UTILIZATION OF POMEGRANATE (*Punica granatum* L.) PEEL AS A NATURAL FOOD COLOURANT

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"UTILIZATION OF POMEGRANATE (*Punica granatum* L.) PEEL AS A NATURAL FOOD COLOURANT"** is a bonafide record of research work done by me during the course of research and that 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.

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LIST OF ABBREVIATIONS

%	Per cent
cm	Centimetre
et al.	And co-workers
g	Gram
Kg	Kilogram
mm	Millimetre
mg	Milligram
mL	Millilitre
µg	Microgram
L	Litre
^{o}C	Degree celsius
RH	Relative Humidity
ppm	Parts per million
viz.	Namely
CRD	Completely Randomised Design
^{o} Brix	Degree Brix
CO_2	Carbon dioxide
Fig.	Figure
NS	Non significant
CD	Critical difference
MAS	Months after storage
TSS	Total Soluble Solids

Sl.	Serial
SO2	Sulfur dioxide
FSSAI	Food Safety and Standards Authority of India

1. INTRODUCTION

The acceptance of food products by customers is influenced by colour, perhaps more than any other element. Since colour is a reliable predictor of both product value and safety, it is frequently associated with quality.

Colour is an important element influencing the acceptance of food by consumers, constituting one of the major food additives. Synthetic colours are generally applied to manipulate food colour; however they are hardly nutrient, blamed for toxic to different extent and some are found to be carcinogenic. Due to the dynamically growing natural, organic, and sustainable food markets, the demand for non-synthetic food colorants continues to increase. Colouring of food with natural pigments is of worldwide interest and is gaining importance because of the awareness of positive health benefits. One such pigment is anthocyanin which is a natural red colourant used in food due to their bright attractive colour, non-toxicity and water solubility. Major limitations to the use of natural food colorants are their low stability in food processing procedures, high cost and limited colour range.

There are many sources of anthocyanin pigments including fruit and vegetable byproduct. Fruit and vegetable processing industry generates 20-50% of raw materials as solid waste, environment friendly disposal of which has become a challenge to the food processors. There is a great need to find out options which have positive values viz., minimization of waste generation and development of potential value added products. One such potential and useful product that can be extracted from processing waste is natural colorant.

Pomegranate (*Punica granatum* L.) peel constitutes about 30-40% portion of the fruit, with the highest antioxidant activity and are rich in many biochemical compounds such as phenolics, flavonoids, ellagitannins, proanthocyanidin compounds, complex polysaccharides, and many minerals (Li *et al*., 2006). But it is discarded in the food industries despite its richness in bioactive compounds.

The studies conducted in the Department of Postharvest Management have identified an efficient and economic method for extraction of natural red pigments including anthocyanin (Mini *et al.*, 2021 and Gayathri, 2023). The standardised processed products can be prepared using pomegranate peel colour extract as a natural food colourant. Utilization of pomegranate peel as an effective food colourant in defined concentrations in preparing various foods would open new avenues for scientific research in the realm of food processing sector. The pigment extracted from the peel of the fruit can find its market in the industry enhancing the product portfolio.

Hence a study on “Utilization of pomegranate (*Punica granatum* L.) peel as a natural food colourant” was done at Department of Postharvest Mangement, College of Agriculture, Vellayani with the objective of standardization of food formulations using pomegranate peel as a natural colourant.

2. REVIEW OF LITERATURE

Consumers first judge the quality of a food product by its colour. The food industry has used colorants for centuries to enhance or restore the original appearance of foods or to ensure uniformity, as indicator of food quality. Colour is a vital constituent of foods because it is one of the first characteristics perceived by the senses and is used by consumers for the rapid identification and ultimate acceptance of food. Since natural, purified anthocyanin extracts may be added to processed foods, the food industry has begun employing them as food colorants. Anthocyanins are naturally occurring compounds widespread in plant derived foodstuffs and therefore abundant in human diet. Research related to natural food colourants, extraction methods of anthocyanin, pomegranate peel as a source of anthocyanin, anthocyanin pigment stability and anthocyanin incorporated functional food products are reviewed in this chapter.

2.1 NATURAL FOOD COLOURS USED IN FOOD INDUSTRY

The public worries regarding the usage of artificial additives have resulted in a significant demand for natural food colorants that can replace synthetic dyes. Due to the dynamically growing natural, organic, and sustainable food markets, the demand for natural food colorants continues to increase. The absence of consistent regulations regarding acceptable food colours, the potential connection between artificial and hyperactive behaviour, the substitution of natural colours for synthetic ones, and the presence of known and unknown harmful illegal dyes in food are the main food safety concerns related to food colouring. A summary of quality requirements for natural food colours would include consistency, safety and stability (Timberlake and Bridle, 1983).

Some of the naturally derived colour pigments, their sources and the colour shades produced are shown below (Downham and Collins, 2000).

Pigment	**Sources**	**Colour shade**
Anthocyanin	Black grape skin, elderberries, black carrots, red cabbage, pomegranate peels	Pink/red to mauve/blue depending on pH
Betanin	Red table beetroot	Pink to red
Bixin/norbixin	*Bixa orellana*	Orange
Curcumin	Turmeric rhizome (roots)	Bright lemon yellow
Carminic acid	Cochineal insect(female)	Orange to red
Capsanthin/capsorubin	Paprika	Red orange
Cholorophyll	Grass ,Lucerne and nettle	Olive green
Crocin	Saffron	Yellow
Lycopene	Tomatoes	Orange red

The number of colorants that are allowed has decreased as a result of concerns raised in recent years about the safety of synthetic colorants. Legislative action and consumer awareness of the use of artificial chemicals in food has led to a major increase in interest in natural colorants (Giusti and Wrostland, 2003).

According to Jenie *et al.* (1994), the use of synthetic colorants for the food or beverages can be toxic and carcinogenic. Due to those negative effects of the use of synthetic dyes, it becomes important to replace synthetic colorants with natural colorants derived from plants.

The natural colorants are biodegradable, environmentally benign, and derived from renewable resources. Their manufacturing process minimizes the likelihood of chemical reactions (Joshi and Preema, 2017).

Any dye, pigment, or other material derived from plants, animals, minerals, or other sources that can be used to color food, medications, cosmetics, or any aspect of the human body is considered natural food coloring. Many things, including seeds, fruits, roots, plants, algae, and insects, are sources of color (Kamatar, 2013).

To enhance their look or compensate for naturally occurring colour variations, food coloring is added to a variety of commodities. The food colours are becoming more prevalent and natural alternatives are becoming more popular than synthetic ones (Oplatowska-Stachowiak and Elliott 2017).

Many compounds found in nature are suitable for food coloring, including oil-soluble carotenoids, chlorophylls, and water soluble anthocyanins and betalains (Jadhav and Bhujbal, 2020).

Natural food pigments have a wide range of chemical and physical characteristics, varying degrees of intrinsic solubility and are susceptible to light, pH changes, and oxidation. The food and beverage industry use more natural colours. Food producers now have faith that a colour provider can create the majority of colours in their natural state, providing the brightness, stability, and usability they need (Affat, 2021).

Natural colorants are safe for human health because they are derived from natural sources. Natural food coloring is inexpensive, simple to prepare, and doesn't give food an odd taste. Not only does using natural coloring enhance the food's appearance, but it also boosts its nutritious content. Additionally, some food colorings have pharmacological properties that include enhancing cognition, reducing seizures, and protecting nerves (Ochiai *et al*., 2007), anti-depressant (Hosseinzadeh and Jahanian, 2010; Akhondzadeh *et al.*, 2004), antioxidant (Akhtari and Harvey, 2023), and anti-tumor (Hadizadeh *et al*., 2010). In order to replace artificial colorings, numerous nations have started extracting and using natural ingredients to make food coloring (Reddy *et al.,* 2005).

2.2 ANTHOCYANINS AS NATURAL COLOURANTS

The vibrant orange, red, purple, and blue hues found in many fruits, vegetables, flowers, leaves, roots, and other plant storage organs are caused by a group of pigments called anthocyanins. They have been consumed for ages without any negative consequences and are water soluble, which makes it easier to incorporate them into watery food systems (Markakis, 1982).

The anthocyanins comprise the largest group of water soluble pigments in the plant kingdom and are especially characteristic of the angiosperms or flowering plants, which themselves provide our major source of food crops. In food plants, anthocyanins are widespread occurring in at least 27 families, 73 genera and a multitude of species (Bridle and Timberlake, 1997).

Red rice anthocyanin extract is a common culinary coloring used in China. Red rice is now accepted by the Chinese Ministry of Health as a modern food additive to complement the color and delicate texture of meat, fish, and soy products in the Chinese diet (Ma *et al*., 2008). Because of their eye-catching orange, red, purple, and blue colors, anthocyanins have a lot of potential for usage as natural colorants. However, they have stability issues. It is possible that highly stable colorant systems already exist in nature; still, they must be recognized and studied for their phytochemical makeup and stability characteristics.

Natural food colouring is becoming more popular worldwide as people become more aware of its many health advantages (Kong *et al*., 2003). One such pigment is anthocyanin which is a natural red colourant used in food due to their bright attractive colour, non-toxicity and water solubility.

In the USA, anthocyanins are allowed to be used as food coloring in fruit (21CFR73.250) or vegetable (21CFR73.260) juice. Only the edible parts of plants are to be used under these categories (Giusti and wrostland, 2003).

Natural colorants called anthocyanins are frequently employed in the food sector in place of artificial colorants. Depending on the molecular structure and pH level, they exhibit a broad range of color tones, from orange to red to purple and blue (Walkowiak-Tomczak and Czapski, 2007).

Anthocyanins mostly exist as glycosides of the corresponding aglycone anthocyanidin chromophores, where the sugar moiety is typically attached at the C or A-ring's 3 or 5 positions. There are just six common antocyanidins, which are extensively distributed and highly significant to human food and health: cyanidin, delphinidin, peonidin, peonidin, pelargonidin, and malvidin. The quantity and location of hydroxyl and methoxy groups on the fundamental anthocyanidin skeleton are what give rise to the variety of anthocyanins. The color becomes more bluish when hydroxyl groups predominate; redness increases when methoxyl groups predominate (Jadhav and Bhujbal, 2020).

2.3 SOURCES OF ANTHOCYANIN

Francis and Markakis (1989) produced one of the most thorough evaluations on anthocyanins as food colorants. In-depth instructions on how to extract and process pigments from grapes and other plant sources, as well as techniques for assessing and testing color stability, are provided.. The data featuring most food plant sources is provided by Mazza & Miniati (1993). These and earlier publications (Markakis, 1982; Francis, 1993) provide an abundance of data on most of the plant species seriously considered as having potential for a supply of anthocyanin food colours.

Kamatar (2013) had listed the commonly used bio colourant and anthiocyanins Oxalis triangularis and banana bract are mentioned as possible sources of food coloring.

The food business uses acylated anthocyanins derived from many edible sources, such as black carrot. Black carrot is a good anthocyanin based natural colour source, but also it has high ratio of monoacylated structures increasing color retention at low pH. Red wine is very good anthocyanin source because it can be reduced to form stable complexes during fermentation. The concentration of the pigment is very important on determining of the colour expressed by anthocyanin.

Kiss *et al*. (2014) measured the anthocyanin content of black currant (*Ribes nigrum.L*), sweet cherry (*Prunus avium L.*), blackberry (*Rubus fruitcosus L.*) and black elder berry (*Sambucus nigra* L.).The sweet cherry had the highest anthocyanin concentration (222.7 mg/kg), as determined by the HPLC techniques.

Anthocyanins are found in the plant organs of many fruits and vegetables, including the roots, leaves, stems, and some grains (Smeriglio *et al*., 2016; Wallace and Giusti, 2019).

The rich source of anthocyanin includes red grapes, eggplant, raspberry, wild blueberries, acai, orange, purple corn, red wine, cherries, chokeberries, elderberries, red currants, strawberries, pomegranate etc. (Li *et al*., 2012; Juroszek *et al*. 2009; Yang *et al*., 2009). According to Sharma *et al*. (2016), guava and jamun had anthocyanin contents of 172 mg/100 g and 138 mg/mg, respectively.

The most prevalent anthocyanidins found in nature are cyanidin, delphinidin, pelargonidin, petunidin, peonidin, and malvidin. According Kong *et al*., (2003) anthocyanin that is most often used and abundant is cyanidin-3-glucoside. Elderberries, pears, figs, cherries, red cabbage, apples, gooseberries, and so on are sources of cyanidin anthocyanins. Sources of peonidin anthocyanins include berries, grapes, cherries, plums, mangos, and plums. Sources of pelaggonidin anthocyanins include sweet potatoes, strawberries, bananas, and red cabbage. Red grape, bilberry, etc. are some of the anthocyanin sources for petunidin and malvidin. Pomegranate, eggplant, green beans, purple carrots, and others are sources of delphinidin anthocyanins. These three types of anthocyanidins are each constituted of about 50% of the flowers, 69% of the fruits, and 80% of the plant leaves phenolic acids (Khoo *et al*. 2017).

Anthocyanin contents reported in various plant sources are listed below:

Sources	Content
Red spinach	6350 ppm
Purple sweet potato	0.24-0.44 mg/g
Red cabbage	11.11-17.80 mg/g
Strawberry	19-55 mg/100g
Wine	0.267 – 1.9 mg/g
Mulberry	19.93 mg/g
Red dragon fruit	0.088 mg/g
Jamblang	1.61 mg/g
Red dragon fruit skin	22.593 ppm
Rambutan skin	41.10 mg/ml
Mangosteen skin	593 ppm
Jamblang skin	0.19 mg/g
Eggplant skin	750 mg/g
Jenitri's skin	0.2387 mg/g
Chokeberry	410-1480 mg/100g
Elderberry	200-1816 mg/100g
Cherry	2-450 mg/100g
Grapes	30-750 mg/100g
Source: Gitelson *et al*. (2006); Djaeni and Utari (2019); Sangadji *et al.* (2017); Ahmadiani *et al.* (2014); Pebrianti *et al*. (2015); Winata and Yunianta (2015); Anggraini *et al*. (2018); Winarti *et al.* (2018); Zulfajri (2018); Lestario *et al*. (2011); Handayani and Rahmawati (2012); Farida and Nisa (2015).	

