mg/g), betaxanthin content (86.35±1.67 mg/g) and total betalain content (692.18±2.52 mg/g). Instrumental colour values of *a** (52.27±0.24), *b** (23.24±0.16), *chroma* (58.75±0.19) and *hue angle* (27.40±0.17°) indicating bright purple-red colour of the pigment concentrate in the MAE method with ethanol (50 %) as solvent. Higher antioxidant activity *viz.,* DPPH (3.40±0.04 µl/ml), FRAP (3.69±0.19 µl/ml) and

ABTS (2.99±0.04 µl/ml), total phenolics (211.37±0.29 mg GAE/100 g) and total flavonoids (124.07±2.53 mg QE/100 g) were observed in the betalain pigment concentrate extracted using 50 per cent ethanol as the solvent by MAE method. The highly significant correlation was observed between the total antioxidant capacity and phenolics content.

A significantly higher recovery percentage of betalain pigment concentrate from red amaranthus (58.73±0.43 %) was observed in acidified aqueous extraction method (1 % citric acid), whereas MAE (50 % ethanol) method recorded significantly higher betacyanin (601.15±2.25 mg/g), betaxanthin content (75.63±0.55 mg/g) and total betalain content (676.78±2.79 mg/g). The red amaranthus pigment concentrate presented the instrumental colour values *viz., L** (41.94±0.06), *a** (33.37±0.22), *b**(56.76±0.02) *chroma* (63.37±0.17) and *hue angle* (58.41±0.02°) showing a darker and reddish colour shade of the pigment concentrate in the MAE method with ethanol (50%) as solvent. Higher antioxidant activity *viz.,* DPPH (1.34±0.01 µl/ml), FRAP (3.69±0.19 µl/ml) and ABTS (0.81±0.01 µl/ml), total phenolics (190.03±0.22 mg GAE/100 g) and total flavonoids (179.07±0.49 mg QE/100 g) in MAE method with ethanol (50 %) as solvent.

Total betalain content, instrumental colour properties, radical scavenging antioxidant activities assessed by DPPH, ABTS and FRAP assays and sensory scores of processed products *i.e.,* guava squash, guava jelly and ash candy decreased significantly throughout the storage period, irrespective of treatments and storage conditions. After three months of storage, the highest retention of total betalain content, highest instrumental colour properties, radical scavenging antioxidant activities and sensory scores was noticed in the products with basella pigment stored under refrigerated condition, whereas the lowest retention of total betalain content, lowest instrumental colour properties, radical scavenging antioxidant activities and sensory scores were in the products added with red amaranthus pigment stored at ambient condition, which indicates that the basella betalain pigment's stability is higher than that of red amaranthus betalain pigment.

A significantly higher recovery percentage of carotenoid pigment concentrate gac fruit seed aril (89.53±0.13 %) was observed in MAE with ethanol method, whereas ethyl lactate extraction method had given significantly higher total carotenoid content (2069.83±4.38 µg/g), β-carotene (689.43±1.04 µg/g), lycopene (1052.31±0.87 µg/g) and lutein content (216.96±0.22 µg/g). Instrumental colour values of *L** (60.44±0.12), *a** (61.35±0.20), *b** (43.24±0.10), *chroma* (65.62±0.10) and *hue angle* (36.64±0.18°) indicating red colour of the carotenoid pigment concentrate from gac fruit seed aril in the ethyl lactate extraction method. Higher antioxidant activity *viz.,* DPPH (0.59±0.05 µl/ml), FRAP (0.63±0.004 µl/ml), ABTS (0.58±0.005 µl/ml) and total phenolics (211.57±0.13 mg GAE/100 g) were observed in ethyl lactate extraction method.

A significantly higher recovery percentage of carotenoid pigment concentrate from marigold petals (93.33±0.27 %) was recorded in ethyl lactate extraction method, whereas ethyl lactate extraction method recorded significantly higher total carotenoid content (2276.93±3.61 µg/g), β-carotene (491.03±0.85 µg/g), lycopene (236.96±0.10 µg/g) and lutein content (1310.02±0.27 µg/g). Instrumental colour values *viz., L** (70.20±0.12), *a** (10.48±0.14), *b** (76.49±0.15) and *hue angle* (82.44±0.19°) showing a darker and reddish colour shade of the carotenoid pigment concentrate were recorded in ethanol extraction method. Higher antioxidant activity *viz.,* DPPH (0.355±0.03 µl/ml), ABTS (0.201±0.002 µl/ml), FRAP (0.557±0.003 µl/ml) and total phenolics (337.61±0.23 mg GAE/100 g) were observed in ethyl lactate extraction method.

Total carotenoid content, instrumental colour properties, radical scavenging antioxidant activities assessed by DPPH, ABTS and FRAP assays and sensory scores of processed products *i.e.,* guava squash, guava jelly and ash candy decreased significantly throughout the storage period, irrespective of treatments and storage conditions. After three months of storage, the highest retention of total carotenoid content, highest instrumental colour properties, radical scavenging antioxidant activities and sensory scores were noticed in the products with marigold pigment stored under refrigerated condition, whereas the lowest retention of total carotenoid content, lowest instrumental colour properties, radical scavenging antioxidant

activities and sensory scores were in the products added with gac fruit pigment stored under ambient condition, which indicates that the marigold carotenoid pigment's stability is higher than that of gac fruit carotenoid pigment.

Future line of work

- Quantity of the pigment to be added in the products to obtain attractive hue needs to be studied. Since in the present study, the guava squash added with gac and marigold pigment did not give colour.
- Toxicity studies to check the safety of the pigment extracted as well as the products added with pigments needs to be taken up for further confirmation.

References

REFERENCES

[Anonymous]. 2021. Natural food colour used in food and beverage product development. Available: https://www.foodresearchlab.com/insights/blog/organic-food/natural-food-colour-used-in-food-and-beverage-product-development/, [30 June 2022].

Abdel-Moemin, A.R. 2016. Effect of Roselle calyces extract on the chemical and sensory properties of functional cupcakes. *Food Sci. Hum. Wellness*. 5: 230-237.

Abdulqader, A., Ali, F., Ismail, A., Esa, N.M. 2019. Antioxidant compounds and capacities of Gac (*Momordica cochinchinensis* Spreng) fruits. *Asian Pac. J. Trop. Biomed.* 9(4): 158-167.

Abers, J.E. and Wrolstad, R.E. 1979. Causative factors of colour determination in strawberry preserves during processing and storage. *J. Food Sci.* 44(1): 75-81.

Adisakwattana, S., Pasukamonset, P., and Chusak, C. 2020. *Clitoria ternatea* beverages and antioxidant usage. In: Preedy, V.R. (ed.), *Pathology*. Academic Press, Cambridge, Massachusetts, pp. 189-196.

Aguilera, Y., Martin-Cabrejas, M.A., and de Mejia, E.G. 2016. Phenolic compounds in fruits and beverages consumed as part of the mediterranean diet: their role in prevention of chronic diseases. *Phytochem Rev.* 15: 405-423.

Albuquerque, B.R., Pinela, J., Barros, L., Oliveira, M.B.P.P., and Ferreira, I.C.F.R. 2020. Anthocyanin-rich extract of jabuticaba epicarp as a natural colorant: Optimization of heat- and ultrasound-assisted extractions and application in a bakery product. *Food Chem*. 316: 126364.

Alim-un-Nisa., S., Hina, S., Mazhar, I., Kalim, I., Ahmad, N., Zahra, S., Masood, M.K., Saeed, Q., Syed, A. and Asif. M. 2018. Stability of Lutein content in

color extracted from marigold flower and its application in candies. *Pak. J. Agric. Res.* 31(1): 15-23.

Altunkaya, A., Gokmen, V., and Skibsted, L.H. 2016. pH dependent antioxidant activity of lettuce (*L. sativa*) and synergism with added phenolic antioxidants. *Food Chem.* 190: 25-32.

Alupului, A., Calinescu, I., and Lavric, V. 2012. Microwave extraction of active principles from medicinal plants. *U.P.B. Sci. Bull. Ser. B.* 74:.129-142.

Aoki, H., Kieu, N.T.M., Kuze, N., Tomisaka, K., and Chuyen, N.V. 2002. Carotenoid pigments in gac fruit (*Momordica cochinchinensis* Spreng.). *Biosci. Biotechnol. Biochem.* 66(11): 2479-2482.

Attia, Gamila, Y., Moussa, M.E.M., Sheashea, E.R. 2013. Characterization of red pigments extracted from red beet (Beta vulgaris L.) and its potential uses as antioxidant and natural food colourants. *Egypt. J. Agric. Res.* 91(3): 1095-1109.

Azabou S., Sebii H., Taheur F.B., Abid Y., Jridi M., and Nasri M. 2020. Phytochemical profile and antioxidant properties of tomato by-products as affected by extraction solvents and potential application in refined olive oils. *Food Biosci.* 36: 100664.

Azima, A.S., Noriham, A., and Manshoor, N. 2017. Phenolics, antioxidants and color properties of aqueous pigmented plant extracts: *Ardisia colorata* var. *elliptica*, *Clitoria ternatea*, *Garcinia mangostana* and *Syzygium cumini*. *J. Funct. Foods*. 38: 232-241.

Aziz, A.A., Padzil, M.A., and Muhamad, I.I. 2018. Effects of incorporating purple-flashed sweet potato in biscuit on antioxidant content, antioxidant capacity, and colour characteristics. *Malaysian J. Anal. Sci*. 22: 665-667.

Azmir, J., Zaidul, I.S.M., and Rahman, M.M. 2013. Techniques for extraction of bioactive compounds from plant materials: A review. *J Food Eng.* 117: 426-436.

Baker, R. and Günter, C. 2004. The role of carotenoids in consumer choice and likely benefits from their inclusion into products for human consumption. *Trends Food Sci. Technol*. 15(10): 484-488.

Barba-Espin, G., Glied-Olsen, S., Dzhanfezova, T., Joernsgaard, B., Lütken, H., and Müller, R. 2018. Preharvest application of ethephon and postharvest UV-B radiation improve quality traits of beetroot (*Beta vulgaris* L. *ssp. vulgaris*) as source of colourant. *BMC Plant Biol.* 18(1): 1-12.

Barrera, F.A., Reynoso, C.R., and Mejia, E.G. 1998. Stability of betalains extracted from garambullo (*Myrtillo cactusgeometrizans*). *Int. J. Food Sci***.** 4:115-120.

Barzana, E., Rubio, D., Santamaria, R. I., Garcia-Correa, O., Garcia, F., Ridaura Sanz, V. E., and López-Munguía, A. 2002. Enzyme-mediated solvent extraction of carotenoids from marigold flower (*Tagetes erecta*). *J. Agric. Food Chem.* 50(16): 4491-4496.

Benzie I.F. and Stezo Y.T. 1999. Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. *J. Agri. Food Chem.* 47: 633-636.

Benzie, I.F.F. and Strain, J.J. 1996. The Ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem.* 239(1): 70-76.

Bharathi, L.K., Singh, H.S., Shivashankar, S., Ganeshamurthy, A.N., and Sureshkumar, P. 2014. Assay of nutritional composition and antioxidant activity of three dioecious Momordica Species of South East Asia. In:

Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 84(1): 31-36.

Bhattacharyya, B. and Johri, B.M. 1998. Amaranthaceae. In: Bhattacharyya, B. and Johri, B.M. (eds), *Flowering plants: Taxonomy and phylogeny*. Narosa Publishing House, Delhi, India, pp. 111-113.

Boldt, J.K., Meyer, M.H., and Erwin, J.E. 2014. Foliar anthocyanins: A horticultural review. *Hortic. Rev*. 42: 209-251.

Boo, H., Hwang, S., Bae, C., Park, S., Hoe, B., and Gorinstein, S. 2012. Extraction and characterization of some natural plant pigments. *Ind. Crops Prod.* 40: 129-135.

Bors, W., Heller, W., Michel, C., and Saran, M. 1990. Flavonoids as antioxidants: Determination of radical-scavenging efficiencies. *Meth. Enzymol.* 186: 343-355.

Brand-Williams, W., Cuvelier, M.E., and Berset, C. 1995. Use of free radical method to evaluate antioxidant activity. *Lebensm. Wiss. Technol.* 28: 25-30.

Cai, Y. and Corke, H. 1999. Amaranthus betacyanin pigments applied in model food systems. *J. Food Sci.* 64(5): 869-873.

Cai, Y., Sun, M., and Corke, H. 2005. HPLC characterization of betalains from plants in the Amaranthaceae. *J. Chromatogr. Sci.* 43: 454-460.

Calva-Estrada, S.J., Jiménez-Fernández, M., and Lugo-Cervantes, E. 2022. Betalains and their applications in food: The current state of processing, stability and future opportunities in the industry. *Food Chem.* 4: 100089.

Calvo, M.M., Dado, D., and Santa-María, G. 2007. Influence of extraction with ethanol or ethyl acetate on the yield of lycopene, β-carotene, phytoene and phytofluene from tomato peel powder. *Eur. Food Res. Technol.* 224: 567-571.

Camel, V. 2001. Recent extraction techniques for solid matrices-supercritical fluid extraction, pressurized fluid extraction and microwave-assisted extraction: Their potential and pitfalls. *Anal.* 126: 1182-1193.

Cante, R.C., Gallo, M., Varriale, L., Garella, I., and Nigro, R. 2022. Recovery of carotenoids from tomato pomace using a hydrofluorocarbon solvent in sub-critical conditions. *Appl. Sci.* 12: 2822.