Dragon fruit peel contains anthocyanin which can be used as a potential natural colorant and source of natural antioxidants. The anthocyanins content in the mixture of two powders viz., dragon fruit peel and soybean varied from 8,00- 387,833 mg/100 g. Dragon fruit peel contains higher anthocyanin than soybean shown by the red colour of the peel (Rosiana *et al.,* 2021).

2.4 POMEGRANATE PEEL AS A SOURCE OF ANTHOCYANIN

Pomegranate (*Punica granatum* L.) peel constitutes about 30-40% portion of the fruit. But peel is discarded in the food industries despite its richness in bioactive compounds (Bertolo *et al*., 2020).

The peel fraction contains about 30% of the total pomegranate fruit anthocyanins (Romeo *et al*., 2015). Many studies have tried to identify and quantify the different types of anthocyanins in pomegranate varieties from several regions of the world, using advanced analytical techniques (Bar-Ya'akov *et al*., 2019).

Fischer *et al*. (2011) isolated 9 anthocyanins from pomegranate peel. Besides the 3-glucosides and 3, 5-diglucosides of delphinidin, cyanidin, and pelargonidin, the other three new ACs, namely cyanidin-pentoside-hexoside, cyanidin-rutinoside, and cyanidin-pentoside were also identified. The anthocyanin accumulation in pomegranate cultivars is influenced by a number of extrinsic environmental factors as well as interactions between them, ranging from intrinsic genetic to diverse extrinsic environmental factors (Zhao *et al.*, 2021).

Zhao *et al*. (2013) reported that the amount of anthocyanin in pomegranate peel varies according on the cultivar, stage of fruit growth, and time of coloration. The predominant phenolic components identified in pomegranate peel are flavonoids, which include quercetin, epicatechin, and catechin, and anthocyanin like cyanidin, pelargonidin, and its derivatives (Singh *et al*., 2018).The peel is primarily rich in phenolic chemicals.

Abid *et al*. (2017), reported that peels from four Tunisian ecotypes were found to contain pelargonidin-3-pentoside, cyanidin-3-rutinoside, cyanidin-3-glucoside, and cyanidin-3-pentoside, according to Liquid Chromatography Tandem Mass Spectrometry (LC-MS-MS) analysis. In a Tunisian PP variety known as pelargonidin-3-glycoside, pelargonidin-3,5-diglycoside, delphinidin-3-glycoside, delphinidin-3,5-diglycoside, cyanidin-3-glycoside, cyanidin-3,5-diglycoside, cyanidin -3-pentoside, and cyanidin-3-rutinoside, eight distinct anthocyanins were identified using HPLC-

DAD-ESI-TOF/MS. “Nana” (Wafa et al., 2017). The peel extract from a Spanish pomegranate cultivar named “Mollar de Elche”, collected from an orchard in Acireale (Italy), showed high levels of cyanidin 3-glucoside (49.36%), pelargonidin 3-glucoside (24.62%) and cyanidin 3,5-diglucoside (12.41%) (Romeo et al., 2015). Other anthocyanins, such as pelargonidin 3,5-diglucoside, delphinidin 3-glucoside and delphinidin 3,5-diglucoside, were detected in much lower concentrations (Romeo et al., 2015).

2.5 METHODS OF ANTHOCYANIN EXTRACTION

Peterson and Jaffe (1969) invented a method that treated grape pomace with water or alcohol containing 200–2,000 ppm SO_2 to produce extracts with rich colour and flavor.

Chiriboga and Francis (1970) found that a combination of maceration and repeated short extractions with acidified methanol produced significant pigment recovery and eliminated the need for extensive extraction durations in their attempt to extract anthocyanin from cranberry pomace.

In order to recover anthocyanins from grape wastes, Philip (1974) proposed extracting the pigments using methanol (or ethanol) that contains 0.1% to 1.0% tartaric acid, and then precipitating the excess tartaric acid as potassium hydrogen tartrate.

Palamidis and Markakis (1975) attempted to use a straightforward technique to separate the anthocyanin from fermented grape skins and investigated the durability of these pigments when applied as colorants to a nonalcoholic carbonated beverage. Hot water and 500 ppm SO_2 were used as the solvents. There was no appreciable difference between these two solvents regarding the total anthocyanin extracted from the grape skins. It was discovered that the anthocyanin-containing dry powder obtained by hot water extraction contained 788 mg of EE per g, whereas the powder obtained by SO2 solution had 905 mg of EE per g. This powder was intended to be used as a beverage colorant. While the overall amount of anthocyanin removed by the two solvents was almost equal, hot water extracted more nonanthocyanin plant material than the room temperature SO2 solution. The artificial synthesis of anthocyanins still represents a challenge and is expensive. However, these compounds can be extracted from vegetal species, especially fruits, where there is high concentration of anthocyanins. The commercial cost of isolating chemically pure anthocyanins is very high (Markakis, 1982).

Anthocyanin yield is dependent on a wide range of variables, including sample type, temperature, pH, type of solvent, and component ratios. A proper quantitative method for anthocyanin extraction has to be identified for different plant matrixes (Tonutare *et al*., 2014).

Numerous studies have been conducted on the extraction techniques used to extract anthocyanins from various natural matrices (Navas *et al*., 2012; Zhang *et al*., 2018; Bagade and Patil, 2021; Azmir *et al.*, 2013; Martín and Asuero, 2021; Xi *et al.* 2019; Ranjha, *et al*. 2021). Conventional techniques, such as maceration and heat-assisted extraction (HAE), do not require sophisticated instrumentation and are easy to apply at the industrial level (Tan *et al*., 2021).

Maran *et al*. (2015) used an aqueous extraction method to recover natural pigment and colors from the pulp of jamun fruit under various extraction conditions, including temperature (40-60^{0}c), time (20-100min), and solid to liquid ratio (1:10-1:15 g/ml). The results showed that an extraction temperature of 44 ^{o}C, an extraction period of 93 min, and a solid-liquid ratio of 1:15 g/ml were the best parameters for increasing the extraction yield of total anthocyanin (10.58 mg/100g) and colours (10618.3 mg/l).

Wahyuningsih *et al*. (2017) reported that anthocyanin can be extracted from red roses by the maceration process carried out in acidic conditions with 0.1 M HCl (ratio 4:1).

Ove *et al*. (2019) conducted a study on extraction and quantification of anthocyanins from banana bracts using different pH and solvent concentrations. Total anthocyanin content of 224mg/kg was extracted from banana bracts using 40% ethanol at a pH of 4.

An efficient and economic method for extraction of natural red pigment, anthocyanin was identified (Mini *et al.*, 2021). Flesh and peels of pomegranate and grape were extracted independently using 100% ethyl alcohol using cold solvent extraction method adopting 1: 1 and 1: 2 w/v solid to solvent ratio for 24 and 48 hrs. Extract yield and pigment content were significantly influenced by raw material, solid to solvent ratio and duration of extraction. Extract yield was higher in peels of grape and pomegranate. With longer extraction times and higher solid to solvent ratios, the percentage yield and pigment concentration increased.

Gayathri, (2023) standardized the extraction procedure for anthocyanin pigment from pomegranate peels of ripe fruits. After surface sanitization and drying in cabinet drier at 50+- 5^0 C for 24 hrs, the filtered infusion mixture can be evaporated under water bath at 60^0C for complete removal of solvent and collection of anthocyanin extract.

The effect of plant preparation methods (eg, fresh, frozen, and freeze-dried puree) and five anthocyanin extraction methods on anthocyanin yield of freeze-dried strawberry puree were compared. The methods were methanol:water:HCl (80:20:1) suggested by Lindoo and Caldwell (1978); the methanol:water:HCl:chloroform method suggested by Neff *et al.*, (1998); the methanol:water:HCl method; and the methanol:HCl:chloroform method, both suggested by Solovchenko *et al.*, (2001); and pH differential method suggested by Lee *et al.*, (2005). The anthocyanin production was not impacted by sample preparation techniques, however it was by the extraction technique. The maximum anthocyanin production was obtained using two chloroform-containing techniques. The pH differential and the other approach using methanol:water:HCl (80:20:1) produced the lowest anthocyanin yields, whereas one method using methanol:water:HCl produced intermediate values. The pH differential approach has the shortest processing time (incubation time); but, the haze it produces could make it difficult to measure anthocyanins spectrophotometrically (Taghavi *et al.*, 2022).

The properties of the sample matrix (such as water activity, plant cell wall rigidity, etc.) and the parameters of the extraction process (pH, solvent, temperature, duration, etc.) are the primary factors influencing the extraction of anthocyanins from berries and fruit residual. As of right now, there are several different ways to extract materials: deep eutectic solvent extraction, ultrasonic assisted extraction, microwave-assisted extraction, supercritical fluid extraction, etc. The new technologies offer clear benefits in terms of extraction rate, energy consumption, extraction time, and environmental protection as compared to conventional solvent extraction (Tan *et al*., 2021).

2.6 STABILITY OF ANTHOCYANINS

Since anthocyanins are highly unstable molecules that are easily broken down, their stability is greatly impacted by the increased number of hydroxyl groups in their structures. Anthocyanin is a chemical that is reactive and this reactivity results from a deficiency of electrons in the flavylium cation nucleus, which makes color degradation easy. It happens as a result of the red flavylium cation changing into an alkali, colorless carbinol, and then finally into a colourless chalcone (Markakis, 1982).

The colour and stability of anthocyanin pigments are dependent on several factors, including structure and concentration of the pigment, pH, temperature, light intensity and quality, presence of copigments, metallic ions, enzymes, oxygen, ascorbic acid, sugars and their degradation products (Mazza & Miniati, 1993).

The anthocyanin stability can be maintained by a copigmentation reaction. It is the interaction between the structure of anthocyanin with other molecules such as metals (Al3+, Fe3+, Sn2+, Cu2+) and organic molecules such as flavonoids (flavones, flavonoons, and flavonols), alkaloid compounds (caffeine) and so on. The presence of co-pigmentation with metals and other organic molecules tends to increase the colour stability of anthocyanins (Hendry and Houghton, 1996).

Because anthocyanin is hydrophilic, it dissolves readily in water (Husna *et al.*, 2013). On the other hand, polar organic solvents including acetone, methanol, ethanol, and chloroform can also dissolve it (Kristiana *et al*., 2012). Many other factors can influence the stability of anthocyanin, which is the presence of the polyphenol oxidation enzyme, temperature, light, and oxygen. Stability of anthocyanins can increase with inter molecular copigmentation (Malien-Aubert *et al.*, 2001).

The acylation of the structure of anthocyanins found in red cabbage, black carrot, red radish, and red sweet potato is said to make them more resistant to heat and pH changes than anthocyanins found in other sources (Bąkowska-Barczak and Kolodziejczyk, 2011).

The stability of anthocyanin isolated from four distinct Berberis subspecies was assessed at a constant 25^{o} C temperature and pH of 2. The findings indicated that B. *integrrima* has the least stable form of anthocyanin and B. *khorasanica* has the most stable form. After 84 days, the percentage of anthocyanin degradation in the Berberis plant studied was 27%, 51%, 67%, and 91% for B. *integerrima*, B. *vulgaris,* B.*khorasanica,* and B. *orthobotrys* respectively (Laleh *et al*., 2006).

Although the stability of anthocyanins can be enhanced by both intra- and intercellular copigmentation as well as preparation of the gel matrix, controlling the pH of a food product should be done to prevent the shift of color. Storage should be done in dark at cool condition (Bordignon-Luiz *et al*., 2007).

Vitis vinifera L.cv. C. Abernet sauvignon was used to investigate how high temperatures affected the anthocyanin content. The total anthocyanin content of the berries at high temperature (maximum 35^{0}C) was less than half that of the control berries at maximum 25^{0} °C (Mori *et al.*, 2007).

Neves *et al*. (2019) reported the ability of anthocyanins found in elderberries to shield cells from oxidative damage.

Anthocyanins are highly sensitive to the change in pH, which results in the shift of color. Using anthocyanins as a natural colorant is suggested at low pH (pH < 4.0). At pH below 4, anthocyanins are primarily in the form of flavylium cation, which is more stable than the other structures (Tan e*t al*., 2021).

Anthocyanin pigment stability and colour are influenced by a number of factors, such as the pigment's concentration and structure, pH, temperature, light quality and intensity, co-pigment presence, metallic ions, enzymes, oxygen, ascorbic acid, sugars and their breakdown products (Herrera-Balandrano *et al*., 2021).

2.6.1 FACTORS AFFECTING ANTHOCYANIN STABILITY

In addition to structural characteristics, other factors that impact anthocyanin color stability include pH, temperature, light, co-pigment content, enzymes, oxygen, and sugars.

The stability and hue of natural pigments are significantly impacted by heat. Nevertheless, anthocyanins withstand heat better than pigments. When temperatures go close to or over 100 °C, anthocyanins begin to degrade (Jackman and Smith, 1996).

The impact of temperature, light and presence of SO_2 on the stability of anthocyanin in soft drinks was examined by Palamidis and Markakis (1975), who demonstrated that a rise in storage temperature significantly speeds up the pigment degradation process in soft drinks.

In addition to structural characteristics, other factors that affect anthocyanin color stability include pH, temperature, light, co-pigment presence, enzymes, oxygen, and sugars (Rodriguez-Saona *et al.*, 1999).

Even in the absence of light, anthocyanins are susceptible to destruction due to the presence of oxygen. This happens as a result of the medium components reacting with them through direct or indirect oxidation. When oxygen is removed from the system through heating, vacuuming, or nitrogen flow, color stability is enhanced.

The pH is another element that influences the stability of anthocyanin. The anthocyanin in samples is more severely destroyed as pH rises. Salts of flevelylium are only stable in extremely acidic environments. At higher pH values, these salts lose their proton and change into an unstable pigment termed quinoidal base. They then instantly link with water to form the colourless molecule known as chromenol.

Little (1977) investigated at how the color pigments in strawberry jam and packaged strawberries changed over time at 37.7 C. The research indicated that the degradation of anthocyanin pigments accelerated with increasing pH.

According to Maccarone *et al.* (1985) research, microwave pasteurization and the addition of glutathione and tartaric acid enhanced the stability of the anthocyanins in blood orange juice. Complexation of anthocyanins with rutin and caffeic acid provided the highest stability.

To obtain the most stable colorant, the source of anthocyanins must be taken into consideration in addition to maintaining the right temperature and pH. Anthocyanin structures vary throughout plant material sources, impacting the stability of the resulting mixtures. The acylation of anthocyanins found in red cabbage, black carrot, red radish, and red sweet potato is said to make them more resistant to heat and pH changes than anthocyanins found in other sources (Jadhav and Bhujbal, 2020).

According to Zhang *et al*. (2022), there was no significant degradation trend of anthocyanins in the temperature range of 4–65 °C and this temperature range have a significant colour enhancing effect on anthocyanin. However, the breakdown of anthocyanins is accelerated when food is processed at temperatures higher than 70 °C (Escher *et al.*, 2020). The structure of the sugar groups and replacements connected to anthocyanins determines their durability at high temperatures; acylated anthocyanins, for instance, exhibit far greater consistency at high temperatures than non-acylated anthocyanins.

2.7 ROLE OF ANTHOCYANIN AS ANTIOXIDANT

According to Khoo *et al*. (2017) anthocyanins have antioxidative and antibacterial properties, enhance neurological and visual function, and offer protection against a range of non-communicable diseases. The existence of the conjugated double bond in the anthocyanin structure can make it very reactive and can function as an antidote to radical compounds or antioxidants (Barrowclough 2015).

Pomegranate peel has the highest antioxidant activity and are rich in many compounds such as phenolics, flavonoids, ellagitannins, proanthocyanidin compounds, complex polysaccharides, and many minerals (Li *et al*., 2006). Iqbal *et al.* (2008) reported that pomegranate peel extract is a potent antioxidant for the stabilization of sunflower oil.

Pomegranate peel polyphenols have remarkable functional characteristics, such as their ability to operate as an antidiabetic (Gautam & Sharma, 2012) and antioxidant (Li *et al*., 2006). The primary cause of phenolics' antioxidant activity is their redox characteristics, which enable them to function as hydrogen donors, reducing agents, singlet oxygen quenchers, and possibly even as metallic chelating agents. Furthermore, the antioxidant activity of the extract is reliant not only on concentration but also on the structure and interactions among the antioxidants due to their synergistic effects (Seram *et al*., 2006).

Pomegranate (*Punica granatum L.*) peel is an excellent source of phenolic and antioxidant compounds, bioactive peptides and polysaccharides (Elfalleh *et al*., 2012). When compared to pomegranate juice, this inedible portion of the fruit has stronger nutraceutical activity because to its strong antioxidant, anti-inflammatory, and antinfective properties (de Silva *et al*., 2014).

Anthocyanin can react with various types of free radicals derived from reactive oxygen, such as peroxyl (ROO), hydroxyl ($^{\cdot}OH$), and singlet oxygen ($O2^{\cdot}$). The free radicals are compounds that can be formed in the strucrure itself by prooxidative enzymes as well as those from the environment such as cigarette smoke, pollution, fat oxidation, exhaust fumes, and exposure to other chemicals (Muttalib *et al.,* 2014).

Barros *et al*. (2014) evaluated the antioxidant activity of the peels of the Moriche palm (*Mauritia flexuosa* L. f.), pomegranates (*Punica granatum* L.), apples (*Malus domestica* Borkh) and grapes (*Vitis vinifera* L.) and to evaluate a juice enriched with the peel extract that which demonstrated the highest antioxidant activity. A direct relationship was observed between the enrichment of guava juice with extract of dried pomegranate peel and an increase in antioxidant activity. The result indicated that for the enrichment of guava juice with antioxidant properties, concentrations above 0.3% extract of dried pomegranate peel should be used. Anthocyanin pigments found in pigmented rice bran can be an excellent source of antioxidants when added to food products. It provides an additional means of making use of the rice milling byproduct.

Forbes-Hernandez *et al*. (2017) reported that anthocyanin has antioxidant activity, it can prevent various degenerative diseases such as cardiovascular disease, cholesterol, colon cancer, and atherosclerosis. The mechanism of anthocyanin in reducing cholesterol levels in the blood is to oxidize LDL (low density lipid).

Pomegranate peel possesses a diverse array of pharmacological and biological properties. Antioxidant, anticancer, and antimutagenic qualities of pomegranate peel are considered to be its main health-promoting characteristics (Abid *et al*., 2017).

Pomegranate peels can be a good source of high-value antioxidants (Essa & Mohamed, 2018). The more phenolic hydroxyl groups that are bound to anthocyanin, the stronger the antioxidant activity (Han *et al*., 2017).

2.8 ANTHOCYANIN INCORPORTATED IN FOOD PRODUCTS

A blueing effect might result from anthocyanin extract absorption onto particle matter, hence its application is limited in somewhat foggy or cloudy liquids. The coloring of acid fruit preparations, jams, and preserves are among the other uses for anthocyanin extracts. Because the recipes for these vary depending on the type and quality of fruitwhether it's frozen, fresh, or sulphited they need to be flexible (Bridle and Timberlake, 1997).

The use of anthocyanins as food colorants not only confers improvements in product appearance but also prevents autoxidation and lipid peroxidation in biological systems (Shipp and Abdel-Aal, 2010). It has been demonstrated that acylated anthocyanins are a possible substitute for artificial colors in food systems. A vast range of colours can be produced, depending on the food matrix's pH and the anthocyanins' chemical structure.

The need for natural food colorants has been growing as a result of consumer pressure, societal shifts, and technical advancements. Natural food coloring agents such as carotenoids, anthocyanins, annatto, and paprika can serve as an alternative for their synthetic counterparts, particularly in single-phase food coloring systems used in drinks and baked goods liquid phase (Mortensen, 2006).

There is a notable retention of grape anthocyanins in the pomace, which could potentially play a significant role in the extraction of these pigments. These pigments are not quantitatively transported to the wine or juice. Italian grape anthocyanin products, sold as Enocianina, or Enocyanin, included a powder and a solution (Garoglio, 1965). Red wine fortification was the main purpose of these goods.

Palamidis and Markakis (1975) tried to extract anthocyanin of fermented grape skins and to study the stability of these pigments in a nonalcoholic carbonated beverage to which they were added as colorants. The anthocyanin extraction solvent utilized to produce the two beverages hot water or 500 ppm SO was the only difference between them. Sodium benzoate 0.05, citric acid 0.1, sucrose 13.0, grape flavor 0.2, and anthocyanin powder 0.7 made up the beverages' composition, measured in grams per 100 milliliters. Additionally, CO2 1.7 volumes were present in the drink. Before combining the syrup with the other ingredients, it was brought to a boil for five minutes. At 0°C, the beverage was fully carbonated. There was a pH of 3.7 in the drinks. The beverage created with hot water anthocyanin extract had a color equivalent to 581 mg of phenol per 100 ml, while the beverage prepared with aqueous SO (500 ppm) anthocyanin extract had a color similar to 640 mg of phenol. The beverages were stored under the conditions, viz., darkness at 3.5 ± 2°C; darkness at 10 ± 2°C; darkness at 20 ± 2°C; darkness at 38 ±1°C; diffuse daylight at 20 ± 2°C; and continuous fluorescent light at 22 ±2°C. Increasing the storage temperature and exposure to light accelerated the pigment destruction in the beverage. In darkness, at 38^{O}C, after 135 days, only 23% of the original amount of hot-water-extracted pigment was left in the beverage, while at 3.5^{0}C, under the same conditions of storage, 92% of the pigment was reatined. A higher stability was noticed for the anthocyanin extracted with 500 ppm SO2 solution than with hot water.

Kanemozhi *et al*. (2020) have utilized pomegranate peel colour as natural colourant in formulation of jelly with no or minimum side effects. Jellies were prepared at pH (5, 7 and 9) and with 2% and 10% concentration of fresh pomegranate peel extract. The study proved the possibility of utilizing pomegranate peel in the development of jelly with no or minimum side effect. The prepared jelly showed excellent properties like colour, taste, odour, flavour, texture and smoothness.

The use of red radish anthocyanin extract for coloring maraschino cherries was evaluated (Wrolstad *et al.*, 2005). The cherries that had been bleached, both primary and secondary, were sweetened to a pH of 3.5 and colored with FD&C Red No. 40 (200 ppm) and two different quantities of radish anthocyanin extracts (60 and 120 mg monomeric anthocyanin per 100 ml syrup). Analyzing the color of the cherries and syrup revealed that maraschino cherries were given a red color that was strikingly similar to FD&C Red No. 40 at pH 3.5 thanks to acylated pelargonidin derivatives that were isolated from red radishes. The colour and pigment durability of cherries and syrup were assessed after a year of storage at 20 °C in the dark and exposure to light (syrup only). Syrups colored with 60 and 120 mg of monomeric anthocyanin per 100 milliliters of syrup had half lives of 29 and 33 weeks, respectively. Increased anthocyanin concentration shielded the colour stability. When brined cherries were stored for six months at room temperature, the colour features of the radish-coloured cherries were very similar to those with FD&C Red No. 40. The strong colour and stability of the pigment were ascribed to the anthocyanin moiety's attachment to hydroxycinnamic and malonic acid acylating groups. These findings indicated that, even under the difficult storage conditions of room temperature and light exposure, radishes would make a great substitute for FD&C Red No. 40 when used for maraschino cherry.

A solution of 150 ppm allura red was used as the final chroma, and anthocyanin pigment was used to color model juice solutions containing 0.1 M citric acid at pH 3. FD&C Red No. 40's intended orange-red color was most closely resembled by the two pelargonidin-based extracts made from red potatoes and red radish. Giusti and Wrolstad, 2003) reported the solutions of black carrot extract (mono-acylated cyanidin derivatives) had a more reddish hue, while meganatural red grape (mono-acylated derivatives of different anthocyanins) and red cabbage (mono- and di-acylated cyanidin derivatives) extracts imparted a more purplish colour.

Giusti and Wrolstad (2003) examined the feasibility of colouring dairy products with pH levels between 4.2 and 4.5 with acylated anthocyanins derived from edible sources. Red cabbage, black carrot, red radish, and grape skin extract were among the extracts examined. The findings demonstrated that at concentrations as low as 5 mg monomeric anthocyanin per 100 g sample, radish and carrot, either alone or in combination, could give these systems the desired red hue. Samples dyed with black carrot had higher colour intensity according to colour measurements and eye inspection than samples coloured with radish. The purple hue of red cabbage was striking, evoking the hue of blueberries.

Barros *et al*. (2014) prepared guava juice enriched with the pomegranate peel extract with the high antioxidant activity. The enrichment of commercially-prepared guava juice did not alter any of the sensory analyses.

Santiago *et al.* (2016) described and assessed jamun fruit peel powder, which was produced by a straightforward drying procedure, as a natural colorant and a source of bioactive chemicals. Because of the high starting concentration of these pigments, even with a decline in anthocyanin levels, the powder retained its quality as a colorant during the stability study. Because of its antioxidant qualities, this substance may also be utilized as a functional ingredient in the creation of food products. Additionally, the powder showed to be high in dietary fiber, making it a useful component for diets low in calories.

Natural antioxidants can be found in soy powder and powdered dragon fruit peel. In order to create an antioxidant beverage, Rosiana *et al.*, (2021) examined the antioxidant components in a mixture of soy powder and powdered dragon fruit peel. Different ratios of soy powder and dragon fruit peel powder were utilized in the research design (0%:100%; 25%:75%; 50%:50%; 75%:25%; 100%:0%). The combination of flavonoids, anthocyanins, and phenol was found to include antioxidant compounds. The beverage's pH ranged from 5,25 to 6,83; its water content ranged from 4,81 to 6,29%; its phenol content ranged from 250,33 to 908,00 mg/100g; its flavonoid ranged from 250,33 to 908,00 mg/100g; its anthocyanin ranged from 8,00 to 387,83 mg/100g; and its IC50 was 17,61 to 18,60.

According to Altunkaya *et al.* (2013), because of its possible health benefits, pomegranate peel powder (PP), a by-product of the pomegranate juice industry high in polyphenols, was investigated for use in the manufacturing of bread. Different amounts of PP (0 to 10 g per 100 g flour) were used to create wheat bread, and the resulting antioxidant levels, represented as Trolox equivalent antioxidant capacity values (TEAC), ranged from 1.8 to 6.8 mmol TEAC per g bread for fresh bread.

The use of anthocyanin rich purple corncob waste as a milk colorant was studied by Jing and Giusti (2005). Violet corn Color was effectively added to milk matrices by ARW. When exposed to heat, milk's proteins and lipids appeared to shield anthocyanins from deterioration. Peonidin-3-(6"-malonylglucoside) is one example of an acylated anthocyanin that was more resilient to heat treatment. It was proposed that a byproduct of purple corncob anthocyanin extraction might be used to create novelty products with novel flavors, colors, and health advantages, as well as to add color to products with a pH range uncommon for anthocyanin uses. The anthocyanin retention of pasteurized milk is enhanced and milk takes on a pleasant "purple" tint when water-insoluble anthocyanin extracts from purple corncob are added.

Addition of pomegranate peel pigment into ice cream at 0.5 and 1.0% (w/w) showed significant improvement in the functional properties viz., antioxidant and α-glucosidase inhibitory activities of the enriched ice creams compared to control sample (Çam *et al*., 2014). Antioxidant activity as EC_{50} and α-glucosidase inhibitory as IC_{50} of 1.0% phenolic enriched ice creams were 133.3 and 22.9 μg/mL, respectively. More than 75% of the panellists accepted the phenolic enriched ice creams in sensory evaluation.

Although soy sauce in dried-tofu coloring is one of the often utilized techniques, the production process of soy sauce created trace levels of methylglyoxal (MGO). Lin *et al*., (2018) evaluated the feasibility of using natural colorants in Taiwan as food colorants on dried-tofu and risk assessment of MGO at the dietary exposure in soy sauce with the objective of making dried tofu more suitable based on the needs of current social food safety and the concept of healthy diet under the food culture of Taiwan. The feasibility of different natural colorants viz., Gardenia Yellow, Curcumin and Radish Red was proved as food colorants on dried-tofu. The combined natural colorants of them was proved as novel colouring materials in the industry of dried tofu in Taiwan. Usage of soy sauce as a colouring agent was proved to be safe.

The effects of pomegranate peel supplementation on the nutritional, physical, and chemical characteristics of muffin cakes were investigated by Topkaya and Isik (2019). The inclusion of pomegranate peel resulted in a significant increase in the levels of total and insoluble dietary fibers, total phenolics, magnesium, calcium, and potassium contents, and total antioxidant activity. Pomegranate peel considerably raised the hardness and lowered the springiness ratings. Both the control cake and the cakes with 5 and 10% pomegranate peel scored similarly for flavor and fragrance.