Castellar, M.R., Obón, J.M., and Fernández-López, J.A. 2006. The isolation and properties of a concentrated red-purple betacyanin food colourant from *Opuntia stricta* fruits. *J. Sci. Food Agric.* 86(1):122-128.

Cejudo-Bastante, M.J., Hurtado, N., Delgado, A., and Heredia, F.J. 2016. Impact of pH and temperature on the colour and betalain content of Colombian yellow pitaya peel (*Selenicereus megalanthus*). *J. Food Sci. Technol.* 53(5): 2405-2413.

Chandrasekhar, J., Madhusudhan, M.C., and Raghavarao, K.S.M.S. 2012. Extraction of anthocyanins from red cabbage and purification using adsorption. *Food Bioprod. Process*. 90: 615-623.

Chandrasekhar, J., Sonika, G., Madhusudhan, M.C., and Raghavarao, K.S.M.S. 2015. Differential partitioning of betacyanins and betaxanthins employing aqueous two-phase extraction. *J. Food Eng.* 144:156-163.

Chaovanalikit, A., Mingmuang, A., Kitbunluewit, T., Choldumrongkool, N., Sondee, J., and Chupratum, S. 2012. Anthocyanin and total phenolics content of

mangosteen and effect of processing on the quality of mangosteen products. *Int. Food Res. J.* 19(3): 1047-1053.

Chaudhary, B. and Mukhopadhyay, K. 2012. *Syzygium cumini* (L.) skeels: A Potential source of nutraceuticals. *Int. J. Pharm. Biol. Sci.* 2(1): 46-53.

Chauhan, N.S., Singh, N.K., and Gupta, J.K. 2017. A review on *Clitoria ternatea* (Linn.): Chemistry and pharmacology, medicinal plants and its therapeutic uses. OMICS Group eBooks, CA, USA.

Chayaratanasin, P., Barbieri, M.A., and Suanpairintr, N. 2015. Inhibitory effect of *Clitoria ternatea* flower petal extract on fructose induced protein glycation and oxidation-dependent damages to albumin *in vitro*. *BMC Complement. Altern. Med*. 15: 27.

Chemat, F., Vian, A.M., Ravi, H.K., Khadhraoui, B., Hilali, S., Perino, S., and Tixier, A.F. 2019. Review of alternative solvents for green extraction of food and natural products: Panorama, principles, applications and prospects. *Molecules.* 24(16): 3007.

Cheok, C.Y., Adzahan, N.M., Rahman, R.A., Abedin, N.H.Z., Hussain, N., Sulaiman, R., and Chong, G.H. 2018. Current trends of tropical fruit waste utilization. *Crit. Rev. Food Sci. Nutr.*, 58: 335-361.

Chhikara, N., Kushwaha, K., Sharma, P., Gat, Y., and Panghal, A. 2019. Bioactive compounds of beetroot and utilization in food processing industry: A critical review. *Food Chem.* 272: 192-200.

Chism, G.W. and Haard, N.F. 1996. Characteristics of edible plant tissues. In: Fennema, O.R. (ed.), *Food Chemistry*, Dekker, New York, pp. 943-1011.

Chong, F.C. and Gwee, X.F. 2015. Ultrasonic extraction of anthocyanin from *Clitoria ternatea* flowers using response surface methodology. *Nat. Prod. Res.* 29:1485-1487.

Chong, P.H., Yusof, Y.A., Aziz, M.G., Mohd Nazli, N., Chin, N.L., and Syed Muhammad, S.K. 2014. Evaluation of solvent extraction of Amaranth betacyanins using multivariate analysis. *Int. Food Res. J.* 21:1569-1573.

Chusak, C., Thilavech, T., Henry, C.J., and Adisakwattana, S. 2018. Acute effect of *Clitoria ternatea* flower beverage on glycemic response and antioxidant capacity in healthy subjects: A randomized crossover trial. *BMC Complement. Altern. Med.* 18: 6.

Chuyen, H.V., Roach, P.D., Golding, J.B., Parks, S.E., and Nguyen, M.H. 2019. Encapsulation of carotenoid-rich oil from Gac peel: Optimisation of the encapsulating process using a spray drier and the storage stability of encapsulated powder. *Powder Technol*. 344:373-379.

Chuyen, H.V., Tran, X.T., Nguyen, M.H., Roach, P.D., Parks, S.E., and Golding, J.B. 2017. Yield of carotenoids, phenolic compounds and antioxidant capacity of extracts from gac peel as affected by different solvents and extraction conditions. *J. Adv. Agric. Technol.* 4(1): 87-91.

Clifford, M. 2000. Anthocyanins - Nature, occurrence and dietary burden: A review. *J. Sci. Food Agric.* 80: 1063-1072.

Coelho, M., Silva, S., Costa, E., Pereira, R.N., Rodrigues, A.S., Teixeira, J.A., and Pintado, M. 2021. Anthocyanin recovery from grape by-products by combining ohmic heating with food-grade solvents: Phenolic composition, antioxidant, and antimicrobial properties. *Molecules*. 26(13): 3838.

Coria-Cayupán, Y. and Nazareno, M.A. 2015. Betalain profile and antioxidant phytochemicals of opuntia fruits from santiago del estero, Argentina. *Acta Hortic.* 1067: 311-317.

Craft, B.D., Kerrihard, A.L., Amarowicz, R. and Pegg, R.B. 2012. Phenol-based antioxidants and the *in vitro* methods used for their assessment. *Compr. Rev. Food Sci. Food Saf.* 11(2): 148-173.

Croitoru, C., Muresan, C., Turturica, M., Stanciuc, N., Andronoiu, D., Dumitrascu, L., Barbu, V., Enachi (Ionita), E., Horincar (Parfene), G., and Râpeanu, G. 2018. Improvement of quality properties and shelf life stability of new formulated muffins based on black rice. *Molecules*. 23: 3047.

Da Silva, L.P., Pereira, E., Prieto, M.A., Simal-Gandara, J., Pires, T.C.S.P., Alves, M.J., Calhelha, R., Barros, L., and Ferreira, I.C.F.R. 2019. *Rubus ulmifolius* Schott as a novel source of food colorant: Extraction optimization of coloring pigments and incorporation in a bakery product. *Molecules*. 24: 2181.

Das, M., Saeid, A., Hossain, M.F., Jiang, G.H., Eun, J.B., and Ahmed, M. 2019. Influence of extraction parameters and stability of betacyanins extracted from red amaranth during storage. *J. Food Sci. Technol.* 56(2): 643-653.

De Mejia, E.G., Zhang, Q., Penta, K., Eroglu, A., and Lila, M.A. 2020. The colors of health: Chemistry, bioactivity, and market demand for colorful foods and natural food sources of colorants. *Annu. Rev. Food Sci. Technol.* 11: 145-182.

Delgado-Vargas, F. and Paredes-López, O. 2002. *Natural Colorants for food and nutraceutical uses* (1st Ed.). CRC Press, Boca Raton, pp. 35-59.

Delgado-Vargas, F., Jiménez, A. R., and Paredes-López, O. 2000. Natural pigments: carotenoids, anthocyanins, and betalains--characteristics, biosynthesis, processing, and stability. *Crit. Rev. Food Sci. Nutr.* 40(3): 173-289.

Delwiche, J.F. 2004. The impact of perceptual interactions on perceived flavor. *Food Qual. Prefer.* 15(2): 137-146.

Deshmukh, S.A. and Gaikwad. D.K. 2014. A review of the taxonomy, ethnobotany, phytochemistry and pharmacology of *Basella alba* (Basellaceae). *J. Appl. Pharm. Sci.* 4: 153-165.

Do, Q.D., Angkawijaya, A.E., Tran-Nguyen, P.L., Huynh, L.H., Soetaredjo, F.E., Ismadji, S., and Ju, Y.H. 2014. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *J. Food Drug Anal.* 22(3): 296-302.

Domingos, L.D., Xavier, A.A.O., Mercadante, A.Z., Petenate, A.J., Jorge R.A., and Viotto, W.H. 2014. Oxidative stability of yogurt with added lutein dye. *J. Dairy Sci.* 97: 616-623.

Dos Santos, C.D., Ismail, M., Cassini, A.S., Marczak, L.D.F., Tessaro, I.C., and Farid, M. 2018. Effect of thermal and high pressure processing on stability of betalain extracted from red beet stalks. *J. Food Sci. Technol.* 55(2): 568-577.

Du, C.T. and Francis, F.J. 1977. Anthocyanins of mangosteen *Garcinia mangostana*. *J. Food Sci***.** 42: 1667.

Duangmal, K., Saicheua, B., and Sueeprasan, S. 2008. Color evaluation of freeze-dried Roselle extract as a natural food colorant in a model system of a drink. *LWT Food Sci. Technol.* 41: 1437-1445.

Eberhardt, M.V., Lee, C.Y., and Liu, R.H. 2000. Antioxidant activity of fresh apples. *Nature.* 405: 903-904.

EFSA [European Food Safety Authority]. 2012. European Food Safety Authority Scientific opinion on the re-evaluation of anthocyanins (E163) as a food additive. *EFSA J.* 10: 24.

Eletr, A.A., Siliha, H.A.E., Elshobargy, G.A., and Galal, G.A. 2017. Evaluation of lycopene extracted from tomato processing waste as a natural antioxidant in some bakery products. *Zagazig J. Agric. Res.* 44(4): 1389-1401.

El-Malah, M.H., Hassanein, M.M.M., Areif M.H., and Al-Amrousi, E.F. 2015. Utilization of Egyptian tomato waste as a potential source of natural antioxidants using solvents, microwave and ultrasound extraction methods. *Am. J. Food Technol.* 10: 14-25.

Escher, G.B., Wen, M., Zhang, L., Rosso, N.D., and Granato, D. 2020. Phenolic composition by UHPLC-Q-TOF-MS/MS and stability of anthocyanins from *Clitoria ternatea* L. (butterfly pea) blue petals. *Food Chem.* 331: 127341.

Eskilsson, C.S. and Björklund, E. 2000. Analytical-scale microwave-assisted extraction. *J. Chromatogr. A.* 902(1): 227-250.

Fathordoobady, F., Mirhosseini, H., Selamat, J., and Manap M.Y.A. 2016. Effect of solvent type and ratio on betacyanins and antioxidant activity of extracts from *Hylocereus polyrhizus* flesh and peel by supercritical fluid extraction and solvent extraction. *Food Chem.* 202: 70-80.

FDA [Food and Drug Administration]. 2014. Code of Federal Regulations, 2014. Food additives permitted for direct addition to food for human consumption, Sub part F: Flavoring agents and related substances. Sec. 172.515: Synthetic flavoring substances and adjuvants. 21(3).

FDA [Food and Drug Administration]. 2018. Q3C – Tables and list guidance for Industry. Available: www.fda.gov/regulatory-information/search-fda-guidance-documents/q3c-tables-and-list-rev-4 , [8 September2021].

FSSAI [Food Safety and Standard Authority of India]. 2011. Food safety and standards (Food products standards and food additives) regulations, 2011. The Ministry of Health and Family Welfare, Government of India.

Fu, X., Wu, Q., Wang, J., Chen, Y., Zhu, G., and Zhu, Z. 2021. Spectral characteristic, storage stability and antioxidant properties of anthocyanin extracts from flowers of Butterfly pea (*Clitoria ternatea* L.). *Molecules*. 26(22): 7000.

Fu, Y., Shi, J., Xie, S.Y., Zhang, T.Y., Soladoye, O.P., and Aluko, R.E. 2020. Red beetroot betalains: Perspectives on extraction, processing, and potential health benefits. *J. Agric. Food Chem.* 68: 11595-11611.

Gamage, G.C.V., Lim, Y.Y., and Choo, W.S. 2021. Anthocyanins from *Clitoria ternatea* flower: Biosynthesis, extraction, stability, antioxidant activity, and applications. *Front. Plant Sci.* 12: 792303.

Gandía-Herrero, F., Escribano, J., and García-Carmona, F. 2016. Biological activities of plant pigments betalains. *Crit. Rev. Food Sci. Nutr.* 56: 937-945.

Garcia-Viguera, C., Zafrilla, P. and Tomás-Barberán, F.A. 1998. The use of acetone as an extraction solvent for anthocyanins from strawberry fruit. *Phytochem. Anal.* 9: 274-277.

Garofulić, I.E., Dragović-Uzelac, V., Jambrak, R.A., and Jukić, M. 2013. The effect of microwave assisted extraction on the isolation of anthocyanins and phenolic acids from sour cherry Marasca (*Prunus cerasus* var. *Marasca*). *J. Food Eng.* 117(4): 437-442.

Garzón, G.A. and Wrolstad, R.E. 2002. Comparison of the stability of pelargonidin-based anthocyanins in strawberry juice and concentrate. *J. Food Sci.* 67(4): 1288-1299.

Gengatharan, A., Dykes, G.A., and Choo, W.S. 2016. Stability of betacyanin from red pitahaya (*Hylocereus polyrhizus*) and its potential application as a natural colourant in milk. *Int. J. Food Sci***.** 51(2): 427-434.

Giusti M.M. and Wrolstad R.E. 2005. Characterization and measurement of anthocyanins by UV-visible spectroscopy. *Handbook of Food Anal. Chem.* 19-31.

Giusti, M.M. and Wrolstad, R.E. 2003. Acylated anthocyanins from edible sources and their applications in food systems. *Biochem. Eng. J.* 14: 217-225.

Gliszczyńska-Swigło A., Szymusiak, H., and Malinowska, P. 2006. Betanin, the main pigment of red beet: Molecular origin of its exceptionally high free radical-scavenging activity. *Food Addit. Contam.* 23:1079-1087.