Urganci, and Fatma (2021) observed the attributes of high-quality biscuits enhanced with pomegranate peel. Pomegranate peel substitution did not cause any differences in crude protein, crude ash and crude fat of the biscuits. With the increase of pomegranate peel rate in formulation, dietary fiber, total phenolics and antioxidant capacity increased significantly thus enriching biscuits with pomegranate peel as functional biscuits.

When added to food products, anthocyanin pigments from colored rice bran have been shown to be an excellent source of antioxidants. The qualities of spray-encapsulated anthocyanins are enhanced by the addition of protein. Fruit juice can have these substances added to it to enhance its beneficial qualities (Mahanta and Baruah, 1992**).**

3. MATERIALS AND METHODS

The present investigation on “Utilization of pomegranate (*Punica granatum* L.*)* peel as a natural food colourant” was undertaken with the objective of standardizing food formulations using pomegranate peel as a natural colourant.The materials used and methodologies adopted for the investigation are described in this chapter.

The experiment was carried out in two different parts:

1. DEVELOPMENT OF PROCESSED PRODUCTS WITH ANTHOCYANIN PIGMENT.
2. EVALUATION OF PIGMENT STABILITY IN PRODUCTS

3.1 DEVELOPMENT OF PROCESSED PRODUCTS WITH ANTHOCYANIN PIGMENT.

Peels of ripe pomegranate fruits of uniform maturity and good quality were collected from processing unit of Dept. of Postharvest Management and were utilized for the experiment.

3.1.1. Extraction of anthocyanin pigment

Anthocyanin pigment was extracted from peel of pomegranate fruits as per the standardized protocol (Mini *et al*., 2021 and Gayathri, 2023). The pomegranate peels collected were surface sanitized using 2ppm ozonised water for 10 minutes, cut into approximate 2 cm^3 size, stored at 4^0C in dark for 24hrs and dried in a cabinet drier at 50+- 5^0 C for 24 hrs. The crushed dehydrated peel pieces were macerated using acidified ethanol with 1% HCl in 2:1 liquid to solid ratio for 48 hrs under room temperature (30-35^0C) & 75-80% relative humidity. The filtered infusion mixture was evaporated under water bath at 60^0C for complete removal of solvent and collection of anthocyanin extract, for incorporation into food products (Plate 1).

Good quality ripe fruit of pomegranate

Peels drying in cabinet drier at $50 \pm 5^{0}C$ for 24 hrs

Colour extract

Plate 1 Preparation of anthocyanin pigment

Three processed products viz., candy, jelly and squash each of solid, semi-solid and liquid nature were selected for incorporation of anthocyanin pigment as natural food colorant.

3.1.2. Preparation of processed products

3.1.2.1 Preparation of squash (liquid product)

Quality lime with good juice content was procured from M/s.Sangamaithri, Kakkamoola, Trivandrum for preparation of squash.

Lime squash was prepared as per the FSSAI standards and anthocyanin extract prepared as per 3.1.1 was incorporated as natural food colour at various concentrations.

T_1 - 1%

T_2 - 2%

T_3 - 3%

T_4- without pigment (control)

The experiment was conducted in Completely Randomised Design and replicated four times.

3.1.2.2 Preparation of Jelly (Semi-solid product)

Jackfruit (*Artocarpus heterophyllus*) was purchased from Instructional Farm Vellayani, Trivandrum. Pectin extract was prepared from the non edible portions of Jackfruit, excluding the outer green spiny portion, and it was utilized for jelly preparation as per the FSSAI Standard.

The extracted anthocyanin pigment was incorporated in jelly at different concentrations.

T_1 - 1%

T_2 - 2%

T_3 - 3%

T_4- without pigment (control)

The experiment was conducted in Completely Randomised Design and replicated four times.

3.1.2.3 Preparation of Ash gourd candy (Solid product)

Mature firm good quality ashgourd (*Benincasa hispida*) was utilized for preparation of candy as per the FSSAI standard and anthocyanin extract was incorporated as natural food colour at various concentrations.

T_1 - 1%

T_2 - 2%

T_3 - 3%

T_4- without pigment (control)

The experiment was conducted in Completely Randomised Design and replicated four times.

3.1.3 Selection of pigment concentration

The prepared products *viz*., candy, jelly and squash incorporated with natural anthocyanin pigment were subjected to chemical, nutritional and sensory quality parameters and the best concentration of colour pigment was selected for each product.

.3.1.3.1 Quality evaluation of the products

3.1.3.1.a. Total soluble solids (TSS) (^{0}Brix)

Total Soluble Solids of squash, jelly and candy was measured using Erma hand refractometer and expressed in degree brix (^{0}Brix).Refractometer of range 28-62^o was used to measure TSS of squash, whereas TSS of jelly and candy was measured using refractometer of range 58-92$^{o.}$

3.1.3.1.b. Acidity (%)

The method described by Sadler and Murphy (2010) was followed to determine acidity of all the three products in per cent.

$$Acidity = \frac{(Titre\ value\ \times Normality\ of\ NaOH(0.1N) X\ Volume\ make\ up(100\ mL) X\ Equivelent\ weight\ of\ \ acid}{Volume\ of\ aliquot\ (10mL) \times\ Weight\ of\ sample\ (5g)}$$

3.1.3.1.c Ascorbic Acid (mg 100g^{-1})

Ascorbic acid content of the products was estimated by the titrimetric method described by Ranganna (1986) using 2, 6-dichloro phenol indophenol (DCPIP) dye and expressed as mg 100g^{-1}.

$$Ascorbic\ acid\ \left(mg\ 100g^{-1}\right) = \frac{Titrevalue(V1) \times Dye\ factor \times Volume\ made\ up(mL)}{Aliquot\ of\ extract\ taken(mL) \times Weightof\ sample(g)}$$

Dye factor = 0.5/V_1 mL

3.1.3.1.d Reducing sugars (%)

The titrimetric method of Lane and Eynon described by Ranganna (1986) was adopted for the estimation of reducing sugar in % in all the three products.

$$Reducing\ Sugar = \frac{Glucose\ Eq.(0.05)\ \times\ Total\ volume\ made\ up(mL)}{Titre\ value(mL)\ \times\ Weight\ of\ the\ sample}$$

3.1.3.1.e Total Sugar (%)

The total sugar content in all the three products was expressed as per cent in terms of invert sugar according to the following formula (Ranganna, 1986).

$$Total\,Sugar = \frac{Glucose\,Eq.(0.05) \times\ Total\,volume\,made\,up(mL) \times Volume\,made\,after\,inversion(mL)}{Titre\,value(mL) \times\ Weight\,of\,pulp\,taken(g) \times Aliquot\,taken\,for\,inversion(mL)} \times\ 100$$

3.1.3.1.f Moisture content (%)

Moisture content of the products such as jelly and candy was determined using moisture analyser, which dries the sample using a halogen lamp and gives the moisture content based on the principle of thermogravimetric analysis.

3.1.3.1.g Total anthocyanin content (mg 100g^{-1})

Estimation of anthocyanin content was done using the spectrophotometric method described by Abdel-Aal *et al*., (2006) with proper modification. One gm of the sample was dipped in 10ml of 85% methanolic HCl for one day and then absorbance was measured on a UV-vis spectrophotometer at 535nm.

$$TOTAL\ OD/100gm\ (A) = \frac{(OD \times Volume\ made\ up \times 100) \times dilution\ factor}{Weight\ of\ sample}$$

$$Total\,anthocyanin\ (mg/100gm) = \frac{A}{98.2}$$

3.1.3.1.h Antioxidant Activity (%)

Total antioxidant activity of the products was determined using 2,2- diphenyl-1-picryhydrazyl (DPPH) radical scavenging assay according to the procedure described by Sharma and Bhat (2009).

$$\%\,inhibition\,of\,DPPH = \frac{\{A\,blank - A\,sample\} \times 100}{A\,blank}$$

Where,

A_{blank} – Absorbance of DPPH solution without sample, read against ethanol blank.

A_{sample} – Absorbance of the test sample after 30 min.

3.1.3.1.i Organoleptic evaluation of the products

Organoleptic scoring/hedonic rating was done by a semi trained panel comprising of 30 members selected from research students and staff members of College of Agriculture, Vellayani. The panel were asked to score the appearance, colour, flavour, texture and taste of the products (squash, jelly and candy) using 9-point hedonic scale (Wichchukit and o'Mahony, 2015) in the order of preference as shown below.

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

The score card used for sensory scoring of processed products is shown in Fig 1.

COLLEGE OF AGRICULTURE, VELLAYANI

DEPARTMENT OF POSTHARVEST MANAGEMENT

Scorecard for Organoleptic evaluation of Coloured squash, jelly and Candy

Instruction: You are given 4 sets of samples. Evaluate them and give score for each.

CRITERIA	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4	REMARKS (Off-flavour /off- taste if any)
APPEARANCE					
COLOUR					
FLAVOUR					
TASTE					
TEXTURE					
OVERALL ACCEPTABILITY					

Scores

Like extremely :9
Like very much :8
Like moderately :7
Like slightly :6
Neither like or dislike :5
Dislike slightly :4
Dislike moderately :3
Dislike very much :2
Dislike extremely :1

Name:

Signature:

Date:

Fig 1. Scorecard for organoleptic evaluation of squash, jelly, candy

3.1.3.1.j Cost of production

Cost of production of one litre / kilogram products was calculated based on the current market price, taking into account all aspects of variable costs involved in the preparation.

Based on the quality parameters, the best concentration of colour pigment was selected for incorporating in each product.

3.2. EVALUATION OF PIGMENT STABILITY IN PRODUCTS

Processed products *viz*., squash, jelly and candy were prepared by incorporating the anthocyanin pigment at the best concentration, selected from 3.1 of the experiment. Stability of pigments was assessed by storing the product independently in glass containers under ambient and refrigerated condition. Observations were taken initially and at monthly intervals for a period of 3 months.

The experiment was conducted in Completely Randomised Design and replicated three times.

3.2.1. Changes in chemical quality parameters during storage

3.2.1.a TSS (^{0}Brix)

Total Soluble Solids (TSS) of the stored products was determined as described in 3.1.3.1.a

3.2.1.b Acidity (%)

Titratable acidity of stored products was determined as described in 3.1.3.1.b.

3.2.1.c Moisture content (%)

Moisture content of the products such as jelly and candy was determined as described in 3.1.3.1.f.

3.2.1.d Reducing sugars (%)

Reducing sugar of the stored products was calculated as described in 3.1.3.1.d.

3.2.1.e Total sugar (%)

Total sugar of stored products was calculated as described in 3.1.3.1.e.

3.2.1.f Ascorbic Acid (mg 100g^{-1})

Ascorbic acid content of the stored products was calculated as described in 3.1.3.1.c.

3.2.1.g Total anthocyanin content (mg 100g^{-1})

Estimation of anthocyanin content during storage was done using the procedure described in 3.1.3.1.g.

3.2.1.h. Antioxidant Activity (%)

Antioxidant activity of the stored products was calculated as described in 3.1.3.1.h.

3.3. STATISTICAL ANALYSIS

The data generated from each experiment were tabulated and analysed statistically using Completely Randomized Design (CRD) and significance was tested using analysis of variance (ANOVA). Experiment on evaluating the stability of the colour extract was analysed by paired t-test to calculate significant difference in treatment means.The General R based Analysis Platform Empowered by Statistics (GRAPES) version 1.0.0 (Gopinath *et al*., 2021) was used to analyse the data. The scores obtained for sensory parameters were statistically analysed using Kruskall-Wallis Chi-square test (Shamrez *et al*.,2013).

4. RESULTS

Results of the experiment titled “Utilization of pomegranate (*Punica granatum* L.*)* peel as a natural food colourant” are presented under the following headings

4.1. Development of processed products with anthocyanin pigment.

4.2. Evaluation of pigment stability in products

4.1 DEVELOPMENT OF PROCESSED PRODUCTS WITH ANTHOCYANIN PIGMENT.

Anthocyanin pigment extracted by the standardised procedure (Mini *et al*., 2021) was incorporated into three processed products *viz* candy, squash and jelly representing solid, liquid and semi solid nature.

4.1.1 Squash

4.1.1.a Preparation of squash (Liquid product)

Lime squash prepared as per the FSSAI standard was incorporated with anthocyanin extract as natural colour at 1%, 2%, 3% and without pigment (0%) as control (Plate 2) .The prepared products were analysed for quality parameters for selection of the best concentration.

4.1.1.b Evaluation of quality parameters

Chemical, nutritional and sensory quality parameters of the squash incorporated with anthocyanin pigment were evaluated.

Chemical and nutritional quality parameters

Chemical and nutritional quality parameters *viz*., TSS, acidity, reducing sugar, total sugar, ascorbic acid, anthocyanin content and antioxidant activity were evaluated.

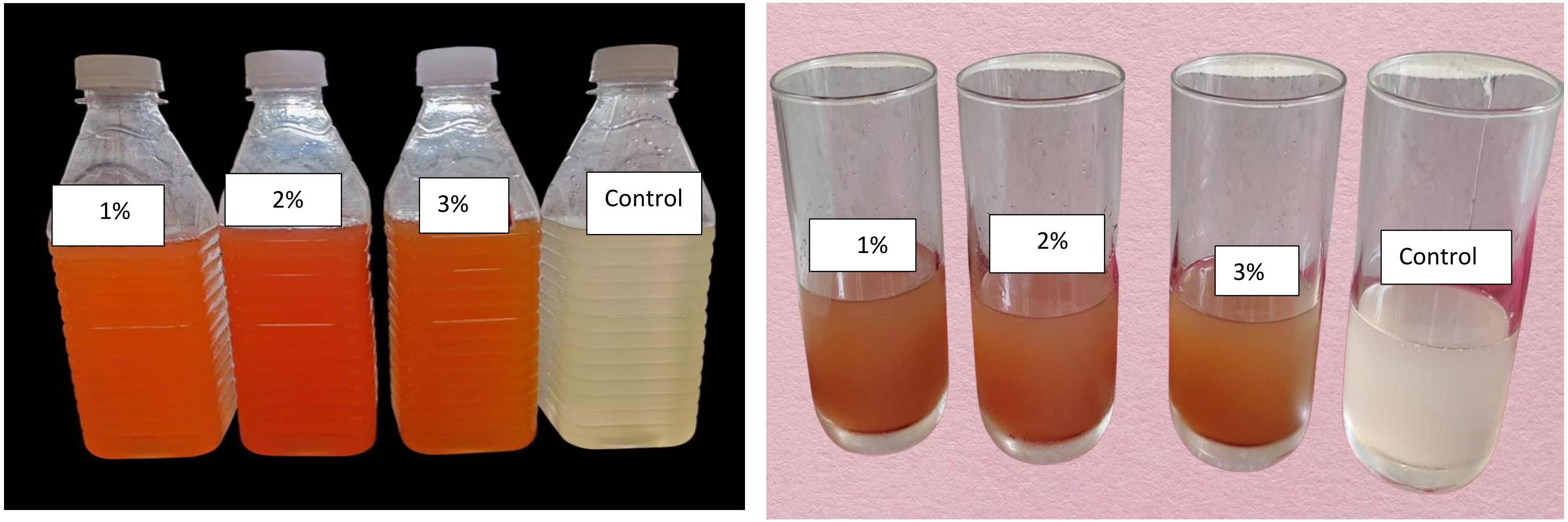


Plate. 2 Squash incorporated with different concentration of anthocyanin pigment

Table.1 Effect of pigment concentration on chemical and nutritional quality parameters of squash.

Pigment concentration in squash	TSS (º Brix)	Acidity (%)	Reducing sugar (%)	Total sugar (%)	Ascorbic acid (mg $100g^{-1}$)	Anthocyanin content (mg $100g^{-1}$)	Antioxidant activity (%)
1%	41.20	1.92	33.33	41.66	416.00	1.92	90.08
2%	41.10	2.03	31.25	44.64	332.80	7.42	89.44
3%	41.80	2.12	23.80	48.07	291.20	10.10	87.80
Control (no pigment)	42.00	1.98	22.72	44.64	416.00	0.76	48.22
S.Em±	**0.118**	**0.105**	**0.767**	**0.155**	**0.163**	**0.115**	**0.159**
CD at 5%	**0.09**	**NS**	**4.12**	**NS**	**48.89**	**0.06**	**2.64**

TSS (^{0}Brix)

TSS of the squash was significantly influenced by pigment concentration .TSS of the colourless squash was the highest (42^0 Brix). Among the coloured squash, the highest TSS (41.80^0 Brix) was recorded in squash incorporated with 3% pigment which was significantly higher than TSS of all other squash.

The lowest TSS (41.10^0 Brix) was recorded in squash incorporated with 2% anthocyanin pigment.

Acidity (%)

There was no significant difference between the acidity of squash due to pigment incorporation. However the lowest acidity (1.92***%***) was recorded in squash incorporated with 1% anthocyanin pigment.

Reducing Sugar (%)

There was significant difference between the reducing sugar of pigment incorporated squash. 1% pigment incorporated squash recorded the highest reducing sugar of 33.33% which was on par with squash incorporated with 2% anthocyanin pigment (31.25 %).

Squash without colour addition showed the lowest reducing sugar of 22.72 % which was on par with the squash added with 3% anthocyanin pigment (23.80%).

Total sugar (%)

There was no significant difference between the total sugar content of squash. However highest total sugar (48.07%) was recorded in squash incorporated with 3% anthocyanin pigment.

Ascorbic acid (mg 100g^{-1})

There was significant difference between the ascorbic acid content of squash. The highest ascorbic acid (416 mg 100g^{-1}) was recorded in squash incorporated with 1% pigment and squash prepared without pigment.

The lowest ascorbic acid (291.20 mg $100g^{-1}$) was recorded in squash incorporated with 3% anthocyanin pigment, which was on par with the squash incorporated with 2% anthocyanin pigment (332.80 mg $100g^{-1}$).

Anthocyanin content (mg $100g^{-1}$)

The anthocyanin content was significantly influenced by pigment concentration. The highest anthocyanin content (10.10 mg $100g^{-1}$) was recorded in squash incorporated with 3% pigment, which was significantly different from all other squash. The next highest anthocyanin content (7.42 mg $100g^{-1}$) was for squash incorporated with 2% pigment. The lowest anthocyanin content (0.76 mg $100g^{-1}$) was recorded in squash without anthocyanin pigment.

Antioxidant activity (%)

There was significant difference between the antioxidant activity of squash (Table 1).The antioxidant activity was highest (90.08%) in squash with 1% pigment which was on par with 2% pigment incorporated squash (89.44%) and squash incorporated with 1% (87.80).The lowest antioxidant activity was recorded in colourless squash (48.22%).

Sensory quality parameters

The prepared squash were analyzed for organoleptic quality parameters and shown in Table 2.

There was no significant difference between the sensory scores of squash with respect to texture.

It was observed that squash incorporated with 2% anthocyanin had the highest mean score for appearance (7.86) and for colour (8.22).

Among the coloured squash, squash incorporated with 1% anthocyanin had the highest mean score for flavor (7.36). Colourless squash recorded highest sensory score for flavor (7.63) and taste (8.09).

When overall acceptability score of coloured squash were computed, squash with 2% colour pigment showed the highest mean score of 7.52 and squash incorporated

Table 2. Effect of pigment concentration on sensory quality parameters of squash

Pigment concentration in Squash	**Sensory quality parameters**											
	Appearance		**Colour**		**Flavour**		**Texture**		**Taste**		**Overall acceptability**	
	Mean score	**Rank**	**Mean score**	**Rank**	**Mean score**	**Rank**	**Mean score**	**Rank**	**Mean score**	**Rank**	**Mean score**	**Rank**
1%	7.04	36.97	7.27	40.11	7.36	48.78	7.50	45.20	7.04	41.09	7.24	41.02
2%	7.86	58.02	8.22	61.50	7.09	46.34	7.59	46.56	6.86	41.97	7.52	49.61
3%	6.50	30.93	6.77	33.50	6.22	31.06	6.95	35.19	6.04	30.63	6.49	32.18
Control (no pigment)	7.59	52.06	6.90	45.40	7.63	58.34	7.63	48.63	8.09	64.29	7.56	55.18
KW value	**18.29**		**15.63**		**13.20**		**4.04**		**21.30**		**10.31**	
$\chi 2$	**7.81**											

with 3% pigment had the lowest score of 6.49. Compared to coloured squash, colourless squash had the highest overall acceptability score (7.56).

4.1.1.c Cost of production

Cost of production of pigment incorporated squash was calculated and compared with that of squash without pigment to analyze whether the production of squash is cost effective or not.

Cost of production of one litre pigment incorporated squash at 1%,2%,3% and without pigment was Rs.63.40/-,Rs.75.80/-,Rs.88.20/- and Rs.51.00/- respectively (Table 3 and Annexure 1).

Table 3.Cost of production of one litre pigment incorporated lime squash

Treatments	**Cost of production Rs./litre**
Squash with 1% pigment incorporation	63.40
Squash with 2% pigment incorporation	75.80
Squash with 3% pigment incorporation	88.20
Squash without pigment(control)	51.00
C.D at 5%	**2.56**

4.1.1.d Selection of the best pigment concentration

Squash prepared by addition of 1% anthocyanin pigment had least acidity (1.92%), maximum reducing sugar (33.33 %) ascorbic acid (416.0 mg $100g^{-1}$) and antioxidant activity (90.08%).

Squash incorporated with 2% and 3% pigment had similar and highest anthocyanin content and antioxidant activity

When sensory parameters were compared, squash with 2% was sensorily superior with higher overall acceptability score of 7.52 compared to squash with 3% pigment (6.49).

Hence, 2% was selected as the best concentration of anthocyanin pigment for incorporation in lime squash.

4.1.2 Jelly

4.1.2.a Preparation of jelly (Semisolid product)

Jelly was prepared from non edible portions of jackfruit as per the FSSAI standard and anthocyanin extract was incorporated as natural food colour at various concentrations of 1%, 2%, 3% and without pigment as control (Plate 3). The prepared products were analysed for quality parameters for selection of the best concentration.

4.1.2.b Evaluation of quality parameters

Chemical and nutritional quality parameters

Chemical and nutritional quality parameters *viz*., TSS, acidity, reducing sugar, total sugar, ascorbic acid, anthocyanin content, antioxidant activity and moisture content of jelly were evaluated and shown in Table 4.

TSS (^{0}Brix)

TSS of the prepared jelly was significantly influenced by pigment concentration. TSS of the colourless jelly and jelly with anthocyanin pigment added at 1% recorded the highest TSS (66 ^{0}Brix) than TSS of all other jellies.

The lowest TSS (65.50^{0}Brix) was recorded in jelly incorporated with 3% anthocyanin pigment.

Acidity (%)

There was significant difference between the acidity of jellies due to pigment incorporation. The lowest acidity (0.89***%***) was recorded in jelly incorporated with 3% anthocyanin pigment .Jelly with 2% anthocyanin pigment incorporation recorded with acidity of 1.02%.

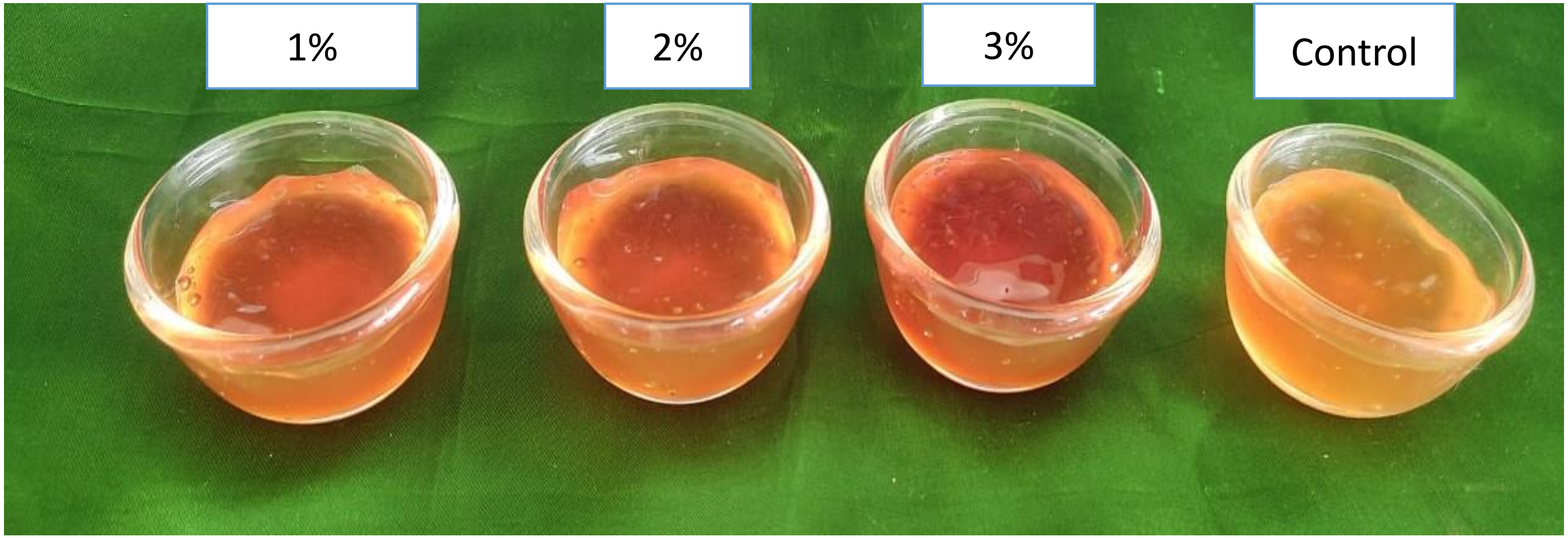


Plate. 3 Jelly incorporated with different concentration of anthocyanin pigment

Jelly with 1% anthocyanin pigment incorporation showed the highest acidity (1.19%).

Reducing Sugar (%)

There was significant difference between the reducing sugar of pigment incorporated jellies. 1% pigment incorporated jelly recorded the highest reducing sugar of 20.89 %.

Jelly with colour addition of 3% anthocyanin pigment showed the lowest reducing sugar of 12.50 % which was on par with colourless jelly.

Total sugar (%)

There was no significant difference between the total sugar content of jellies. However highest total sugar (55.23 %) was recorded in jelly incorporated with 1% anthocyanin pigment.

Ascorbic acid (mg 100g^{-1})

There was significant difference between the ascorbic acid content of jellies. The highest ascorbic acid (118.45mg 100g^{-1}) was recorded in jelly without pigment addition. The lowest ascorbic acid was recorded in 3% pigment incorporated jelly (60.60 mg 100g^{-1}) which was on par with jelly incorporated with 1% anthocyanin pigment (71.62 mg 100g^{-1}).

Anthocyanin content (mg 100g^{-1})

The anthocyanin content was significantly influenced by pigment concentration. Among the jellies, the highest anthocyanin content (5.52 mg 100g^{-1}) was recorded in jelly incorporated with 3% pigment incorporated jellies which was on par with jelly incorporated with 2% (4.47 mg 100g^{-1}).The lowest anthocyanin content (0.69 mg 100g^{-1}) was recorded in jelly without anthocyanin pigment.

Table 4. Effect of pigment concentration on chemical and nutritional quality parameters of jelly

Pigment concentration in jelly	**TSS (^{0}Brix)**	**Acidity (%)**	**Reducing sugar (%)**	**Total sugar (%)**	**Ascorbic acid (mg $100g^{-1}$)**	**Anthocyanin content (mg $100g^{-1}$)**	**Antioxidant activity (%)**	**Moisture content (%)**
1%	66.00	1.19	20.89	55.23	71.62	3.56	43.45	25.33
2%	65.70	1.02	15.46	50.74	63.36	4.47	39.78	25.83
3%	65.50	0.89	12.50	40.50	60.60	5.52	41.00	25.90
Control (no pigment)	66.00	1.11	13.16	46.92	118.45	0.69	32.28	25.96
S.Em±	**0.196**	**0.065**	**0.147**	**0.135**	**0.189**	**0.069**	**0.134**	**0.153**
CD at 5%	**0.09**	**0.19**	**1.50**	**NS**	**17.96**	**0.070**	**5.23**	**0.26**

Antioxidant activity (%)

There was significant difference between the antioxidant activity of jellies (Table 4).The antioxidant activity was highest (43.45%) in jelly with 1% pigment which was on par with 3% pigment incorporated jelly (41.00%) and jelly incorporated with 2% anthocyanin pigment (39.78%).The lowest antioxidant activity was recorded in colourless jelly (32.28%).