Gonfa, T., Teketle, S., and Kiros, T. 2020. Effect of extraction solvent on qualitative and quantitative analysis of major phyto-constituents and *in-vitro* antioxidant activity evaluation of *Cadaba rotundifolia* Forssk leaf extracts. *Cogent Food Agric.* 6: 1853867.

González-Gómez, D., Lozano, M., Fernández-León, M.F., Bernalte, M.J., Ayuso, M.C., and Rodríguez, A.B. 2010. Sweet cherry phytochemicals: Identification and characterization by HPLC-DAD/ESI-MS in six sweet-cherry cultivars grown in Valle del Jerte (Spain). *J. Food Compost. Anal.* 23(6): 533-539.

Gopalakrishnan, C., Shankaranarayanan, D., Kameswara, L., and Nazimudern, S.K. 1980. Effect of mangostin, a xanthone from *Garcinia mangostana* Linn in immunopathological and inflammatory reactions. *Indian J. Exp. Biol*., 18: 843-846.

Grotewold, E. 2006. The genetics and biochemistry of floral pigments. *Annu. Rev. Plant Biol.* 57: 761-780.

Guimarães, R., Barreira, J.C.M., Barros, L., Carvalho, A.M., and Ferreira I.C.F.R. 2011. Effects of oral dosage form and storage period on the antioxidant properties of four species used in traditional herbal medicine. *Phyther. Res.* 25: 484-492.

Hariadi, H., Sunyoto, M., Nurhadi, B., and Karuniawan, A. 2018. Comparison of phytochemical characteristics pigmen extract (Antosianin) sweet purple potatoes powder (*Ipomoea batatas* L) and clitoria flower (*Clitoria ternatea*) as natural dye powder. *J. pharmacogn. phytochem.* 7(4): 3420-3429.

Herbach, K.M., Stintzing, F.C., and Carle, R. 2006. Betalain stability and degradation - Structural and chromatic aspects, *J. Food Sci.* 71(4): 41-50.

Isabelle, M., Lee, B.L., Ling, M.T., Koh, W.P., Huang, D., and Ong, C.N. 2010. Antioxidant activity and profiles of common fruits in Singapore. *Food Chem.* 123: 77-84.

Ishida, B.K. and Chapman, M.H. 2009. Carotenoid extraction from plants using a novel, environmentally friendly solvent. *J. Agric. Food Chem.* 57**:** 1051-1059.

Jaafar, N.F., Ramli, M.E., and Salleh, R.M. 2020. Optimum extraction condition of *Clitorea ternatea* flower on antioxidant activities, total phenolic, total flavonoid and total anthocyanin contents. *Trop. Life Sci. Res.* 31: 1-17.

Jaeschke, D. P., Rech, R., Marczak, L.D.F., and Mercali, G.D. 2017. Ultrasound as an alternative technology to extract carotenoids and lipids from *Heterochlorella luteoviridis*. *Bioresour. Technol.* 224: 753-757.

Jain, T. 2009. Microwave assisted extraction for phytoconstituents: An overview. *Asian J. Chem*. 2:19-25.

Jamil, N., Zairi, M.N.M., Naim, N., and Pa'ee, F. 2018. Influences of environmental conditions to phytoconstituents in *Clitoria ternatea* (butterfly pea flower): A review. *J. Sci. Technol*. 10: 208-228.

Jang, M. and Kim, G. 2014. Antioxidant activity and HPLC analysis of lycopene, β carotene and α-Tocopherol from GEUK (*Momordica cochinchinensis* Spreng) fruit. *J. Int. Sci. Publ.: Agric. Food.* 2: 430-438.

Jeyaraj, E.J., Lim, Y.Y., and Choo, W.S. 2020. Extraction methods of butterfly pea (*Clitoria ternatea*) flower and biological activities of its phytochemicals. *J. Food Sci. Technol.* 58(6): 2054-2067.

Jeyaraj, E.J., Lim, Y.Y., and Choo, W.S. 2021. Effect of organic solvents and water extraction on the phytochemical profile and antioxidant activity of *Clitoria ternatea* flowers. *ACS Food Sci. Technol.* 1: 1567-1577.

Jiménez-López, C., Caleja, C., Prieto, M.A., Sokovic, M., Calhelha, R.C., Barros, L., and Ferreira, I.C.F.R. 2019. Stability of a cyanidin-3-O-glucoside extract obtained from *Arbutus unedo* L. and incorporation into wafers for colouring purposes. *Food Chem*. 275: 426-438.

Jiménez-Zamora, A., Delgado-Andrade, C., and Rufián-Henares, J.A. 2016. Antioxidant capacity, total phenols and color profile during the storage of selected plants used for infusion. *Food Chem.* 199: 339-346.

Joshi V. and Devi M.P. 2014. Optimization of extraction treatment and concentration of extract on yield and quality of anthocyanins from plum var. 'Santa Rosa'. *Indian J. Nat. Prod. Resour.* 5(2): 171-175.

Kamalambigeswari, R. and Rebecca J. L. 2016. Extraction of antioxidant lutein from various flowers. *Int. J. Pharm. Sci. Rev. Res.* 39(1): 122-124.

Kamkaen, N. and Wilkinson, J.M. 2009. The antioxidant activity of *Clitoria ternatea* flower petal extracts and eye gel. *Phytother. Res.* 23(11): 1624-1625.

Kanatt, S.R. 2020. Development of active/intelligent food packaging film containing Amaranthus leaf extract for shelf life extension of chicken/fish during chilled storage. *Food Packag. Shelf Life* . 24: 100506.

Kang, H.J., Ko, M.J., and Chung, M.S. 2021. Anthocyanin structure and pH dependent extraction characteristics from blueberries (*Vaccinium corymbosum*) and chokeberries (*Aronia melanocarpa*) in subcritical water state. *Foods.* 10(3): 527.

Kanner, J., Harel, S., and Granit, R. 2001. Betalains - A new class of dietary antioxidants. *J. Agric. Food Chem.* 49: 5178-5185.

Kara, S. and Ercelebi, A.E. 2013. Thermal degradation kinetics of anthocyanins and visual colour of Urmu mulberry (*Morus nigra* L.) *J. Food Eng.* 116: 541-547.

Kaufmann, B. and Christen, P. 2002. Recent extraction techniques for natural products: Microwave-assisted extraction and pressurised solvent extraction. *Phytochem. Anal*. 13: 105-113.

Kayın, N., Atalay, D., Türken Akçay, T., and Erge, H.S. 2019. Color stability and change in bioactive compounds of red beet juice concentrate stored at different temperatures. *J. Food Sci. Technol.* 56(11): 5097-5106.

Kazuma, K., Noda N., and Suzuki, M. 2003. Malonylated flavonol glycosides from the petals of *Clitoria ternatea. Phytochemistry*. 62(2): 229-237.

Kearsley M.W., and Katsaboxakis, K.Z. 1980. Stability and use of natural colours in foods red beet powder, copper chlorophyll powder and cochineal. *Int. J. Food Sci. Technol.* 15:501-514.

Kha, T.C., Nguyen, M.H., and Roach, P.D. 2010. Effects of spray drying conditions on the physicochemical and antioxidant properties of the Gac (*Momordica cochinchinensis*) fruit aril powder. *J. Food Eng*. 98: 385-392.

Khan, M.I. and Giridhar, P. 2014. Enhanced chemical stability, chromatic properties and regeneration of betalains in *Rivina humilis* L. berry juice. *LWT Food Sci. Technol.* 58: 649-657.

Khan, M.I., Harsha, P.S.C.S., Chauhan, A.S., Vijayendra, S.V.N., Asha, M.R. and Giridhar, P. 2015. Betalains rich *Rivina humilis* L. berry extract as natural colorant in product (fruit spread and RTS beverage) development. *J. Food Sci. Technol.* 52 (3): 1808-1813.

Khoo, H.E., Azlan, A., Tang, S.T., and Lim, S.M., 2017. Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food Nutr. Res.* 61: 1361779.

Kim J.S. and Lee J.H. 2016. Antioxidant and anti-inflammatory activity of phloroglucinol from seaweeds. *Fed. Am. Soc. Exp. Biol.* 30: 1174.12.

Kosai, P., Sirisidthi, K., Jiraungkoorskul, K., and Jiraungkoorskul, W. 2015. Review on ethnomedicinal uses of memory boosting herb, butterfly pea, *Clitoria ternatea*. *J. Nat. Remedies*. 15: 71-76.

Kua, Y.L., Gan, S., Morris A., and Ng, H.K. 2018. Simultaneous recovery of carotenes and tocols from crude palm olein using ethyl lactate and ethanol. *J. Phys.: Conf. Ser.* 989: 012005.

Kua, Y.L., Gan, S., Ng, H.K., and Morris A. 2014. The potential of ethyl lactate as a green solvent to extract carotenoids and vitamin E from crude palm oil. *Proceedings of the 3rd International symposium on processing of foods, vegetables and fruits*, 11-13 August 2014, Kuala Lumpur Teaching Centre, University of Nottingham, Malaysia, pp. 244-251.

Kubola, J. and Siriamornpun, S. 2011. Phytochemicals and antioxidant activity of different fruit fractions (peel, pulp, aril and seed) of Thai gac (*Momordica cochinchinensis* Spreng). *Food Chem.* 127: 1138-1145.

Kumar, S.S. and Giridhar, P. 2017. Stabilization of bioactive betalain pigment from fruits of *Basella rubra* L. through maltodextrin encapsulation. *Madridge J. Food Technol.* 2(1): 73-77.

Kumar, S.S., Manoj, P., and Giridhar, P. 2015. A method for red-violet pigments extraction from fruits of Malabar spinach (*Basella rubra*) with enhanced antioxidant potential under fermentation. *J. Food Sci. Technol*. 52(5):3037-3043.

Kumar, S.N.A., Ritesh, S.K., Sharmila, G. and Muthukumaran, C. 2017. Extraction optimization and characterization of water soluble red purple pigment from floral bracts of *Bougainvillea glabra*. *Arab. J. Chem.* 10: 2145-2150.

Kumar, S.S., Arya, M., Chauhan, A.S., and Giridhar, P. 2020. *Basella rubra* fruit juice betalains as a colorant in food model systems and shelf-life studies to determine their realistic usability. *J. Food Process. and Preserv.* 44(8): 1-9.

Kumkong, N., Banjongsinsiri, P., Laohakunjit, N., Vatanyoopaisarn, S., and Thumthanaruk, B. 2020. Influence of natural colour blends of freeze-dried Gac

aril and pulp on the quality of whey protein-mixed gelatin-based chewables. *Heliyon*. 6: e05817.

Lakshan, S.A.T., Jayanath, N.Y., Abeysekera, W.P.K.M., and Abeysekera, W.K.S.M. 2019. A commercial potential blue pea (*Clitoria ternatea* L.) flower extract incorporated beverage having functional properties. *Evid. Based Complement. Alternat. Med.* 2916914.

Lakshmi, Raju, B.D.P., Madhavi, T., and Sushma, N.J. 2014. Identification of bioactive compounds by FTIR analysis and *in vitro* antioxidant activity of *Clitoria ternatea* leaf and flower extracts. *Ind. Am. J. Pharm. Res.* 4: 3894-3903.

Laqui-Vilca, C., Aguilar-Tuesta, S., Mamani-Navarro, W., Montaño-Bustamante, J., and Condezo-Hoyos, L. 2018. Ultrasound-assisted optimal extraction and thermal stability of betalains from colored quinoa (*Chenopodium quinoa* Willd) hulls. *Ind. Crops Prod.* 111: 606-614.

Lavanya, V., Thamaraiselvi, S. P. and Uma, D. 2019. Studies on extraction of betalain pigments by different solvents and assessing antioxidant activity of *Bougainvillea spectabilis* and *Celosia argentea* flowers. *Madras Agric. J.* 106(1-3): 104-108.

Le, A.V., Huynh, T.T., Parks, S.E., Nguyen, M.H., and Roach, P.D. 2018. Bioactive composition, antioxidant activity, and anticancer potential of freeze-dried extracts from defatted gac (*Momordica cochinchinensis* Spreng) seeds. *Medicines* (Basel). 5(3): E104.

Legendre, P. 2005. Species associations: The Kendall coefficient of concordance revisited. *J. Agric. Biol. Environ. Stat.* 10(2): 226–245.

Li, C., Hu, C., Wang, R., Wang, H., Ma, Q., and Chen, S. 2019. Protective effect of sakuranetin in brain cells of dementia model rats. *Cell Mol. Biol.* 65: 54-58.

Li, H., Deng, Z., Liu, R., Zhu, H., Draves, J., Marcone, M., Sun, Y., and Tsao, R. 2015. Characterization of phenolics, betacyanins and antioxidant activities of the seed, leaf, sprout, flower and stalk extracts of three Amaranthus species. *J. Food Compos. Anal.* 37: 75-81.

Liu, S., Zheng, X., Pan, J., Peng, L., Cheng, C., Wang, X., Zhao, C., Zhang, Z., Lin, Y., XuHan, X., and Lai, Z. 2019. RNA-sequencing analysis reveals betalains metabolism in the leaf of *Amaranthus tricolor* L. *PLoS One*. 14(4): e0216001.

Lobo, F.A.T., Silva, V., Domingues, J., Rodrigues, S., Costa, V., Falcão, D., and Araújo, L.K.G. 2018. Inclusion complexes of yellow bell pepper pigments with β-cyclodextrin: Preparation, characterisation and application as food natural colorant. *J. Sci. Food Agric.* 98: 2665-2671.