Moisture content (%)

There was significant difference between the moisture content of anthocyanin pigment incorporated jelly as in Table 4.

Moisture content of jelly was lowest in jelly with 1% anthocyanin pigment incorporation (25.33%).

The highest moisture content was observed in jelly without pigment incorporation (25.96%) which was on par with moisture content of 2% anthocyanin pigment incorporated jelly (25.83%) and 1% pigment incorporated jelly (25.90%).

Sensory quality parameters

The prepared jellies were analyzed for organoleptic quality parameters and shown in Table 5.

There was no significant difference between the sensory scores of jellies with respect to flavor and taste.

It was observed that jelly incorporated with 3% anthocyanin had the highest mean score for appearance (8.30) and for color (8.70). Jelly incorporated with 2% anthocyanin had the highest mean score for texture (8.05).

When overall acceptability of coloured jellies as computed, jelly with 3% colour pigment and 2 % colour pigment showed highest mean score of 8.13 and jelly without pigment had the lowest score of 7.44.

4.1.2.c Cost of production

Cost of production of pigment incorporated jelly was calculated and compared with that of jelly without pigment to analyze whether the production of jelly is cost effective or not.

Cost of production of one kilogram pigment incorporated jelly at 1%,2%,3% and without pigment was Rs.92.40/-, Rs104.80/-, Rs.115.20/- and Rs.80.00/- respectively (Table 6 and Annexure 2).

Table 6.Cost of production of one kilogram jelly

Treatments	**Cost of production (Rs./kg)**
Jelly with 1% pigment incorporation	92.40
Jelly with 2% pigment incorporation	104.80
Jelly with 3% pigment incorporation	115.20
Jelly without pigment(control)	80.00
C.D at 5%	**3.96**

Table 5. Effect of pigment concentration on sensory quality parameters of jelly

Pigment concentration in jelly	**Sensory quality parameters**											
	Appearance		**Colour**		**Flavour**		**Taste**		**Texture**		**Overall acceptability**	
	Mean score	**Rank**	**Mean score**	**Rank**	**Mean score**	**Rank**	**Mean score**	**Rank**	**Mean score**	**Rank**	**Mean score**	**Rank**
1%	7.70	35.85	7.90	36.70	7.70	35.55	8.35	46.22	8.00	45.90	7.93	39.05
2%	7.95	43.37	8.05	40.57	8.25	49.27	8.35	46.67	8.05	47.77	8.13	47.55
3%	8.30	51.40	8.70	58.92	8.00	42.05	7.90	38.12	7.75	37.95	8.13	47.22
Control (no pigment)	7.45	31.37	7.25	25.80	7.60	35.12	7.55	30.97	7.35	30.37	7.44	28.18
KW value	**9.66**		**23.75**		**5.48**		**6.94**		**8.33**		**9.32**	
$\chi 2$	**7.815**											

4.1.2.d Selection of best concentration of jelly

Jelly incorporated with 1% pigment had highest reducing sugar (20.89%), total sugar (55.23 %) and antioxidant activity (43.45%).

When sensory parameters were compared, jelly with 2% and 3% was sensorily superior with higher overall acceptability score of 8.13 compared to jelly with 1% pigment (7.93).

Jelly prepared with 3% anthocyanin pigment had least acidity (0.89%) maximum anthocyanin content (5.52 mg $100g^{-1}$) and antioxidant activity (41%).

Hence 3% was selected as the best concentration of anthocyanin pigment for incorporation in jelly for further storage analysis.

4.1.3 Candy

4.1.3.a Preparation of candy (Solid product)

Anthocyanin extract was incorporated in ash gourd candy as natural colour at 1%, 2% and 3% (Plate 4). The prepared candies were analysed for quality parameters and compared with the candy prepared without colouring pigment as control for selection of the best concentration.

4.1.3.b Evaluation of quality parameters

Chemical and nutritional quality parameters

Chemical and nutritional quality parameters *viz*., TSS, acidity, reducing sugar, total sugar, ascorbic acid, anthocyanin content, antioxidant activity and moisture content of candy were evaluated and shown in Table 7.

Total Soluble Solids (TSS) (^{0}Brix)

TSS of the ashgourd candy was significantly influenced by pigment concentration.

Plate 4: Candy incorporated with different concentration of anthocyanin pigment

TSS of the coloured candy with anthocyanin pigment added at 3% was the highest (76.50 ^{0}Brix) and was significantly different from TSS content of all other candies.

The lowest TSS (75^{0}Brix) was recorded in candy incorporated with 1% anthocyanin pigment and the candy without colour incorporation.

Acidity (%)

There was significant difference between the acidity of candies due to pigment incorporation. The lowest acidity (0.20*%*) was recorded in candy without anthocyanin pigment, which was on par with all other coloured candies, except the candy with 3% anthocyanin pigment incorporation with the highest acidity content (0.40%).

Reducing Sugar (%)

There was no significant difference between the reducing sugar content of candies. However, 1% pigment incorporated candy and candy without pigment showed the highest reducing sugar of 25.00 % and the lowest reducing sugar content (19.23%) was recorded in candy incorporated with 3% anthocyanin pigment. .

Total sugar (%)

There was significant difference between the total sugar content of candies.

The highest total sugar (78.12%) was recorded in candy incorporated with 3% anthocyanin pigment, which was significantly different from all other candies.The 2% anthocyanin pigment incorporated candies showed the lowest total sugar (56.81%).

Table 7. Effect of pigment concentration on chemical and nutritional quality parameters of candy

Pigment concentration in candy	**TSS (^{0}Brix)**	**Acidity (%)**	**Reducing sugar (%)**	**Total sugar (%)**	**Ascorbic acid (mg $100g^{-1}$)**	**Anthocyanin content (mg $100g^{-1}$)**	**Antioxidant activity (%)**	**Moisture content (%)**
1%	75.00	0.26	25.00	69.44	45.23	3.93	53.21	8.44
2%	75.50	0.26	23.80	56.81	65.00	4.71	59.09	8.03
3%	76.50	0.40	19.23	78.12	65.00	6.24	79.53	8.62
Control (no pigment)	75.00	0.20	25.00	69.44	39.28	2.22	47.95	7.84
S.Em±	**0.156**	**0.012**	**0.195**	**0.259**	**0.152**	**0.069**	**0.132**	**0.264**
CD at 5%	**0.38**	**0.07**	**NS**	**0.50**	**12.51**	**0.06**	**0.05**	**0.44**

Ascorbic acid (mg 100g^{-1})

There was significant difference between the ascorbic acid content of candies.

The highest ascorbic acid (65.00 mg 100g^{-1}) was recorded in candy with 2% and 3% anthocyanin pigment addition.

The lowest ascorbic acid content was recorded in candy without pigment (39.28 mg 100g$^{-1)}$, which was on par with the candy incorporated with 1% anthocyanin pigment (45.23 mg 100g^{-1}).

Anthocyanin content (mg 100g^{-1})

The anthocyanin content was significantly influenced by pigment concentration (Table 7).

Among the candies, the highest anthocyanin content (6.24 mg 100g^{-1}) was recorded in candy incorporated with 3% pigment, which was significantly different from all other candies.

The lowest anthocyanin content (2.22 mg 100g^{-1}) was recorded in candy without anthocyanin pigment. Among the coloured candies, the lowest anthocyanin content was recorded in candies with 1% anthocyanin content (3.93 mg 100g^{-1}).

Antioxidant activity (%)

There was significant difference between the antioxidant activity of candies (Table 7).

The antioxidant activity was highest (79.53%) in candy with 3% anthocyanin pigment, which was significantly different from all other candies. The lowest antioxidant activity was recorded in candy without anthocyanin pigment (47.95%).

Among the coloured candies, the lowest antioxidant activity was observed in candy with 1% anthocyanin content (53.88%).

Moisture (%)

There was significant difference between the moisture content of candies (Table 7).

The moisture content was highest (8.62%) in candy with 3% anthocyanin pigment. The lowest moisture content was recorded in candy without pigment incorporation (7.84%).

Sensory quality parameters

The colour incorporated and colourless candies were analyzed for organoleptic quality parameters and is shown in Table 8.

There was no significant difference between the sensory scores of candies with respect to flavor, texture and taste.

The highest mean scores for appearance (8.60) and colour (8.60) were observed for colourless candy. Candy with 1% anthocyanin pigment incorporation had least score for appearance (7.60).

The candy with 3% anthocyanin pigment incorporation had the lowest score (7.60) for colour.

When overall acceptability score of coloured candies as computed, candy without pigment incorporation showed the highest score (8.30).

The candy with 2 % pigment incorporation recorded the highest mean score of 8.00 among the coloured candies. The lowest overall acceptability was for 3% pigment incorporated candy (7.70).

4.1.3.c Cost of production

Cost of production of pigment incorporated candy was calculated and compared with that of candy without pigment to analyze whether the production of pigment incorporated candy is cost effective or not.

Cost of production of one kilogram pigment incorporated candy at 1%,2%,3% and without pigment was Rs.133.90/-, Rs146.30/-, Rs.153.70/- and Rs.121.50/- respectively(Table 9 & Annexure 3).

Table 8. Effect of pigment concentration on sensory quality parameters of the candies

Pigment concentration in candies	Sensory quality parameters											
	Appearance		Colour		Flavour		Texture		Taste		Overall acceptability	
	Mean score	Rank	Mean score	Rank	Mean score	Rank	Mean score	Rank	Mean score	Rank	Mean score	Rank
1%	7.60	33.60	7.70	34.55	8.20	43.90	8.00	39.75	8.10	41.35	7.90	37.90
2%	7.80	37.90	7.90	39.10	8.20	42.35	8.10	41.30	8.00	38.10	8.00	39.72
3%	7.70	34.20	7.60	31.25	7.70	31.32	7.80	36.55	7.70	33.27	7.70	30.72
Control (no pigment)	8.60	56.30	8.60	57.10	8.20	44.42	8.20	44.40	8.30	49.27	8.30	53.65
KW value	**14.08**		**16.42**		**4.85**		**1.35**		**6.19**		**10.30**	
$\chi 2$	**7.81**											

Table 9. Cost of production of one kilogram pigment incorporated candy

Treatments	Rs./kg
Candy with 1% pigment incorporation	133.90
Candy with 2% pigment incorporation	146.30
Candy with 3% pigment incorporation	153.70
Candy without pigment(control)	121.50
C.D at 5%	2.74

4.1.3.d Selection of best concentration of candy

Candy prepared without addition of anthocyanin pigment had least acidity (0.20%), which was on par with candy prepared using 2% and 3% anthocyanin pigment concentration (0.26).

Candy incorporated with 1% pigment had lowest anthocyanin content(3.93 mg $100g^{-1}$) reducing sugar (25.00 g $100g^{-1}$) and total sugar (69.44 g $100g^{-1}$).Candy prepared with 3% anthocyanin pigment recorded highest antioxidant activity (79.53%).Candy with 2% anthocyanin pigment incorporation showed highest ascorbic acid (65.00 mg 100 g^{-1}).

When sensory parameters were analysed, candy without pigment incorporation showed highest mean score (8.30) followed by candy with 2% anthocyanin pigment (8.00) compared to all other candies with pigment incorporation.

Hence 2% was selected as the best concentration of anthocyanin pigment for incorporation in ashgourd candy for further storage analysis.

4.2 Evaluation of pigment stability in products

Stability of all the three pigment incorporated products was analysed by storing them in glass bottles under ambient and refrigerated condition (4-7^0 C) for a period of three months.

4.2.1 Effect of storage on pigment stability in squash

Lime squash incorporated with 2% anthocyanin pigment was selected for storage analysis and observations were taken initially and at monthly intervals for a period of three months. Stability of pigments was assessed by storing the 2% anthocyanin pigment incorporated squash in glass containers under ambient and refrigerated condition.

4.2.1.a Chemical and nutritional quality parameters

The following chemical and nutritional quality parameters were analysed initially and at monthly intervals for three months.

TSS (^{O} Brix)

TSS of anthocyanin incorporated squash as influenced by storage period and storage condition is shown in (Table 10).

TSS of coloured squash was significantly influenced only by storage period. TSS of 2% anthocyanin pigment incorporated lime squash was decreased from 44.06 ^{O} Brix at the time of storage to 43.25 ^{O} Brix at three months after storage.

Though there was no significant difference between the storage conditions, TSS of the squash was higher in ambient storage (43.68 ^{O} Brix) compared to that under refrigerated storage (43.65^O Brix).

Acidity (%)

Acidity of 2% anthocyanin incorporated squash as influenced by storage conditions, storage period and their interactions is shown in Table 11.

Table 10. Effect of storage on TSS (^{O} Brix) of squash

Storage conditions (S)	TSS (^{O} Brix) Months After Storage(M)				Mean (S)
	0	1	2	3	
Ambient Storage	44.17	43.75	43.57	43.25	43.68
Refrigerated Storage	43.95	43.82	43.60	43.25	43.65
Mean (M)	44.06	43.78	43.58	43.25	
S.Em±	**S-0.065**	**M-0.245**	**MxS-0.156**		
CD at 5%	**S - NS M- 0.134 MxS- NS**				

Table11. Effect of storage on acidity (%) of squash

Storage conditions (S)	Acidity (%) Months After Storage(M)				Mean (S)
	0	1	2	3	
Ambient Storage	1.88	1.98	2.1	2.49	2.11
Refrigerated Storage	1.82	1.88	1.98	2.1	1.94
Mean (M)	1.85	1.93	2.04	2.3	
S.Em±	**S-0.013**	**M-0.149**	**MxS-0.163**		
CD at 5%	**S -0.051 M- 0.072 MxS- 0.102**				

Acidity of 2% anthocyanin pigment incorporated lime squash increased from 1.85 % at the initial period of storage to 2.3 % at three months after storage.

Acidity of the pigment incorporated squash was less (1.94%) in refrigerated storage compared to that under ambient storage (2.11%).

Reducing Sugar (%)

Reducing sugar of anthocyanin incorporated squash as influenced by storage period and storage condition is shown in Table 12.

Reducing sugar of the colored squash was significantly influenced only by storage period.

Reducing sugar of the squash was higher in refrigerated storage (35.24 %) compared to that in ambient storage (33.04 %), though there was no significant difference between them.

Reducing sugar of 2% anthocyanin pigment incorporated lime squash was decreased from 35.76 % at the initial period of storage to 33.40 % at three months after storage .But there was no significant difference between the reducing sugar of squash at first and third month of storage.