Lombardelli, C., Benucci, I., Mazzocchi, C., and Esti, M.A. 2021. Novel process for the recovery of betalains from unsold red beets by low-temperature enzyme-assisted extraction. *Foods.* 10(2): 236.

Lores, M., Pájaro, M., Álvarez-Casas, M., Domínguez, J., and García-Jares, C. 2015. Use of ethyl lactate to extract bioactive compounds from *Cytisus scoparius*: Comparison of pressurized liquid extraction and medium scale ambient temperature systems. *Talanta.* 140: 134-142.

Loypimai, P., Moongngarm, A., and Chottanom, P. 2016. Thermal and pH degradation kinetics of anthocyanins in natural food colorant prepared from black rice bran. *J. Food Sci. Technol.* 53(1): 461-470.

Lozano, J.E. and Ibarz, A. 1997. Colour changes in concentrated fruit pulp during heating at high temperatures. *J. Food Eng.* 31: 365-373.

Ludin, A.A., Al-Alwani, M.A., and Mohamad, A.B. 2018. Utilization of natural dyes from *Zingiber officinale* leaves and *Clitoria ternatea* flowers to prepare new photosensitisers for dye-sensitised solar cells. *Int. J. Electrochem. Sci.* 13(8): 7451-7465.

Luzardo-Ocampo, I., Ramírez-Jiménez, A.K., Yañez, J., Mojica, L., and Luna-Vital, D.A. 2021. Technological applications of natural colorants in food systems: A review. *Foods.* 10: 634.

Mai, D.S. and Tan, L.V. 2013. Study the anthocyanin extraction from the rind of mangosteen (*Garcinia mangostana*) in Vietnam. In: *International Conference on Environment, Energy and Biotechnology*, 51: 28-31.

Manzoor, M.F., Ahmad, N., Ahmed, Z., Siddique, R., Zeng, X., Rahaman, A., Aadil, R.M., and Wahab, A. 2019. Novel extraction techniques and pharmaceutical activities of luteolin and its derivatives. *J. Food Biochem*. 43(9): e12974.

Marpaung, A.M., Andarwulan, N., Hariyadi, P., and Faridah, D.N. 2017. The colour degradation of anthocyanin-rich extract from butterfly pea (*Clitoria ternatea* L.) petal in various solvents at pH 7. *Nat. Prod. Res.* 31: 2273-2280.

Mazza, G. and Miniati, E. 1993. Introduction. In: Mazza, G. and Miniati, E. (eds), *Anthocyanins in Fruits, Vegetables, and Grains.* CRC Press, Boca Raton, pp. 1-28.

Megawati, D.S., Fardhyanti, D., Widjanarko, Hanifah, G.M., Bungsu' and Rizky, M.H.F. 2020. Kinetics of soursop leaves antioxidant extraction using microwave-assisted extraction. *IOP Conf. Ser.: Earth Environ. Sci.* 572(1): 012041.

Mehmood, A., Ishaq, M., Zhao, L., Yaqoob, S., Safdar, B., Nadeem, M., Munir, M., and Wang, C. 2019. Impact of ultrasound and conventional extraction techniques on bioactive compounds and biological activities of blue butterfly pea flower (*Clitoria ternatea* L.). *Ultrason. Sonochem.* 51: 12-19.

Mordente, A., Guantario, B., and Meucci, E. 2011. Lycopene and cardiovascular diseases: An update. *Curr. Med. Chem*. 18: 1146-1163.

Mortensen, A. 2006. Carotenoids and other pigments as natural colorants. *Pure Appl. Chem.* 78(8): 1477-1491.

Mozetic, B., Trebse, P., Simcic, M., and Hribar, J. 2004. Changes of anthocyanins and hydroxycinnamic acids affecting the skin colour during maturation of sweet cherries (*Prunus avium* L.). *LWT Food Sci. Technol.* 37: 123-128.

Mukherjee, P.K., Kumar, V., Kumar, N.S., and Heinrich M. 2008. The Ayurvedic medicine *Clitoria ternatea* - From traditional use to scientific assessment. *J. Ethnopharmacol*. 120: 291-301.

Myojin C., Enami, N., Nagata, A., Yamaguchi, T., Takamura, H., and Matoba, T. 2008. Changes in the radical-scavenging activity of bitter gourd (*Momordica charantia* L.) during freezing and frozen storage with or without blanching. *J. Food Sci.* 73: 546-550.

Natesh, N.H., Ijenyo, M.O., Asiedu, S.K., Vasantha Rupasinghe, H.P., and Abbey, L. 2021. Plant growth and nutritional quality attributes of *Basella alba* applied with variable rates of nitrogen fertilizer at different planting dates under Canadian maritime climatic conditions. *Int. J. Agron.* 2021: 1-11.

Natnoi, S. and Pirak, T. 2019. Effect of ultrasonic-assisted extraction on the properties, antioxidant and inflammatory activities of carotenoids from gac (*Momordica cochinchinensis*) fruit pericarp. *Cogent Food Agric.* 5: 1696512.

Neagu, C. and Barbu, V. 2014. Principal component analysis of the factors involved in the extraction of beetroot betalains. *J. Agroaliment. Processes Technol*. 20(4): 311-318.

Neda, G.D., Rabeta, M.S., and Ong, M.T. 2013. Chemical composition and anti-proliferative properties of flowers of *Clitoria ternatea*. *Int. Food. Res. J*. 20: 1229-1234.

Ngamwonglumlert, L., Devahastin, S., and Chiewchan, N. 2017. Natural colourants: Pigment stability and extraction yield enhancement via utilization of appropriate pretreatment and extraction methods. *Crit. Rev. Food Sci. Nutr.* 57(15): 3243-3259.

Nie, J., Chen, D., Ye, J., Lu, Y., and Dai, Z. 2021. Optimization and kinetic modeling of ultrasonic-assisted extraction of fucoxanthin from edible brown algae *Sargassum fusiforme* using green solvents. *Ultrason. Sonochem.* 77: 105671.

Nisha, R.B. and Narayanan, R. 2020. Effect of different solvents on recovery of total monomeric anthocyanin from mangosteen peel (*Garcinia mangostana* L.). *Int. J. Chem. Stud.* 8(1): 9-11.

Nithianantham, K., Ping, K.Y., and Latha, L.Y. 2013. Evaluation of hepatoprotective effect of methanolic extract of *Clitoria ternatea* (Linn.) flower against acetaminophen-induced liver damage. *Asian Pac. J. Trop. Dis*. 3: 314-319.

Nour, V., Stampar, F., Veberic, R. and Jakopic, J. 2013. Anthocyanins profile, total phenolics and antioxidant activity of black currant ethanolic extracts as

influenced by genotype and ethanol concentration. *Food Chem.* 141(2): 961-966.

Oancea S., Stoia M., and Coman D. 2012. Effects of extraction conditions on bioactive anthocyanin content of *Vaccinium Corymbosum* in the perspective of food applications. *Procedia Eng.* 42**:** 489-495.

Oancea, S., Moiseenco, F., and Traldi, P. 2013. Total phenolic and anthocyanin profile of Romanian wild and cultivated blueberries by direct infusion ESI-IT-MS/MS. *Rom. Biotechnol. Lett*. 18(3): 8350-8360.

Odriozola-Serrano, I., Soliva-Fortuny, R., Hernández-Jover, T., and Martín-Belloso, O., 2009. Carotenoid and phenolic profile of tomato juices processed by high intensity pulsed electric fields compared with conventional thermal treatments. *Food Chem.* 112: 258-266.

Oplatowska-Stachowiak, M. and Elliott, C.T. 2015. Food colours: Existing and emerging food safety concerns. *Crit. Rev. Food Sci. Nutr.* 57(3): 524-548.

Orozco-Villafuerte, J., Escobar-Rojas, A., Buendía-González, L., García-Morales, C., Hernandez-Jaimes, C., and Alvarez-Ramirez, J. 2019. Evaluation of the protection and release rate of bougainvillea (*Bougainvillea spectabilis*) extracts encapsulated in alginate beads. *J. Dispers. Sci. Technol*. 40(7): 1065-1074.

Otálora, M.C., Barbosa, H.J, Perilla, J.E., Osorio, C., and Nazareno, M.A. 2019. Encapsulated betalains (*Opuntia ficus-indica*) as natural colorants Case study: Gummy candies. *LWT Food Sci. Technol.* 103: 222-227.

Othman A., Ismail A., Ghani N.A., and Adenan I. 2007. Antioxidant capacity and phenolic content of cocoa beans. *Food Chem.* 100: 1523-1530.

Paciulli, M., Medina-Meza, I.G., Chiavaro, E., and Barbosa-Cánovas, G.V. 2016. Impact of thermal and high pressure processing on quality parameters of beetroot (*Beta vulgaris* L.) *LWT Food Sci. Technol.* 68: 98-104.

Palapol, Y., Ketsa, S., Stevenson, D., Cooney, J.M., Allan, A.C., and Ferguson, I.B. 2009. Colour development and quality of mangosteen (*Garcinia mangostana* L.) fruit during ripening and after harvest. *Postharvest Biol. Technol.* 51: 349-353.

Pandey, G., Pandey, V., Pandey P.R., and Thomas, G. 2018. Effect of extraction solvent temperature on betalain content, phenolic content, antioxidant activity and stability of beetroot (*Beta vulgaris* L.) powder under different storage conditions. *Plant Arch.* 18(2): 1623-1627.

Pandya, D., Akbari, S., and Bhatt, H. 2017. Standardization of solvent extraction process for Lycopene extraction from tomato pomace. *J. Appl. Biotechnol. Bioeng*. 2(1): 12-16.

Panse, V.G. and Sukhatme, P.V., 1985. Statistical methods for agricultural workers. Indian Council of Agricultural Research publication, New Delhi. 87-89.

Pasqueta, V., Cherouvrier, J.R., Firas, F., and Picot, L. 2011. Study on the microalgal pigments extraction process: Performance of microwave assisted extraction. *Process. Biochem.* 46: 59-67.

Pataro, G., Carullo, D., Falcone, M., and Ferrari, G. 2020. Recovery of lycopene from industrially derived tomato processing by-products by pulsed electric fields-assisted extraction. *Innov. Food Sci. Emerg. Technol*. 63: 102369.

Pawar, N., Shinde, M., and Junna, L. 2018. Stabilization of food colourant and antimicrobial activity in fruit extracts of *Basella rubra*. L. *Int. J. Pharmacogn. Phytochem. Res.* 10(1): 43-47.

Pazmiño-Durán, A.E., Giusti, M.M., Wrolstad, R.E., and Glória, M.B.A. 2001. Anthocyanins from *Oxalis triangularis* as potential food colorants. *Food Chem.* 75: 211-216.

Pereira, F.S., Agostini D.L.S., Santo, R.D.E., deAzevedo, E.R., Bonagamba, T.J., Job, A.E., and Gonzalez, E.R.P. 2011. A comparative solid state ^{13}C NMR and thermal study of CO_2 capture by amidines PMDBD and DBN. *Green Chem*. 13(8): 2146-2153.

Pham, T.N., Nguyen, D.C., Lam, T.D., Van Thinh, P., Le, X.T., Nguyen, and D.V.V. 2019. Extraction of anthocyanins from Butterfly pea (*Clitoria ternatea* L. Flowers) in Southern Vietnam: Response surface modeling for optimization of the operation conditions. In: *Proceedings of the 2018 the 6th International Conference on Mechanical Engineering, IOP Conference Series: Materials Science and Civil Engineering*. Iop Publishing Ltd., Bristol.

Philip, T. and Berry, J.W. 1975. Nature of lutein acylation in marigold (*Tagetes erecta*) flowers. *J. Food Sci.* 40(5): 1089-1090.

Prado, L.A.S., Shen, Y., Ardoin. R., Osorio, L.F., Cardona, J., Xu, Z., and Prinyawiwatkul, W. 2019. Effects of different solvents on total phenolic and total anthocyanin contents of *Clitoria ternatea* L. petal and their anti-cholesterol oxidation capabilities. *Int. J. Food. Sci. Technol.* 54: 424-431.

Prakash-Maran, J., Manikandan, S., and Mekala, V. 2013. Modeling and optimization of betalain extraction from *Opuntia ficus-indica* using Box-Behnken design with desirability function. *Ind. Crops Prod.* 49: 304-311.

Prieto-Santiago, V., Cavia, M.M., Alonso-Torre, S.R., and Carrillo, C. 2020. Relationship between color and betalain content in different thermally treated beetroot products. *J. Food Sci. Technol.* 57(9): 3305-3313.

Putra, T.N.M., Zainol, M.K., MohdIsa, N.S., and MohdMaidin, N. 2021. Chemical characterization of ethanolic extract of Butterfly pea flower (*Clitoria ternatea*). *Food Res.* 5(4): 127-134.

Rabeta, M.S. and An Nabil, Z.A. 2013. Total phenolic compounds and scavenging activity in *Clitoria ternatea* and *Vitex negundo* Linn. *Int. Food Res. J.* 20(1): 495-500.

Rafi, M.M., Yadav, P.N., and Reyes, M. 2007. Lycopene inhibits LPS-induced proinflammatory mediator inducible nitric oxide synthase in mouse macrophage cells. *J. Food Sci.* **72**: 69-74.

Ramli, N.S., Ismail, P., and Rahmat, A. 2014. Influence of conventional and ultrasonic-assisted extraction on phenolic contents, betacyanin contents, and antioxidant capacity of red dragon fruit (*Hylocereus polyrhizus*). *Sci. World J.* 2014: 1-7.

Ranneh, Y., Ali, F., Zarei, M., Akim, A.M., Hamid, H.A., and Khazaai, H. 2018. Malaysian stingless bee and Tualang honeys: A comparative characterization of total antioxidant capacity and phenolic profile using liquid chromatography-mass spectrometry. *LWT Food Sci. Technol.* 89: 1-9.

Rashid, M.T., Hashim, M.M., Wali, A., Guo, L., Jian, X., and Ma, H. 2018. Effect of storage on physicochemical, microbial analysis, and sensory characteristics of diet guava squash. *J. Food Safety Food Qual.* 69(1):19-25.

Rastogi, A. and Shukla, S. 2013. Amaranth: A new millennium crop of nutraceutical values. *Crit. Rev. Food Sci. Nutr.* 53(2): 109-125.

Reddy, C.V.K., Sreeramulu, D., and Raghunath, M., 2010. Antioxidant activity of fresh and dry fruits commonly consumed in India. *Int. Food Res. J.* 43(1): 285-288.

Ren S., Lei H., Wang L., Bu Q., Chen S., Wu J., Julson J., and Ruan R. 2012. Biofuel production and kinetics analysis for microwave pyrolysis of Douglas fir sawdust pellet. *J. Anal. Appl. Pyrolysis.* 94: 163-169.

Reshmi, S.K., Aravindhan, K.M., and Suganya Devi, P. 2012. Antioxidant analysis of betacyanin extracted from *Basella alba* fruit. *Int. J. Pharmtech Res.* 4: 900-913.

Ribeiro, H.S., Ax, K., and Schubert, H. 2003. Stability of lycopene emulsions in food systems. *J. Food Sci.* 68(9): 2730-2734.

Rocha, R., Pinela, J., Abreu, R.M.V., Añibarro-Ortega, M., Pires, T.C.S.P., and Saldanha, A.L. 2020. Extraction of anthocyanins from red raspberry for natural food colorants development: processes optimization and *in vitro* bioactivity. *Process.* 8: 1447.

Rodriguez-Amaya, D.B. 1997. Carotenoids and food preservation: The retention of pro-vitamin A carotenoid in prepared, processed and storage food. *Office of Health and Nutrition, US Agency for International Development.* Washington, DC.

Rodriguez-Amaya, D.B. 2017. Natural food pigments and colorants. In: Mérillon, J.M. and Ramawat, K. (eds), *Bioactive molecules in food*. Springer, Cham, pp. 1-35.

Rodríguez-Sánchez, J.A., Cruz M.T., Victoria, Y., and Barragán-Huerta, B.E. 2017. Betaxanthins and antioxidant capacity in *Stenocereus pruinosus*: Stability and use in food. *Int. Food Res. J.* 91: 63-71.

Roobab, U., Aadil, R.M., Madni, G.M., and Bekhit, A.E.D. 2018. The impact of nonthermal technologies on the microbiological quality of juices: A review. *Compr. Rev. Food Sci. Food Saf.* 17:437-457.

Saadedin, S., Al-Zaidi, I.H.M., and Al-Awadi, S.J.A. 2017. Solvents extraction efficiency for lycopene and β-carotene of gac fruit (*Momordica Cochinchinensis* Spreng) cultivated in Iraq. *Biosci. Res.* 14(4): 788-800.

Saini, R.K. and Keum, Y.S. 2018. Carotenoid extraction methods: A review of recent developments. *Food Chem.* 240: 90-103.

Sanchez-Gonzalez, N., Jaime-Fonseca, M.R., Martin-Martinez, E.S., and Zepeda, L.G. 2013. Extraction, stability, and separation of betalains from *Opuntia joconostle* cv. using response surface methodology. *J. Agric. Food Chem.* 61(49): 11995-12004.

Sánchez-Moreno, C., Plaza, L., De Ancos, B., and Cano, P., 2003. Vitamin C, pro-vitamin A carotenoid: and other carotenoids in high-pressurized orange juice during refrigerated storage. *J. Agric. Food Chem.* 51: 647-653.

Sapkale, G.N., Patil, S.M., Surwase, U.S., and Bhatbhage, P.K. 2010. A Review: Super-critical fluid extraction. *Int. J. Chem. Sci*. 8(2): 729-743.

Schieber, A. and Carle, R. 2005. Occurrence of carotenoid *cis*-isomers in food: Technological, analytical, and nutritional implications. *Trends Food Sci. Technol.* 16: 416-422.

Shen, Y., Ardoin, R., Osorio, L.F., Cardona, J., Prado, L.A.S., and Osorio, L.F. 2019. Effects of different solvents on total phenolic and total anthocyanin contents of *Clitoria ternatea* L. petal and their anti-cholesterol oxidation capabilities. *Int. J. Food Sci. Technol.* 54: 424-431.

Shen, Y., Du, L., Zeng, H., Zhang, X., Prinyawiwatkul, W., Alonso-Marenco, J.R., and Xu, Z. 2016. Butterfly pea (*Clitoria ternatea*) seed and petal extracts decreased *Hep-2* carcinoma cell viability. *Int. J. Food Sci. Technol.* 51: 1860-1868.

Sigwela, V., De Wit, M., du Toit, A., Osthoff, G., and Hugo, A. 2021. Bioactive betalain extracts from cactus pear fruit pulp, beetroot tubers, and amaranth leaves. *Molecules.* 26(16): 5012.

Singh, K., Kumar, P., and Singh, N.V. 2020. Natural dyes: An emerging ecofriendly solution for textile industries. *Pollut. Res.* 39: 80-86.

Sinha, K., Saha, P.D., and Ramya, V. 2012. Improved extraction of natural blue dye from butterfly pea using microwave assisted methodology to reduce the effect of synthetic blue dye. *Int. J. Chem. Technol.* 4: 57-65.

Siriamornpun, S., Kaisoon, O., and Meeso, N. 2012. Changes in colour, antioxidant activities and carotenoids (lycopene, β-carotene, lutein) of marigold flower (*Tagetes erecta* L.) resulting from different drying processes. *J. Funct. Foods.* 4: 757-766.

Sivakumar, V., Vijaeeswarri, J., and Lakshmi, A.J. 2011. Effective natural dye extraction from different plant materials using ultrasound. *Ind. Crops Prod.* 33(1): 116-122.

Sivaranjan, V.V. and Balachandran, I. 1994. Ayurvedic drugs and their plant sources. Oxford and IBH Publishers Pvt., Ltd., New Delhi.

Skrede, G., Wrolstad, R., and Durst, R. 2000. Changes in anthocyanins and polyphenolics during juice processing of highbush blueberries *(Vaccinium corymbosum* L.). *J. Food Sci.* 65: 357-364.

Slimen, I.B., Najar, T., and Abderrabba, M. 2017. Chemical and antioxidant properties of betalains. *J. Agric. Food Chem.* 65: 675-689.

Srichaikul, B. 2018. Ultrasonication extraction, bioactivity, antioxidant activity, total flavonoid, total phenolic and antioxidant of *Clitoria ternatea* Linn. flower extract for anti-aging drinks. *Pharmacogn. Mag.* 14(56): 322-327.

Stintzing, F.C. and Carle, R. 2004. Functional properties of anthocyanins and betalains in plants, food and in human nutrition. *Trends Food Sci. Technol.* 15: 19-38.

Stintzing, F.C. and Carle, R. 2007. Betalains – emerging prospects for food scientists *Trends Food Sci. Technol.* 18(10): 514-525.

Stone, H., Bleibaum, R.N., and Thomas, H.A. 2020. Sensory evaluation practices (5th Ed.). Academic Press, pp. 79-116.

Strack, D., Vogt, T., and Schliemann, W. 2003. Recent advances in betalain research. *J. Phytochem*. 62: 247-269.

Strati, I.F. and Oreopoulou, V. 2011. Effect of extraction parameters on the carotenoid recovery from tomato waste, *Int. J. Food Sci. Tech.* 46: 23-29.

Subramanian, M.S. and Prathyusha, P. 2011. Pharmaco-phytochemical characterization of *Clitoria ternatea* Linn. *Int. J. Pharmtech. Res.* 3: 606-612.

Sumarny, R., Sofiah, S., Nurhidayati, L., and Fatimah. 2014. Antioxidant activity of mangosteen (*Garcinia mangostana* L.) fruit rind extract in oral solution dosage form. In: *International Symposium on Medicinal Plants & Traditional Medicine*, Tawangmangu, Central Java Indonesia.

Sutor-Świeży, K., Antonik, M., Dziedzic, E., Bieniasz, M., Mielczarek, P., Popenda, Ł., Pasternak, K., Tyszka-Czochara, M., and Wybraniec, S. 2022. Structural studies on diverse betacyanin classes in matured pigment-rich fruits of *Basella alba* L. and *Basella alba* L. var. '*Rubra*' (Malabar Spinach). *Int. J. Mol. Sci*. 23(19):11243.

Suttirak, W. and Manurakchinakorn, S. 2012. *In vitro* antioxidant properties of mangosteen peel extract. *J. Food. Sci. Technol*. 12: 3546-3558.

Szabo, K., Teleky, B.E., Ranga, F., Roman, I., Khaoula, H., Boudaya, E., Ltaief, A.B., Aouani,W., Thiamrat, M., and Vodnar, D.C. 2022. Carotenoid recovery from tomato processing by-products through green chemistry. *Molecules*. 27: 3771.

Tabio-García, D., Paraguay-Delgado, F., Sánchez-Madrigal, M.Á., Quintero-Ramos, A., Espinoza-Hicks, J.C., Meléndez-Pizarro, C.O., Ruiz-Gutiérrez, M.G., and Espitia-Rangel, E. 2021. Optimisation of the ultrasound-assisted extraction of betalains and polyphenols from *Amaranthus hypochondriacus* var. Nutrisol. *Ultrason. Sonochem.* 77: 105680.

Tan, J.B.L. and Lim, Y.Y. 2015. Critical analysis of current methods for assessing the *in vitro* antioxidant and antibacterial activity of plant extracts. *Food Chem.* 172: 814-822.

Terahara, N., Saito, N., Honda, T., Toki, K., and Osajima, Y. 1990. Structure of ternatin A_1, the largest ternatin in the major blue anthocyanins from *Clitoria ternatea* flowers. *Tetrahedron Lett.* 31(20): 2921-2924.

Terahara, N., Toki, K., and Saito, N. 1998. Eight new anthocyanins, ternatins C1–C5 and D3 and preternatins A3 and C4 from young *Clitoria ternatea* flowers. *J. Nat.* 61:1361-1367.

Tesoriere, L., Allegra, M., Gentile, C., and Livrea, M.A. 2009. Betacyanins as phenol antioxidants: Chemistry and mechanistic aspects of the lipoperoxyl radical-scavenging activity in solution and liposomes. *Free Radical Res.* 43(8): 706-717.

Thiyajai, P. and Koyama, T. 2022. Binary ethanol-water solvents affect betalain contents and health-promoting properties of red *Celosia argentea* inflorescence extracts. *Int. Food Res. J.* 29(1): 67-77.

Thuy, N.M., Ben, T.C., Minh, V.Q., and Tai, N.V. 2021. Effect of extraction techniques on anthocyanin from butterfly pea flowers (*Clitoria ternatea* L.) cultivated in Vietnam. *J. Appl. Biol. Biotechnol.* 9(6): 173-180.

Tinrat, S., Akkarachaneeyakorn, S., and Singhapol, C. 2014. Evaluation of antioxidant and antimicrobial activities of *Momordica Cochinchinensis* Spreng (Gac fruit) ethanolic extract. *Int. J. Pharm. Sci.* 5(8): 3163.

Tran, X.T., Parks, S.E., Roach, P.D., Golding, J.B., and Nguyen, M.H. 2016. Effects of maturity on physicochemical properties of Gac fruit (*Momordica cochinchinensis* Spreng.). *Food Sci. Nutr.* 4(2): 305-314.

Truong D.H., Nguyen D.H., Ta N.T., Bui A.V., Do T.H., and Nguyen H.C. 2019. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of *Severinia buxifolia*. *J. Food Qual.* 2019: 8178294.

Tsai, P., McIntosh, J., Pearce, P., Camden, B., and Jordon, B.R. 2002. Anthocyanin and antioxidant capacity in Roselle (*Hibiscus Sabdariffa*) extract. *Food Res. Int.* 35(4): 351-356.

Venkatesan, T., Choi, Y.W., and Kim, Y.K. 2019. Impact of different extraction solvents on phenolic content and antioxidant potential of *Pinus densiflora* bark extract. *Biomed. Res. Int.* 3520675.

Verma, K., Manorama, and Pophaly, S.D. 2018. Natural food colours. *Plant Arch.* 18(1): 1159-1162.

Vieira, D.A.D., Caliari, M., Souza, E.R.B., and Soares, M.S.Jr. 2020. Methods for and pigments extraction and determination of color in tomato for processing cultivars. *Food Sci. Technol.* 40(1): 11-17.

Vuong, L.T., Franke, A.A., Custer, L.J., and Murphy, S.P. 2006. *Momordica cochinchinensis* Spreng. (gac) fruit carotenoids reevaluated. *J. Food Compos. Anal.* 19(6-7): 664-668.

Wang, H., Cao, G., and Prior, R.L. 1997. Oxygen radical absorbing capacity of anthocyanins. *J. Agric. Food Chem.* 45(2): 304–309.

Wang, Z., Li, S., Ge, S., and Lin, S. 2020. Review of distribution, extraction methods, and health benefits of bound phenolics in food plants. *J. Agric. Food Chem.* 68(11): 3330-3343.

Wani, S.A., Bishnoi, S., and Kumar, P. 2016. Ultrasound and microwave assisted extraction of diosgenin from fenugreek seed and fenugreek-supplemented cookies. *J. Food Meas. Charact.* 10: 527-532.