Total sugar (%)

Total sugar of anthocyanin incorporated squash as influenced by storage period, storage condition and their interaction is shown in Table 13.

Total sugar of the colored squash was significantly influenced by storage period, storage condition and their interaction.

Total sugar of the squash was higher in refrigerated storage (39.46%) compared to that in ambient storage (38.69 %).

Total sugar of 2% anthocyanin pigment incorporated lime squash decreased from 41.78% at the initial period of storage to 37.29 % at three months after storage. But there was no significant difference between the total sugar content at 1st and 2nd month of storage.

Table 12. Effect of storage on reducing sugar (%) of squash

Storage conditions (S)	**Reducing sugar (%)**				**Mean (S)**
	Months After Storage(M)				
	0	1	2	3	
Ambient Storage	34.77	32.81	32.31	32.29	33.04
Refrigerated Storage	36.76	34.86	34.66	34.52	35.24
Mean (M)	35.76	33.83	33.48	33.40	
S.Em±	**S-0.456**	**M-0.659**	**MxS-0.923**		
CD at 5%	**S -NS M- 1.23 MxS- NS**				

Table 13. Effect of storage on total sugars (%) of squash

Storage conditions (S)	**Total sugar (%)**				**Mean (S)**
	Months After Storage(M)				
	0	**1**	**2**	**3**	

Ambient Storage	41.85	38.33	37.33	37.26	38.69
Refrigerated Storage	41.72	40.32	38.48	37.33	39.46
Mean (M)	41.78	39.32	37.90	37.29	
S.Em$\pm$	**S-0.365**	**M-0.231**	**MxS-0.234**		
CD at 5%	**S -0.111 M- 1.845 MxS- 2.609**				

Ascorbic acid (mg $100g^{-1}$)

Ascorbic acid content of 2% anthocyanin incorporated squash as influenced by storage is shown in Table 14.

Ascorbic acid of coloured squash was significantly influenced only by storage period.

Though there was no significant difference between the storage condition, ascorbic acid content of the squash was higher in refrigerated storage (49.12 mg $100g^{-1}$) compared to that in ambient storage (48.03 mg $100g^{-1}$).

Ascorbic acid content was significantly reduced from 0^{th} month of storage (52.01 mg $100g^{-1}$) to 1^{st} month (44.07 mg $100g^{-1}$).But there was no significant difference between the ascorbic acid content of squash at 1^{st} and 3^{rd} month of storage.

There was no significant difference between the interactions.

Anthocyanin content (mg $100g^{-1}$)

Anthocyanin content of 2% pigment incorporated squash as influenced by storage condition, storage period and their interaction is shown in Table 15.

Anthocyanin content of the coloured squash was significantly influenced by storage condition, storage period, and their interaction.

Anthocyanin content of the squash was higher in refrigerated storage (6.99 mg $100g^{-1}$) compared to that under ambient storage (6.58 mg $100g^{-1}$).

Anthocyanin content of 2% pigment incorporated lime squash gradually reduced from 7.17 mg $100g^{-1}$ at the initial period of storage to 6.38 mg $100g^{-1}$ at three months after storage.

Antioxidant activity (%)

Antioxidant activity of 2% pigment incorporated squash as influenced by storage period, storage condition and their interaction is shown in Table 16.

Antioxidant activity of the colored squash was significantly influenced by storage period, storage condition and their interaction. Antioxidant activity of the squash was higher in refrigerated storage (87.56%) compared to that under ambient storage (87.27%).

Table 14. Effect of storage on ascorbic acid (mg $100g^{-1}$) of squash

Storage conditions (S)	**Ascorbic acid (mg $100g^{-1}$)**				**Mean (S)**
	Months After Storage(M)				
	0	**1**	**2**	**3**	
Ambient Storage	54.02	46.77	47.53	47.43	48.03
Refrigerated Storage	50.00	41.38	41.28	40.13	49.12

Mean (M)	52.01	44.07	44.40	43.78	
S.Em±	**S-1.283**	**M- 1.815**	**MxS-2.567**		
CD at 5%	**S -NS M- 5.855 MxS- NS**				

Table 15. Effect of storage on anthocyanin content (mg $100g^{-1}$) of squash

Storage conditions (S)	**Anthocyanin (mg $100g^{-1}$)**				**Mean (S)**
	Months After Storage(M)				
	0	**1**	**2**	**3**	
Ambient Storage	7.00	6.63	6.39	6.33	6.58
Refrigerated Storage	7.35	7.24	6.96	6.44	6.99
Mean (M)	7.17	6.93	6.67	6.38	
S.Em±	**S-0.026**	**M-0.034**	**MxS-0.038**		
CD at 5%	**S -0.053 M- 0.075 MxS- 0.106**				

Table 16. Effect of storage on antioxidant activity (%) of squash

Storage conditions (S)	**Antioxidant activity (%)**	**Mean (S)**

	Months After Storage (M)				
	0	1	2	3	
Ambient Storage	87.39	87.30	87.24	87.18	87.56
Refrigerated Storage	87.89	87.75	87.34	87.27	87.27
Mean (M)	87.64	87.52	87.29	87.22	
S.Em±	**S-0.238**		**M-1.256**	**MxS-0.562**	
CD at 5%	**S -0.006 M- 0.210 MxS-0.017**				

Antioxidant activity of anthocyanin incorporated squash decreased during storage from 87.64 % at the time of storage to 87.22% at three months after storage. But there was no significant difference between the antioxidant content of coloured squash at initial and first month of storage and between second and third month of storage.

4.2.1.b Sensory quality parameters

The squash incorporated with 2% anthocyanin pigment stored in glass bottles were kept in ambient and low temperature condition and analysed during storage for various sensorial attributes by using 9 point hedonic scale .The sensory scores and mean ranks of stored squash recorded with respect to appearance and colour for a period of three months at monthly intervals are presented in Table 17.

There was significant reduction in colour and appearance scores of pigment incorporated squash during storage.

Mean score for appearance was reduced from 8.1 at the time of storage to 5.6 at three months after storage under ambient condition. The corresponding scores under refrigerated storage were 8.3 and 6.2.

The mean score for colour reduced from 8.4 to 5.6 from 0^{th} to 3^{rd} month of storage under ambient conditions, whereas the scores under refrigerated storage reduced from 8.3 to 7.0.

The sensory scores were high for samples stored under refrigerated condition.

Sensory scores of 2% pigment incorporated squash during storage								
	Appearance				**Colour**			
	Ambient		**Refrigerated**		**Ambient**		**Refrigerated**	
Months After Storage(S)	**Mean score**	**Rank**	**Mean score**	**rank**	**Mean score**	**Rank**	**Mean score**	**rank**
0	8.1	9.7	8.3	11.3	8.4	9.5	8.3	13.75
1	7.3	8.5	7.7	10.5	7.7	7.25	8	11.5
2	6.4	8	6.9	9.4	7	6.35	7.9	10.25
3	5.6	7.9	6.2	8.3	5.6	5.8	7	8.65
KW value	**9.85**		**9.96**		**8.84**		**10.63**	
	7.81							

Table 17.Effect of storage on sensory parameters of coloured squash

4.2.2 Effect of storage on pigment stability in jelly

Jackfruit jelly incorporated with 3% anthocyanin pigment was selected for the storage analysis and observations were taken initially and at monthly intervals for a period of three months. Stability of pigments was assessed by storing the 3% anthocyanin pigment incorporated jelly in glass containers under ambient and refrigerated condition.

4.2.2.a Chemical and nutritional quality parameters

The following chemical and nutritional quality parameters were analysed initially and at monthly intervals for three months.

TSS (^{O} Brix)

TSS of anthocyanin incorporated jelly as influenced by storage condition, storage period and their interactions is shown in Table 18.

TSS of colored jelly was significantly influenced by storage period, storage condition and their interactions.

TSS of the jelly was higher in refrigerated storage (65.52 ^{O} Brix) compared to that in ambient storage (65.47 ^{O} Brix).

TSS of 3% anthocyanin pigment incorporated jelly decreased from 65.92 ^{O} Brix at the time of storage to 64.72 ^{O} Brix at three months after storage.

Acidity (%)

Acidity of anthocyanin incorporated jelly was influenced by storage period is shown in Table 19.

Acidity of the colored jelly was significantly influenced by storage period.

Acidity of the jelly was lower in refrigerated storage (1.11%) compared to that in ambient storage (1.12%), though there was no significant difference between them.

Table 18.Effect of storage on TSS (^{O} Brix) of jelly

Storage conditions (S)	**TSS (^{O} Brix)**				**Mean (S)**
	Months After Storage(M)				
	0	**1**	**2**	**3**	
Ambient Storage	65.87	65.67	65.50	64.87	65.47
Refrigerated Storage	65.97	65.90	65.65	64.57	65.52
Mean (M)	65.92	65.78	65.57	64.72	
S.E m±	**S-0.474**	**M-0.533**	**MxS-0.129**		
CD at 5%	**S -0.077 M- 0.109 MxS- 0.154**				

Table 19.Effect of storage on acidity (%) of jelly

Storage conditions (S)	**Acidity (%)**				**Mean (S)**
	Months After Storage(M)				
	0	1	2	3	
Ambient Storage	1.11	1.05	1.06	1.08	1.12
Refrigerated Storage	1.20	1.15	1.18	1.11	1.11
Mean (M)	1.19	1.09	1.12	1.06	

S.E m±	**S-0.053**	**M-0.169**	**MxS-0.156**
CD at 5%	**S -NS M- 0.066 MxS- NS**		

Acidity of 3% anthocyanin pigment incorporated jelly decreased gradually from 1.19% at the initial period of storage to 1.06 % at three months after storage.

Reducing Sugar (%)

Reducing sugar of anthocyanin incorporated jelly as influenced by storage period, storage condition and their interaction is shown in Table 20.

Reducing sugar of the colored jelly was significantly influenced by storage condition, storage period and their interaction.

Reducing sugar of the jelly was higher in refrigerated storage (15.96 %) compared to that in ambient storage (14.86 %).

Reducing sugar of 3% anthocyanin pigment incorporated jelly decreased from 16.39 % at the initial period of storage to 13.99 % at three months after storage.

Total sugar (%)

Total sugar of anthocyanin incorporated jelly as influenced by storage condition, storage period and interaction is shown in Table 21.

Total sugar of the colored jelly was significantly influenced by storage condition, storage period, and their interaction.

Total sugar of the jelly was higher in refrigerated storage (44.66 %) compared to that in ambient storage (42.00 %).

Total sugar of 3% anthocyanin pigment incorporated jelly decresed from 52.44 % at the initial period of storage to 39.26 % at three months after storage.

Table 20.Effect of storage on reducing sugar (%) of jelly

Storage conditions (S)	**Reducing sugar (%)**				**Mean (S)**
	Months After Storage(M)				
	0	**1**	**2**	**3**	
Ambient Storage	16.12	15.54	15.15	12.65	14.86
Refrigerated Storage	16.66	16.25	15.62	15.34	15.96
Mean (M)	16.39	15.89	15.38	13.99	
S.E m±	**S-0.569**	**M-1.286**	**MxS-1.371**		
CD at 5%	**S -0.111 M- 0.156 MxS- 0.221**				

Table 21.Effect of storage on total sugar (%) of jelly

Storage conditions (S)	**Total sugar (%)**				**Mean (S)**
	Months After Storage(M)				
	0	1	2	3	
Ambient Storage	48.07	47.21	38.02	34.72	42.00

Refrigerated Storage	56.81	52.08	44.64	43.81	44.66
Mean (M)	52.44	49.64	41.33	39.26	
S.E m±	**S-1.238**	**M-1.987**	**MxS-1.389**		
CD at 5%	**S -1.354 M- 1.915 MxS- 2.708**				

Ascorbic acid (mg 100g^{-1})

Ascorbic acid content of anthocyanin incorporated jelly as influenced by storage condition, storage period and their interaction is shown in Table 22.

Ascorbic acid of coloured jelly was significantly influenced by storage condition, storage period and their interaction.

Ascorbic acid content of the jelly was higher in refrigerated storage (57.45 mg 100g^{-1}) compared to that in ambient storage (50.94 mg 100g^{-1}). Ascorbic acid of 3% anthocyanin pigment incorporated jelly was reduced from initial period of storage (63.01 mg 100g^{-1}) to third month of storage (44.40 mg 100g^{-1}).

Anthocyanin content (mg 100g^{-1})

Anthocyanin content of pigment incorporated jelly as influenced by storage condition, storage period and their interaction is shown in Table 23.

Anthocyanin content of the coloured jelly was significantly influenced by storage condition, storage period, and their interaction.

Anthocyanin content of the jelly was higher in refrigerated storage (5.31 mg 100g^{-1}) compared to that in ambient storage (5.10 mg 100g^{-1}).

Anthocyanin content of 3% pigment incorporated jelly reduced from 5.38 mg 100g^{-1} at the initial period of storage to 5.04 mg 100g^{-1} at three months after storage. But there was no significant difference between the anthocyanin content of jelly at 2nd and 3rd month.

Table 22.Effect of storage on ascorbic acid of jelly

Storage conditions (S)	**Ascorbic acid (mg 100g^{-1})**				**Mean (S)**
	Months After Storage(M)				
	0	**1**	**2**	**3**	
Ambient Storage	59.91	55.09	47.31	41.46	50.94
Refrigerated Storage	66.11	59.91	53.78	50.00	57.45
Mean (M)	63.01	57.50	51.89	44.40	
S.E m±	**S-1.263**	**M-1.856**	**MxS-0.765**		
CD at 5%	**S -2.784 M- 3.937 MxS- 5.568**				

Table 23.Effect of storage on anthocyanin content (mg 100g^{-1}) of jelly

Storage conditions (S)	Anthocyanin (mg 100g^{-1})				Mean (S)
	Months After Storage(M)				
	0	**1**	**2**	**3**	
Ambient Storage	5.22	5.15	5.05	5.01	5.10
Refrigerated Storage	5.55	5.53	5.10	5.08	5.31
Mean (M)	5.38	5.34	5.07	5.04	
S.E m±	**S-0.562**		**M-0.491**	**MxS-0.592**	
CD at 5%	**S -0.028 M- 0.039 MxS- NS**				

Antioxidant activity (%)

Antioxidant activity of 3% anthocyanin pigment incorporated jelly as influenced by storage condition, storage period and their interaction is shown in Table 24.