Waszkowiak, K. and Gliszczyńska-Świgło, A. 2016. Binary ethanol-water solvents affect phenolic profile and antioxidant capacity of flaxseed extracts. *Eur. Food Res. Technol.* 242(5): 777-786.

Wen, C., Zhang, J., and Zhang, H. 2018. Advances in ultrasound assisted extraction of bioactive compounds from cash crops: A review. *Ultrason. Sonochem*. 48: 538-549.

Wrolstad, R.E., Durst, R.W., and Lee, J. 2005. Tracking color and pigment changes in anthocyanin products. *Trends Food Sci. Technol.* 16: 423-428.

Wu, W., Lu, M., and Yu, L. 2011. A new environmentally friendly method for astaxanthin extraction from *Xanthophyllomyces dendrorhous*. *Eur. Food Res. Technol.* 232(3): 463-467.

Wu, X., Beecher, G.R., Holden, J.M., Haytowitz, D.B., Gebhardt, S.E., and Prior, R.L. 2006. Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. *J. Agric. Food Chem.* 54: 4069-4075.

Xianquan, S., Shi, J., Kakuda, Y., and Yueming, J. 2005. Stability of lycopene during food processing and storage. *J. Med. Food.* 8(4): 413-422.

Yadav, A., Chandra, S., Singh J., and Kumar, V. 2013. Effect of storage conditions on physico-chemical, microbial and sensory quality of ready-to-serve banana beverage. *Madras Agric. J.* 100(1-3): 747-750.

Yang, J., Paulino, R., Janke-Stedronsky, S., and Abawi, F. 2007. Free-radical-scavenging activity and total phenols of noni (*Morinda citrifolia* L.) juice and powder in processing storage. *Food Chem.* 102: 302-308.

Yong M., Ning H., and Liu H., 2006. Exploitation and composition of pumpkin powder. *Food Sci. Technol.* 6: 299-301.

Yoshida, K., Mori, M., and Kondo, T. 2009. Blue flower colour development by anthocyanins: From chemical structure to cell physiology. *Nat. Prod. Rep.* 26: 884-915.

Zadernowski R., Czaplicki, S., and Naczk, M. 2009. Phenolic acid profiles of mangosteen fruits (*Garcinia mangostana*). *Food Chem*. 112(3): 685-689.

Zakynthinos, G. and Varzakas, T. 2016. Carotenoids: From plants to food industry. *Curr. Res. Nutr. Food Sci.* 4: 38-51.

Zang, Z., Chou, S., Tian, J., Lang, Y., Shen, Y., Ran, X., Gao, N., and Li, B. 2021. Effect of whey protein isolate on the stability and antioxidant capacity of blueberry anthocyanins: A mechanistic and in vitro simulation study. *Food Chem.* 336: 127700.

Zhou, H.Y. and Liu, C.Z. 2006. Microwave-assisted extraction of solanesol from tobacco leaves. *J. Chromatogr. A*. 1129: 135-139.

Zhu, D., Ji, B. Eum, H. L., and Zude, M. 2009. Evaluation of non enzymatic browning in thermally processed apple juice by front face fluorescence spectroscopy. *Food Chem*. 133: 272-279**.**

Zia, S., Khan, M.R., Shabbir, M.A., Maan, A.A., Khan, M.K., Nadeem, M., Khalil, A.A., Din, A., and Aadil, R.M. 2020. An inclusive overview of advanced thermal and nonthermal extraction techniques for bioactive compounds in food and food-related matrices. *Food Rev. Int.* 38: 1166-1196.

Zielinski, A.A.F., Haminiuk, C.W.I., Alberti, A., Nogueira, A., Demiate, I.M., and Granato, D. 2014. A comparative study of the phenolic compounds and the *in vitro* antioxidant activity of different Brazilian teas using multivariate statistical techniques. *Int. Food Res. J.* 60: 246-254.

Zin, M.M., Anucha, C.B., and Bánvölgyi, S. 2020a. Recovery of phytochemicals via electromagnetic irradiation (microwave-assisted-extraction): Betalain and phenolic compounds in perspective. *Foods*. 9(7): 918.

Zin, M.M., Márki, E., and Bánvölgyi, S. 2020b. Conventional extraction of betalain compounds from beetroot peels with aqueous ethanol solvent, *Acta Aliment.* 49(2): 163-169.

Zorić, Z., Pelaić, Z., Pedisić, S., Garofulić, I.E., Kovačević, D.B., and Dragović–Uzelac, V. 2017. Effect of storage conditions on phenolic content and antioxidant capacity of spray dried sour cherry powder. *LWT Food Sci. Technol.* 79: 251-259.

Appendices

APPENDIX – I

Score card for sensory evaluation of pigmented guava squash

Name of the judge:

Date:

Characteristics	Scores					
	Ambient condition			Refrigerated condition		
	T_1	T_2	T_3	T_1	T_2	T_3
Appearance						
Colour						
Flavour						
Body and consistency						
After-taste						
Overall acceptability						

9-Hedonic scale	
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Signature

APPENDIX – II

Score card for sensory evaluation of pigmented guava jelly

Name of the judge:

Date:

Characteristics	Scores					
	Ambient condition			Refrigerated condition		
	T_1	T_2	T_3	T_1	T_2	T_3
Visual appearance and transparency						
Colour						
Flavour						
After-taste						
Overall acceptability						

9-Hedonic scale	
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Signature

APPENDIX – III

Score card for sensory evaluation of pigmented ash gourd candy

Name of the judge:

Date:

Characteristics	Scores					
	Ambient condition			Refrigerated condition		
	T_1	T_2	T_3	T_1	T_2	T_3
Visual appearance						
Colour						
Flavour						
Texture						
After-taste						
Overall acceptability						

9-Hedonic scale	
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Signature

EVALUATION AND UTILIZATION OF PLANT PIGMENTS AS NATURAL FOOD COLOURANTS

By

NETRAVATI

(2019-22-020)

ABSTRACT OF THE THESIS

Submitted in partial fulfillment of the requirement for the degree of

Doctor of Philosophy in Horticulture

Faculty of Agriculture

Kerala Agricultural University, Thrissur

DEPARTMENT OF POSTHARVEST MANAGEMENT

COLLEGE OF AGRICULTURE

VELLANIKKARA, THRISSUR – 680 656

KERALA, INDIA

2023

ABSTRACT

Employing natural colours is the current marketing trend because of consumers' concern about the safety of artificial food dyes, reinforced by possible health benefits of the natural pigments. Replacement of synthetics with natural colourants, however, is challenging because natural colourants are usually less stable, more costly, not as easily utilized as artificial colours, requires more material to achieve equivalent colour strength and have limited range of hues. With this background, the present study was formulated to evaluate the efficacy of different eco-friendly solvents in the extraction of anthocyanin, betalain and carotenoid pigments from plant sources and subsequently, application of the extracted pigment in different categories of food products to study their stability with the title "Evaluation and utilization of plant pigments as natural food colourants" which was carried out at Department of Postharvest Management, College of Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur, Kerala during 2019-22. The main objectives of the study were to standardize the process for extraction of plant pigments from selected fruits (mangosteen), vegetables (red amaranthus, basella, gac fruit), and flowers (butterfly pea, marigold) and to assess application of extracted pigments in food products (guava squash, guava jelly and ash gourd candy).

The present study was focused on the extraction of anthocyanin, betalain and carotenoid pigments. Two crops for each pigment group were taken *viz.*, butterfly pea flowers and mangosteen fruit rind for anthocyanin pigment; basella berries and red amaranthus leaves for betalain pigment; gac fruit seed aril and marigold flower petals for carotenoid pigment.

Clitoria ternatea L. produces edible flowers that are rich sources of brilliant blue-coloured anthocyanins called "ternatins". Among the different extraction methods used, acidified aqueous extraction method gave significantly higher recovery percentage of pigment concentrate (74.03±0.84 %) and aqueous extraction method resulted in higher total monomeric anthocyanin content (TMAC) (7925.29±36.07 mg/L), whereas instrumental colour values indicating brilliant-blue colour *viz.., a** (46.90±0.77), *b** (-63.25±0.13), *chroma* (78.74±0.38) and *hue angle* (306.56±0.50),

higher antioxidant activity *viz.,* DPPH (3.49±0.59 µl/ml), FRAP (3.99±1.10 µl/ml) and ABTS ((2.42±0.01 µl/ml), total phenolics (29.78±1.79 mg GAE/100 g), total flavonoids (20.13±0.40 mg QE/100 g) were recorded in microwave assisted extraction (MAE) method with aqueous medium.

The inedible dark purple rind of mangosteen (*Garcinia mangostana* L.) fruit is a rich source of anthocyanin pigment that can be used for food application if extracted by using eco-friendly solvents. Microwave assisted extraction method with acidified solvent recorded significantly higher recovery percentage of pigment concentrate (55.03±2.98 %), instrumental colour values indicating purple-red colour *viz.., L** (3.17±0.38), *a** (59.53±0.85), *b** (5.09±0.52), *chroma* (60.80±1.00) and *hue angle* (28.06±0.33°), higher antioxidant activity *viz.,* DPPH (2.29±0.24 µl/ml), FRAP (2.83±0.21 µl/ml) and ABTS (2.02±0.03 µl/ml), total phenolics (32.25±0.30 mg GAE/100 g) and total flavonoids (40.02±3.52 mg QE/100 g), whereas acidified solvent extraction method resulted in higher TMAC (17652.54±139.47 mg/L).

Basella (*Basella alba* var. *rubra* L.) berries with dark purple skin and deep red violet flesh are potential sources of betalain. Among the different extraction methods used, MAE (50 % ethanol) method recorded significantly higher betacyanin (605.83±4.10 mg/g), betaxanthin content (86.35±1.67 mg/g) and total betalain content (692.18±2.52 mg/g), instrumental colour values *viz.., a** (52.27±0.24), *b** (23.24±0.16), *chroma* (58.75±0.19) and *hue angle* (27.40±0.17), higher antioxidant activity *viz.,* DPPH (3.40±0.04 µl/ml), FRAP (3.69±0.19 µl/ml) and ABTS (2.99±0.04 µl/ml), total phenolics (211.37±0.29 mg GAE/100 g) and total flavonoids (124.07±2.53 mg QE/100 g), whereas higher recovery percentage of pigment concentrate (53.13±0.41 %) was recorded in acidified aqueous extraction method (1 % citric acid).

Red amaranthus (*Amaranthus tricolor* L.) plants contain large amounts of betalain, including betaxanthin and betacyanin along with flavonoids, alkaloids, and other elements. Among the different extraction methods used, MAE (50 % ethanol) method recorded significantly higher betacyanin (601.15±2.25 mg/g), betaxanthin content (75.63±0.55 mg/g) and total betalain content (676.78±2.79 mg/g),

instrumental colour values *viz.., L** (41.94±0.06), *a** (33.37±0.22), *chroma* (63.37±0.17) and *hue angle* (58.41±0.02), higher antioxidant activity *viz.,* DPPH (1.34±0.01 µl/ml), FRAP (3.69±0.19 µl/ml) and ABTS (0.81±0.01 µl/ml), total phenolics (190.03±0.22 mg GAE/100 g) and total flavonoids (179.07±0.49 mg QE/100 g), whereas higher recovery percentage of pigment concentrate (58.73±0.43 %) was recorded in acidified aqueous extraction method (1 % citric acid).

Gac fruit (*Momordica cochinchinensis* Spreng) seed aril has been reported as a rich source of bioactive compounds which include carotenoid, phenolic and flavonoid compounds. Among the different extraction methods used, ethyl lactate extraction method gave significantly higher total carotenoid content (2069.83±4.38 µg/g), beta-carotene (689.43±1.04 µg/g), lycopene (1052.31±0.87 µg/g) and lutein content (216.96±0.22 µg/g), instrumental colour values *viz.., L** (60.44±0.12), *a** (61.35±0.20), *b** (43.24±0.10), *chroma* (65.62±0.10) and *hue angle* (36.64±0.18), higher antioxidant activity *viz.,* DPPH (0.59±0.05 µl/ml), FRAP (0.63±0.004 µl/ml), ABTS (0.58±0.005 µl/ml) and total phenolics (211.57±0.13 mg GAE/100 g), whereas higher recovery percentage of pigment concentrate (89.53±0.13 %) was recorded in MAE with ethanol extraction method.

Marigold (*Tagates erecta* L.) flowers are the most concentrated common sources of carotenoid, with lutein, a dihydroxylated compound, accounting for 85 per cent of the total carotenoid present in the flower and are one of the most important sources of carotenoid for application in the food industry. Among the different extraction methods used, ethyl lactate extraction method recorded significantly higher recovery percentage of pigment concentrate (93.33±0.27 %), total carotenoid content (2276.93±3.61 µg/g), beta-carotene (491.03±0.85 µg/g), lycopene (236.96±0.10 µg/g) and lutein content (1310.02±0.27 µg/g), higher antioxidant activity *viz.,* DPPH (0.355±0.03 µl/ml), FRAP (0.557±0.003 µl/ml), ABTS (0.201±0.002 µl/ml) and total phenolics (337.61±0.23 mg GAE/100 g), whereas higher instrumental colour values *viz., L** (70.20±0.12), *a** (10.48±0.14), *b** (76.49±0.15) and *hue angle* (82.44±0.19) were recorded in ethanol extraction method.

Anthocyanin, betalain and carotenoid pigments' stability in processed products *viz.,* guava squash, guava jelly and ash gourd candy coloured with anthocyanin, betalain and carotenoid pigments were evaluated for a period of three months under ambient and refrigerated storage conditions. The retention of the pigment content (TMAC, total betalain and total carotenoid content), antioxidant properties *viz.,* DPPH, FRAP and ABTS, instrumental colour properties and sensory scores of processed products was higher in the coloured guava squash, guava jelly and ash gourd candy stored under refrigerated condition as compared to ambient condition.

The findings of the study indicated higher recovery percentage of pigment concentrates having highest pigment content and antioxidant properties with bright hues of their respective pigments. The proposed extraction methods have been shown to be safer, economic, convenient and eco-friendly. They serve as foundational techniques for the commercial extraction of anthocyanin, betalain and carotenoid pigments that can be up-scaled as green-extraction techniques at the industrial level. Further, the products *viz.,* guava squash, guava jelly and ash gourd candy added with the extracted pigments (anthocyanin, betalain and carotenoid) not only increase the aesthetic value but also the nutritive properties with other qualities intact.

സംഗ്രഹം

കൃത്രിമ ഭക്ഷണ ചായങ്ങളുടെ സുരക്ഷയെക്കുറിച്ചുള്ള ഉപഭോക്താക്കളുടെ ആശങ്കയും പ്രകൃതിദത്ത നിറങ്ങളുടെ ആരോഗ്യപരമായ ഗുണങ്ങളും കാരണം പ്രകൃതിദത്ത നിറങ്ങൾ ഉപയോഗിക്കുന്നത് നിലവിലെ വിപണിയിലെ പ്രവണതയാണ്. എന്നിരുന്നാലും, കൃത്രിമ നിറങ്ങൾക്ക് പകരം പ്രകൃതിദത്ത നിറങ്ങൾ ഉപയോഗിക്കുക എന്നത് ശ്രമകരമാണ്. കാരണം പ്രകൃതിദത്ത നിറങ്ങൾ സാധാരണയായി സ്ഥിരത കുറഞ്ഞതും, കൂടുതൽ ചെലവേറിയതും കൃത്രിമ നിറങ്ങൾ പോലെ എളുപ്പത്തിൽ ഉപയോഗിക്കാൻ ബുദ്ധിമുട്ടുള്ളതുമാണ്. കൃത്രിമ നിറങ്ങൾക്ക് സമാനമായ വർണ്ണ ശക്തി കൈവരിക്കുന്നതിനായി പ്രകൃതിദത്ത നിറങ്ങൾക്ക് കൂടുതൽ അസംസ്കൃത പദാർത്ഥങ്ങൾ ആവശ്യമാണ്. കൂടാതെ പരിമിതമായ നിറങ്ങൾ മാത്രമാണ് പ്രകൃതിദത്തമായി ലഭ്യമായിട്ടുള്ളത്. ഈ പശ്ചാത്തലത്തിൽ, സസ്യ സ്രോതസ്സുകളിൽ നിന്ന് ആന്തോസയാനിൻ, ബെറ്റാലെയ്ൻ, കരോട്ടിനോയിഡ് എന്നീ പിഗ്മെന്റുകൾ വേർതിരിച്ചെടുക്കുന്നതിൽ വ്യത്യസ്ത പരിസ്ഥിതി സൗഹൃദ ലായകങ്ങളുടെ ഫലപ്രാപ്തി വിലയിരുത്തുന്നതിനും തുടർന്ന് വേർതിരിച്ചെടുത്ത പിഗ്മെന്റ് അവയുടെ സ്ഥിരത പഠിക്കുന്നതിനായി വിവിധ ഭക്ഷ്യപദാർത്ഥങ്ങളിൽ പ്രയോഗിക്കുന്നതിനുമായി "പ്രകൃതിദത്ത ഭക്ഷ്യ വർണ്ണങ്ങളായി സസ്യങ്ങളുടെ പിഗ്മെന്റുകളുടെ മൂല്യനിർണ്ണയവും ഉപയോഗവും" എന്ന തലക്കെട്ടോടെ 2019-22 കാലയളവിൽ കേരള കാർഷിക സർവ്വകലാശാലയുടെ കീഴിലുള്ള വെള്ളാനിക്കര കാർഷിക കോളേജിലെ പോസ്റ്റ്ഹാർവെസ്റ്റ് മാനേജ്മെന്റ് ഡിപ്പാർട്ട് മെന്റിൽ പഠനം നടത്തി. തിരഞ്ഞെടുത്ത പഴങ്ങൾ (മാംഗോസ്റ്റീൻ), പച്ചക്കറികൾ (ചുവന്ന ചീര, ബസെല്ല, ഗാക് പഴം), പൂക്കൾ (ശംഖുപുഷ്പം, ചെണ്ടുമല്ലി) എന്നിവയിൽ നിന്ന് സസ്യങ്ങളുടെ പിഗ്മെന്റുകൾ വേർതിരിച്ചെടുക്കുന്നതിനുള്ള പ്രക്രിയയെ ക്രമപ്പെടുത്തുക, വേർതിരിച്ചെടുത്ത പിഗ്മെന്റുകളുടെ ഭക്ഷ്യേതര ഉൽപ്പന്നങ്ങളിലുള്ള (പേരക്ക സ്കാഷ്, പേരക്ക ജെല്ലി, കുമ്പളങ്ങ മിഠായി) പ്രായോഗികത വിലയിരുത്തുക എന്നിവയായിരുന്നു പഠനത്തിന്റെ പ്രധാന ലക്ഷ്യങ്ങൾ.

ആന്തോസയാനിൻ, ബെറ്റാലെയ്ൻ, കരോട്ടിനോയിഡ് എന്നീ പിഗ്മെന്റുകൾ വേർതിരിച്ചെടുക്കുന്നതിനെ

കേന്ദ്രീകരിച്ചായിരുന്നു നിലവിലെ പഠനം. ഓരോ പിഗ്മെന്റ് വിഭാഗത്തിനും രണ്ട് വിളകൾ തിരഞ്ഞെടുത്തു. അതായത്, ആന്തോസയാനിൻ പിഗ്മെന്റിനായി ശംഖുപുഷ്പവും മാങ്കോസ്റ്റീൻ പഴത്തിന്റെ തൊലിയും; ബെറ്റാലൈൻ പിഗ്മെന്റിനായി ബാസെല്ലയുടെ പഴങ്ങളും ചുവന്ന ചീരയുടെ ഇലകളും; കരോട്ടിനോയിഡ് പിഗ്മെന്റിനായി ഗാക് പഴത്തിന്റെ വിത്തിന്റെ അല്ലികളും, ചെണ്ടുമല്ലി പുഷ്പ ദളങ്ങളും തിരഞ്ഞെടുത്തു.

ക്ലിറ്റോറിയ ടർനേഷ്യ എൽ. (ശംഖുപുഷ്പം) എന്ന് വിളിക്കപ്പെടുന്ന തിളങ്ങുന്ന നീല നിറത്തിലുള്ള പൂക്കൾ "ടെർനാറ്റിൻസ്" ആന്തോസയാനിനുകളുടെ സമ്പന്നമായ ഉറവിടങ്ങളാണ്. ഉപയോഗിച്ച വ്യത്യസ്ത വേർതിരിച്ചെടുക്കൽ രീതികളിൽ, അസിഡിഫൈഡ് അകവസ് എക്സ്ട്രാക്ഷൻ രീതി പിഗ്മെന്റ് കോൺസെൻട്രേറ്റിന്റെ (74.03±0.84 %) ഉയർന്ന വീണ്ടെടുക്കൽ ശതമാനവും അകവസ് വേർതിരിച്ചെടുക്കൽ രീതി ഉയർന്ന മൊണോമെറിക് ആന്തോസയാനിൻ മൂല്യവും (7925.29±36.07 മില്ലി ഗ്രാം/ലിറ്റർ) നൽകി. എന്നാൽ തിളക്കമുള്ള-നീല നിറം സൂചിപ്പിക്കുന്ന വർണ്ണ മൂല്യങ്ങൾ, അതായത് എ* (46.90±0.77), ബി* (-63.25±0.13), ക്രോമ (78.74±0.38), ഹ്യൂ ആംഗിൾ (306.56±0.50), ഉയർന്ന ആന്റിഓക്സിഡന്റ് പ്രവർത്തനം അതായത് DPPH (3.49±0.59 മൈക്രോ ലിറ്റർ/ മില്ലി ലിറ്റർ), FRAP (3.99±1.10 മൈക്രോ ലിറ്റർ/ മില്ലി ലിറ്റർ), ABTS ((2.42±0.01 മൈക്രോ ലിറ്റർ/ മില്ലി ലിറ്റർ), മൊത്തം ഫിനോളിക്സ് (29.78±1.79 മില്ലി ഗ്രാം GAE/100 ഗ്രാം), ആകെ ഫ്ളേവനോയ്ഡുകൾ (20.13±0.40 മില്ലി ഗ്രാം QE/100 ഗ്രാം) എന്നിവ ജല മാധ്യമം ഉപയോഗിച്ചുള്ള മൈക്രോവേവ് അസിസ്റ്റഡ് എക്സ്ട്രാക്ഷൻ രീതിയിൽ രേഖപ്പെടുത്തി.

മാംഗോസ്റ്റീൻ (*ഗാർസിനിയ മാങ്കോസ്റ്റാന* എൽ.) പഴത്തിന്റെ ഭക്ഷ്യയോഗ്യമല്ലാത്ത ഇരുണ്ട പുറംതോട് ആന്തോസയാനിൻ പിഗ്മെന്റിന്റെ സമ്പന്നമായ ഉറവിടമാണ്. ഇത് പരിസ്ഥിതി സൗഹൃദ ലായകങ്ങൾ ഉപയോഗിച്ച് വേർതിരിച്ചെടുത്താൽ ഭക്ഷണപദാർത്ഥങ്ങളിൽ പ്രയോഗിക്കുന്നതിനായി ഉപയോഗിക്കാം. അസിഡിഫൈഡ് ലായനി ഉപയോഗിച്ചുള്ള മൈക്രോവേവ് അസിസ്റ്റഡ് എക്സ്ട്രാക്ഷൻ രീതി, പിഗ്മെന്റ് കോൺസെൻട്രേറ്റിന്റെ (55.03±2.98 %) ഉയർന്ന വീണ്ടെടുക്കൽ ശതമാനം രേഖപ്പെടുത്തി. പർപ്പിൾ-ചുവപ്പ് നിറത്തെ സൂചിപ്പിക്കുന്ന ഇൻസ്ട്രുമെന്റൽ കളർ

മൂല്യങ്ങൾ, അതായത്., എൽ* (3.17±0.38), എ* (59.53±0.85), ബി* (5.09±0.52), ക്രോമ (60.80±1.00), ഹ്യൂ ആംഗിൾ (28.06±0.33°), ഉയർന്ന ആന്റിഓക്സിഡന്റ് പ്രവർത്തനം, അതായത്, DPPH (2.29±0.24 മൈക്രോ ലിറ്റർ/ മില്ലി ലിറ്റർ), FRAP (2.83±0.0.21മൈക്രോ ലിറ്റർ/ മില്ലി ലിറ്റർ), ABTS (2.02±0.03 മൈക്രോ ലിറ്റർ/ മില്ലി ലിറ്റർ), ഉയർന്ന മൊത്തം ഫിനോളിക്സ് (32.25±0.30 മില്ലി ഗ്രാം GAE/100 ഗ്രാം), മൊത്തം ഫ്ലേവനോയ്ഡുകൾ (40.02±3.52 മില്ലി ഗ്രാം QE/100 ഗ്രാം) എന്നിവയും രേഖപ്പെടുത്തി. അതേസമയം അസിഡിഫൈഡ് സോൾവെന്റ് എക്സ്ട്രാക്ഷൻ രീതിയിലാണ് ഉയർന്ന മൊണോമെറിക് ആന്തോസയാനിൻ മൂല്യം (17652.54±139.47 മില്ലി ഗ്രാം) രേഖപ്പെടുത്തിയത്.

കടും പർപ്പിൾ നിറത്തിലുള്ള തൊലിയും കടും ചുവപ്പ് വയലറ്റ് മാംസവുമുള്ള ബസെല്ല (*ബസെല്ല ആൽബ* വെർ. *റുബ്ര*) പഴങ്ങൾ ബീറ്റലൈനിന്റെ ഉറവിടങ്ങളാണ്. ഉപയോഗിച്ച വ്യത്യസ്ത വേർതിരിച്ചെടുക്കൽ രീതികളിൽ, മൈക്രോവേവ് അസിസ്റ്റഡ് എക്സ്ട്രാക്ഷൻ രീതി (50 % എത്തനോൾ) ഗണ്യമായി ഉയർന്ന ബെറ്റാസയാനിൻ (605.83±4.10 മില്ലി ഗ്രാം/ ഗ്രാം), ബീറ്റാക്സാന്തിൻ (86.35±1.67 മില്ലി ഗ്രാം/ ഗ്രാം), മൊത്തം ബീറ്റാലൈൻ (692.18±2.52 മില്ലി ഗ്രാം/ ഗ്രാം) എന്നിവ രേഖപ്പെടുത്തി. ഇൻസ്ട്രുമെന്റൽ കളർ മൂല്യങ്ങളായ എ * (52.27±0.24), ബി * (23.24±0.16), ക്രോമ (58.75±0.19), ഹ്യൂ ആംഗിൾ (27.40±0.17), ഉയർന്ന ആന്റിഓക്സിഡന്റ് പ്രവർത്തനം, DPPH (3.440±0.04 മൈക്രോ ലിറ്റർ / മില്ലി ലിറ്റർ), FRAP (3.69±0.19 മൈക്രോ ലിറ്റർ / മില്ലി ലിറ്റർ), ABTS (2.99±0.04 മൈക്രോ ലിറ്റർ / മില്ലി ലിറ്റർ), ഉയർന്ന മൊത്തം ഫിനോളിക്സ് (211.37±0.29 mg GAE/100 g) കൂടാതെ മൊത്തം ഫ്ലേവനോയ്ഡുകൾ (124.07±2.53 മില്ലി ഗ്രാം QE/100 ഗ്രാം) എന്നിവയും രേഖപ്പെടുത്തി. അതേസമയം പിഗ്മെന്റ് കോൺസെൻട്രേറ്റിന്റെ (53.13±0.41 %) ഉയർന്ന വീണ്ടെടുക്കൽ ശതമാനം അസിഡിഫൈഡ് അകവസ് എക്സ്ട്രാക്ഷൻ രീതിയിൽ (1 % സിട്രിക് ആസിഡ്) രേഖപ്പെടുത്തിയിട്ടുണ്ട്.

ചുവന്ന ചീര (*അമരാന്തസ് ട്രൈകളർ* എൽ.) സസ്യങ്ങളിൽ ഫ്ളേവനോയ്ഡുകൾ, ആൽക്കലോയിഡുകൾ, മറ്റ് മൂലകങ്ങൾ എന്നിവയ്ക്കൊപ്പം ബീറ്റാക്സാന്തിൻ, ബെറ്റാസയാനിൻ എന്നിവയുൾപ്പെടെ വലിയ അളവിൽ ബെറ്റാലൈൻ അടങ്ങിയിട്ടുണ്ട്. ഉപയോഗിച്ച വ്യത്യസ്ത വേർതിരിച്ചെടുക്കൽ രീതികളിൽ, മൈക്രോവേവ് അസിസ്റ്റഡ് എക്സ്ട്രാക്ഷൻ രീതിയിൽ (50 % എത്തനോൾ) ഗണ്യമായി ഉയർന്ന

ബീറ്റാസയാനിൻ (601.15±2.25 മില്ലി ഗ്രാം / ഗ്രാം), ബീറ്റാക്സാന്തിൻ (75.63±0.55 മില്ലി ഗ്രാം / ഗ്രാം), മൊത്തം ബീറ്റാലൈൻ (676.78±2.79 മില്ലി ഗ്രാം / ഗ്രാം) എന്നിവ രേഖപ്പെടുത്തി. ഇൻസ്ട്രുമെന്റൽ കളർ മൂല്യങ്ങളായ എൽ* (41.94±0.06), എ* (33.37±0.22), ക്രോമ (63.37±0.17), ഹ്യൂ ആംഗിൾ (58.41±0.02), ഉയർന്ന ആന്റിഓക് സിഡന്റ് പ്രവർത്തനം അതായത് DPPH (1.34±0.01 മൈക്രോ ലിറ്റർ /മില്ലി ലിറ്റർ), FRAP (3.69±0.19 മൈക്രോ ലിറ്റർ /മില്ലി ലിറ്റർ), ABTS (0.81±0.01 മൈക്രോ ലിറ്റർ /മില്ലി ലിറ്റർ), ഉയർന്ന മൊത്തം ഫിനോളിക്സ് (190.03±0.22 mg GAE/100 g) കൂടാതെ മൊത്തം ഫ്ലേവനോയ്ഡുകൾ (179.07±0.49 മില്ലി ഗ്രാം QE/100 ഗ്രാം) എന്നിവയും രേഖപ്പെടുത്തി. അതേസമയം പിഗ്മെന്റ് കോൺസെൻട്രേറ്റിന്റെ ഉയർന്ന വീണ്ടെടുക്കൽ ശതമാനം (58.73±0.43 %) അസിഡിഫൈഡ് അകവസ് എക്സ്ട്രാക്ഷൻ രീതിയിൽ (1 % സിട്രിക് ആസിഡ്) രേഖപ്പെടുത്തിയിട്ടുണ്ട്.

കരോട്ടിനോയിഡ്സ്, ഫിനോളിക്സ്, ഫ്ലേവനോയ്ഡ്സ് എന്നീ സംയുക്തങ്ങൾ ഉൾപ്പെടുന്ന ബയോ ആക്റ്റീവ് സംയുക്തങ്ങളുടെ സമ്പന്നമായ ഉറവിടമാണ് ഗാക് പഴത്തിൻറെ (*മൊമോർഡിക്ക കൊച്ചിൻചൈനൻസിസ്* സ്പ്രെങ്.) വിത്തിന്റെ അല്ലികൾ. ഉപയോഗിച്ച വ്യത്യസ്ത വേർതിരിച്ചെടുക്കൽ രീതികളിൽ, എഥൈൽ ലാക്റ്റേറ്റ് ഉപയോഗിച്ചുള്ള വേർതിരിച്ചെടുക്കൽ രീതിയിൽ ഗണ്യമായി ഉയർന്ന മൊത്തം കരോട്ടിനോയിഡ് (2069.83±4.38 മൈക്രോ ഗ്രാം /ഗ്രാം), ബീറ്റാ-കരോട്ടിൻ (689.43±1.04 മൈക്രോ ഗ്രാം /ഗ്രാം), ലൈക്കോപീൻ (1052.31±0.87 മൈക്രോ ഗ്രാം /ഗ്രാം), ലുട്ടീൻ (216.96±0.22 മൈക്രോ ഗ്രാം /ഗ്രാം), ഇൻസ്ട്രുമെന്റൽ കളർ മൂല്യങ്ങളായ എൽ* (60.44±0.12), എ* (61.35±0.20), ബി* (43.24±0.10), ക്രോമ (65.62±0.10), ഹ്യൂ ആംഗിൾ 36.64±0.18), ഉയർന്ന ആന്റിഓക്സിഡന്റ് പ്രവർത്തനം, അതായത് DPPH (0.59±0.05 മൈക്രോ ലിറ്റർ/ മില്ലി ലിറ്റർ), FRAP (0.63±0.004 മൈക്രോ ലിറ്റർ/ മില്ലി ലിറ്റർ), ABTS (0.58±0.005 മൈക്രോ ലിറ്റർ/ മില്ലി ലിറ്റർ), കൂടാതെ മൊത്തം ഫിനോളിക്സ് (211.57±0.13 മില്ലി ഗ്രാം GAE/100 ഗ്രാം) എന്നിവ രേഖപ്പെടുത്തി. അതേസമയം പിഗ്മെന്റ് കോൺസെൻട്രേറ്റിന്റെ ഉയർന്ന വീണ്ടെടുക്കൽ ശതമാനം (89.53 ± 0.13 %) എത്തനോൾ വേർതിരിച്ചെടുക്കൽ രീതി ഉപയോഗിച്ച് മൈക്രോവേവ് അസിസ്റ്റഡ് എക്സ്ട്രാക്ഷൻ രീതിയിൽ രേഖപ്പെടുത്തിയിട്ടുണ്ട്.

കരോട്ടിനോയിഡിന്റെ സുലഭമായ സ്രോതസ്സുകളാണ് ചെണ്ടുമല്ലി (*ടാജിറ്റസ് എറെക്ട* എൽ.) പൂക്കൾ. ഭക്ഷ്യ

വ്യവസായത്തിലെ കരോട്ടിനോയിഡിന്റെ ഏറ്റവും പ്രധാനപ്പെട്ട ഉറവിടങ്ങളിൽ ഒന്നായ ല്യൂട്ടിൻ എന്ന ഡൈഹൈഡ്രോക് സിലേറ്റഡ് സംയുക്തമാണ് പൂവിലുള്ള മൊത്തം കരോട്ടിനോയിഡിന്റെ 85 ശതമാനവും അടങ്ങിയിട്ടുള്ളത്. ഉപയോഗിച്ച വ്യത്യസ്ത വേർതിരിച്ചെടുക്കൽ രീതികളിൽ, എഥൈൽ ലാക്റ്റേറ്റ് വേർതിരിച്ചെടുക്കൽ രീതിയിലാണ് ഉയർന്ന പിഗ്മെന്റ് കോൺസൺട്രേറ്റ് (93.33±0.27 %), മൊത്തം കരോട്ടിനോയിഡ് (2276.93±3.61 മൈക്രോ ഗ്രാം /ഗ്രാം), ബീറ്റാ കരോട്ടിൻ (491.03±0.0.85 മൈക്രോ ഗ്രാം /ഗ്രാം), ലൈക്കോപീൻ (236.96±0.10 മൈക്രോ ഗ്രാം /ഗ്രാം), ല്യൂട്ടിൻ (1310.02±0.27 മൈക്രോ ഗ്രാം /ഗ്രാം), ഉയർന്ന ആന്റിഓക്സിഡന്റ് പ്രവർത്തനം, അതായത്, DPPH (0.355±0.03 മൈക്രോ ലിറ്റർ/ മില്ലി ലിറ്റർ), FRAP (0.557±0.003 മൈക്രോ ലിറ്റർ/ മില്ലി ലിറ്റർ), ABTS (0.201±0.002 മൈക്രോ ലിറ്റർ/ മില്ലി ലിറ്റർ), മൊത്തം ഫിനോളിക്സ് (337.61±0.23 മില്ലി ഗ്രാം GAE/100 ഗ്രാം) എന്നിവ രേഖപ്പെടുത്തിയത്. അതേസമയം ഉയർന്ന ഇൻസ്ട്രുമെന്റൽ കളർ മൂല്യങ്ങൾ, എൽ* (70.20±0.12), എ* (10.48±0.14), ബി* (76.159) കൂടാതെ ഹ്യൂ ആംഗിളും (82.44±0.19) എത്തനോൾ എക്സ്ട്രാക്ഷൻ രീതിയിൽ രേഖപ്പെടുത്തി.

ആന്തോസയാനിൻ, ബെറ്റാലെയ്ൻ, കരോട്ടിനോയിഡ് എന്നീ പിഗ്മെന്റുകളുടെ സ്ഥിരത ഭക്ഷ്യാല്പന്നങ്ങളായ പേരക്ക സ്കാഷ്, പേരക്ക ജെല്ലി, കുമ്പളങ്ങ കാൻഡി എന്നിവയിൽ മൂന്ന് മാസത്തേക്ക് അന്തരീക്ഷത്തിലും ശീതീകരിച്ച സംവിധാനത്തിലും സംഭരിച്ചു വിലയിരുത്തി. പിഗ്മെന്റ് ഉള്ളടക്കം (മൊണോമെറിക് ആന്തോസയാനിൻ മൂല്യം, മൊത്തം ബീറ്റാലൈൻ, മൊത്തം കരോട്ടിനോയിഡ്), ആന്റിഓക്സിഡന്റ് പ്രവർത്തനങ്ങൾ, അതായത് ഡിപിപിഎച്ച്, എഫ്ആർഎപി, എബിടിഎസ്, ഇൻസ്ട്രുമെന്റൽ കളർ മൂല്യങ്ങൾ, സംസ്കരിച്ച ഉൽപ്പന്നങ്ങളുടെ സെൻസറി സ്കോറുകൾ എന്നിവ ആംബിയന്റ് അവസ്ഥയുമായി താരതമ്യപ്പെടുത്തുമ്പോൾ ശീതീകരിച്ച അവസ്ഥയിൽ സൂക്ഷിച്ചിരിക്കുന്ന പിഗ്മെന്റ്സ് ചേർത്ത പേരക്ക സ്കാഷ്, പേരക്ക ജെല്ലി, കുമ്പളങ്ങ കാൻഡി എന്നിവയിൽ കൂടുതലാണ്.

പഠനത്തിന്റെ കണ്ടെത്തലുകൾ സൂചിപ്പിക്കുന്നത് പിഗ്മെന്റ് കോൺസൺട്രേറ്റിന്റെ ഉയർന്ന വീണ്ടെടുക്കൽ ശതമാനവും ഉയർന്ന പിഗ്മെന്റ് ഉള്ളടക്കവും അതത് പിഗ്മെന്റുകളുടെ തിളക്കമുള്ള നിറങ്ങളുള്ള ആന്റിഓക് സിഡന്റ് ഗുണങ്ങളുമാണ്. നിർദ്ദിഷ്ട വേർതിരിച്ചെടുക്കൽ

രീതികൾ സുരക്ഷിതവും സാമ്പത്തികവും സൗകര്യപ്രദവും പരിസ്ഥിതി സൗഹൃദവുമാണെന്ന് തെളിയിക്കപ്പെട്ടിട്ടുണ്ട്. വ്യാവസായിക തലത്തിൽ ഗ്രീൻ എക്സ്ട്രാക്ഷൻ ടെക് നിക്കുകളായി ഉയർത്താൻ കഴിയുന്ന ആന്തോസയാനിൻ, ബെറ്റാലെയ്ൻ, കരോട്ടിനോയിഡ് പിഗ്മെന്റുകൾ എന്നിവയുടെ വാണിജ്യപരമായ വേർതിരിച്ചെടുക്കുന്നതിനുള്ള അടിസ്ഥാന സാങ്കേതികതകളായി അവ പ്രവർത്തിക്കുന്നു. കൂടാതെ, വേർതിരിച്ചെടുത്ത പിഗ്മെന്റുകൾ (ആന്തോസയാനിൻ, ബെറ്റാലെയ്ൻ, കരോട്ടിനോയിഡ്) ചേർക്കുന്ന പേരക്ക സ്കാഷ്, പേരക്ക ജെല്ലി, കുമ്പളങ്ങ കാൻഡി എന്നിവയ്ക്ക് ദൃഷ്ടി മൂല്യം മാത്രമല്ല, പോഷകഗുണങ്ങളും ഉണ്ട്.