Antioxidant activity of the colored jelly was significantly influenced by storage condition, storage period and their interaction. Antioxidant activity of the jelly was higher in refrigerated storage (49.98%) compared to that in ambient storage (42.00%).

Antioxidant activity of anthocyanin incorporated jelly decreased during storage from 58.51 % at the time of storage to 39.07% at three months after storage.

Moisture content (%)

Moisture content of anthocyanin incorporated jelly as influenced by storage period, storage condition and their interaction is shown in Table 25.

There was no significant difference between the moisture content of 3% anthocyanin pigment incorporated jelly during storage condition. Moisture content was lower in ambient storage (27.32%) compared to that in refrigerated storage (27.81%).

There was a significant difference between the storage period. Moisture content decreased from 28.10% at the initial period of storage to 26.95% at three months after storage.

4.2.2.b Sensory quality parameters

The jelly incorporated with 3% anthocyanin pigment in glass bottles were stored in ambient and low temperature condition and analyzed during storage for

Table 24.Effect of storage on antioxidant (%) of jelly

Storage conditions (S)	**Antioxidant (%) Months After Storage(M)**				**Mean (S)**
	0	**1**	**2**	**3**	
Ambient Storage	64.42	53.21	42.42	39.9	49.98
Refrigerated Storage	48.61	40.78	40.39	38.24	42.00
Mean (M)	58.51	46.99	41.40	39.07	
S.E m±	**S-0.829**	**M-1.253**	**MxS-1.486**		
CD at 5%	**S -0.735**	**M- 1.039**	**MxS-1.469**		

Table 25.Effect of storage on moisture content (%) of jelly

Storage conditions (S)	**Moisture (%)**				**Mean (S)**
	Months After Storage(M)				
	0	**1**	**2**	**3**	
Ambient Storage	27.90	27.50	27.30	26.60	27.32
Refrigerated Storage	28.30	28.15	27.50	27.30	27.81
Mean (M)	28.10	27.82	27.40	26.95	
S.E m±	**S-0.897**	**M-0.654**	**MxS-0.263**		
CD at 5%	**S -NS M- 0.383 MxS-NS**				

various sensorial attributes by using 9 point hedonic scale .The sensory scores and mean ranks of stored jelly recorded with respect to appearance and colour for a period of three months at monthly intervals are presented in Table 26.

There was significant reduction in colour and appearance scores of pigment incorporated jelly during storage.

Mean score for appearance was reduced from 8.3 at the time of storage to 6.5 at three months after storage under ambient condition. The corresponding scores under refrigerated storage were 8.4 and 7.0.

The mean score for colour reduced from 8.4 to 6.0 from 0^{th} to 3^{rd} month of storage under ambient conditions, whereas the scores under refrigerated storage reduced from 8.6 to 7.0.

The sensory scores were high for samples stored under refrigerated condition.

4.2.3 Evaluation of pigment stability in candy

Ashgourd candy incorporated with 2% anthocyanin pigment was selected for the storage analysis and observations were taken initially and at monthly intervals for a period of three months. Stability of pigments was assessed by storing the 2% anthocyanin pigment incorporated candy in glass containers under ambient and refrigerated condition.

4.2.3.a Chemical and nutritional quality parameters

The following chemical and nutritional quality parameters were analysed initially and at monthly intervals for three months.

TSS (^{O} Brix)

TSS of anthocyanin incorporated candy as influenced by storage period is shown in Table 27.

Table 26.Effect of storage on sensory parameters of coloured jelly

Sensory scores of 3% pigment incorporated jelly during storage								
Month After Storage(S)	**Appearance**				**Colour**			
	Ambient		**Refrigerated**		**Ambient**		**Refrigerated**	
	Mean score	**Rank**	**Mean score**	**rank**	**Mean score**	**Rank**	**Mean score**	**rank**
0	8.3	10.5	8.4	11.25	8.4	9.5	8.6	11.5
1	7.4	10	7.8	11	7.9	9.3	8	11.2
2	7	8.4	7.3	10.25	7.6	9	7.8	11
3	6.5	5.2	7	9.6	6	7.9	7	10.2
KW value	**7.98**		**10.96**		**8.56**		**9.63**	
χ^2	**7.81**							

Table 27.Effect of storage on TSS (^{O} Brix) of candy

Storage conditions (S)	TSS (^{O} Brix)				Mean (S)
	Months After Storage(M)				
	0	1	2	3	
Ambient Storage	71.70	71.12	71.7	71.12	71.58
Refrigerated Storage	71.85	71.47	71.90	71.57	71.69
Mean (M)	71.87	71.72	71.52	71.12	
S.E m±	**S-0.049**	**M-0.732**	**MxS-0.241**		
CD at 5%	**S -NS M- 0.135 MxS- NS**				

Table 28.Effect of storage on acidity (%) of candy

Storage conditions (S)	Acidity (%)				Mean (S)
	Months After Storage (M)				
	0	1	2	3	
Ambient Storage	0.75	1.02	0.76	0.92	0.94
Refrigerated Storage	0.84	1.15	0.78	1.13	0.90
Mean (M)	0.76	0.81	0.97	1.14	
S.E m±	**S-0.056**	**M-0.087**	**MxS-0.146**		

CD at 5%	S -NS M- 0.069 MxS- NS

TSS of coloured candy was significantly influenced only by storage period. TSS of 2% anthocyanin pigment incorporated candy decreased from 71.87 ^{O} Brix at the time of storage to 71.12 ^{O} Brix at three months after storage.

Acidity (%)

Acidity of anthocyanin incorporated candy was influenced by storage period which is shown in Table 28.

Though there was no significant difference between the storage conditions, acidity was lower in refrigerated storage (0.90%) than in ambient storage (0.94%).

Acidity of 2% anthocyanin pigment incorporated candy increased from 0.76 % at the initial period of storage to 1.14 % at three months after storage.

Reducing Sugar (%)

Reducing sugar of anthocyanin incorporated candy as influenced by storage period, storage condition and their interactions is shown in Table 29.

Reducing sugar of the coloured candy was significantly influenced by storage condition, storage period and their interaction.

Reducing sugar of the candy was higher in refrigerated storage (43.97%) compared to that in ambient storage (43.06 %).

Reducing sugar of 2% anthocyanin pigment incorporated candy decreased from 50.12 % at the initial period of storage to 36.85 % at three months after storage.

Total sugar (%)

Total sugar content of anthocyanin incorporated candy as influenced by storage condition, storage period and interaction is shown in Table 30.

Total sugar of the coloured candy was significantly influenced by storage condition, storage period, and their interaction.

Table 29. Effect of storage on reducing sugar (%) of candy

Storage conditions (S)	**Reducing sugar (%)**				**Mean (S)**
	Months After Storage(M)				
	0	**1**	**2**	**3**	
Ambient Storage	50.25	40.94	50.00	41.66	43.06
Refrigerated Storage	46.02	35.02	45.55	38.68	43.97
Mean (M)	50.12	45.78	41.30	36.85	
S.E m±	**S-0.956**		**M-0.156**	**MxS-0.562**	
CD at 5%	**S -0.305 M- 0.432 MxS- 0.61**				

Table 30.Effect of storage on total sugar (%) of candy

Storage conditions (S)	**Total sugar (%)**				**Mean (S)**
	Months After Storage(M)				
	0	**1**	**2**	**3**	
Ambient Storage	56.17	44.64	56.17	54.56	48.75

Refrigerated Storage	54.44	39.76	55.15	53.62	54.87
Mean (M)	56.17	54.79	49.60	46.69	
S.E m±	**S-1.736**		**M-1.259**	**MxS-0.587**	
CD at 5%	**S – 0.149 M- 0.211 MxS- 0.298**				

Total sugar of the candy was higher in refrigerated storage (54.87 %) compared to that in ambient storage (48.75 %).

Total sugar of 2% anthocyanin pigment incorporated candy decreased from 56.17 %at the initial period of storage to 46.69 % at three months after storage.

Ascorbic acid (mg 100g^{-1})

Ascorbic acid content of anthocyanin incorporated candy as influenced by storage condition, storage period and their interactions is shown in Table 31.

Ascorbic acid of coloured candy was significantly influenced by storage condition, storage period and their interaction.

Ascorbic acid of the candy was higher in refrigerated storage (44.56 mg 100g^{-1}) compared to that under ambient storage (44.46 mg 100g^{-1}). Ascorbic acid of 2% anthocyanin pigment incorporated candy was seen reduced from initial period of storage (47.90 mg 100g^{-1}) to third month of storage (41.04 mg 100g^{-1}).

Anthocyanin content (mg 100g^{-1})

Anthocyanin content of pigment incorporated candy as influenced by storage condition, storage period and their interaction is shown in Table 32.

Anthocyanin content of the coloured candy was significantly influenced by storage condition, storage period, and their interaction.

Anthocyanin content of the candy was higher in refrigerated storage (7.55 mg 100g^{-1}) compared to that under ambient storage (7.48 mg 100g^{-1}).

Anthocyanin content of 2% pigment incorporated candy gradually reduced from 7.64 mg $100g^{-1}$ at the initial period of storage to 7.37 mg $100g^{-1}$ at three months after storage.

Antioxidant activity (%)

Antioxidant activity of anthocyanin incorporated candy as influenced by storage condition, storage period and their interaction is shown in Table 33.

Table 31. Effect of storage on ascorbic acid (mg $100g^{-1}$) of candy

Storage conditions (S)	**Ascorbic acid (mg $100g^{-1}$)**				**Mean (S)**
	Months After Storage(M)				
	0	**1**	**2**	**3**	
Ambient Storage	48.44	45.83	47.36	45.86	44.46
Refrigerated Storage	47.21	36.36	47.22	45.80	46.56
Mean (M)	47.90	47.21	45.84	41.04	
S.E m±	**S-1.796**		**M-1.487**		**MxS-0.963**
CD at 5%	**S -0.015 M- 0.021 MxS- 0.03**				

Table 32.Effect of storage on anthocyanin content (mg $100g^{-1}$) of candy

Storage conditions (S)	**Anthocyanin(mg $100g^{-1}$)**				**Mean (S)**
	Months After Storage(M)				
	0	**1**	**2**	**3**	

Ambient Storage	7.61	7.42	7.68	7.52	7.48
Refrigerated Storage	7.55	7.34	7.61	7.41	7.55
Mean (M)	7.64	7.58	7.47	7.37	
S.E m±	**S-0.246**		**M-0.463**	**MxS-0.589**	
CD at 5%	**S - 0.008 M-0.011 MxS-0.015**				

Table 33. Effect of storage on antioxidant activity (%) of candy

Storage conditions (S)	**Antioxidant activity (%)**				**Mean (S)**
	Months After Storage(M)				
	0	**1**	**2**	**3**	
Ambient Storage	76.33	54.23	63.70	59.32	65.34
Refrigerated Storage	78.33	63.99	76.87	61.48	68.22
Mean (M)	77.60	70.01	62.73	56.77	
S.E m±	**S-0.189**	**M-0.853**	**MxS-0.431**		
CD at 5%	**S -0.362 M-0.512 MxS-0.724**				

Table 34.Effect of storage on moisture content (%) of candy

Storage conditions (S)	**Moisture (%)**	**Mean (S)**

	Months After Storage(M)				
	0	**1**	**2**	**3**	
Ambient Storage	8.95	6.78	8.80	20.75	7.28
Refrigerated Storage	7.28	6.15	18.50	22.30	17.60
Mean (M)	8.87	12.91	13.75	14.22	
S.E m±	**S-0.725**	**M-0.654**		**MxS-0.431**	
CD at 5%	**S -0.206 M- 0.292 MxS-0.413**				

Antioxidant activity of the coloured candy was significantly influenced by storage condition, storage period and their interaction. Antioxidant activity of the candy was higher in refrigerated storage (68.22%) compared to that in ambient storage (65.34%).

Antioxidant activity of anthocyanin incorporated candy was decreased during storage from 77.60 % at the time of storage to 56.77% at three months after storage.

Moisture content (%)

Moisture content of anthocyanin incorporated candy as influenced by storage period, storage condition and their interaction is shown in Table 34.

There was significant difference between the moisture content of 2% anthocyanin pigment incorporated candy between storage period, storage condition and their interaction. Moisture content was lower in ambient storage(7.28%) compared to that in refrigerated storage (17.60%).There was an increase in moisture content from initial month of storage(8.87%) to final month (14.22%).

4.2.3.b Sensory quality parameters

The candy with 2% anthocyanin pigment stored in glass bottles were kept in ambient and low temperature condition and analysed during storage for various sensorial attributes by using 9 point hedonic scale .The sensory scores and mean ranks of stored candy recorded with respect to appearance and colour for a period of three months at monthly intervals are presented in Table 35.

There was significant reduction in colour and appearance scores of pigment incorporated candy during storage.

Mean score for appearance was reduced from 8.6 at the time of storage to 7.4 at three months after storage under ambient condition. The corresponding scores under refrigerated storage were 8.7 and 7.6.

The mean score for colour reduced from 8.5 to 7.5 from 0^{th} to 3^{rd} month of storage under ambient conditions, whereas the scores under refrigerated storage reduced from 8.6 to 7.9.

The sensory scores were high for samples stored under refrigerated condition.

Sensory scores of 2% pigment incorporated candy during storage								
	Appearance				Colour			
	Ambient		Refrigerated		Ambient		Refrigerated	
Month After Storage(S)	Mean score	Rank	Mean score	rank	Mean score	Rank	Mean score	rank
0	8.6	9	8.7	10	8.5	11	8.6	12
1	8.2	8.5	8.3	9.5	8.1	9.8	8.2	10
2	7.8	8	7.9	9	7.8	7.5	8.1	9
3	7.4	6.55	7.6	8	7.5	6	7.9	8
KW value	**8.99**		**9.89**		**7.86**		**9.56**	
χ^2	**7.81**							

Table 35.Effect of storage on sensory parameters of coloured candy

5. DISCUSSION

The results obtained from the investigation on "Utilization of pomegranate (*Punica granatum* L.) peel as a natural food colourant" are discussed in this chapter under following headings:

5.1. Development of processed products with anthocyanin pigment.

5.2. Evaluation of pigment stability in products

5.1 Development of processed products with anthocyanin pigment.

With the growing demand for natural and healthier food options, the use of natural food colours is becoming increasingly popular in the food industry. Many approved synthetic food colorants have been prohibited as they proved to be toxic. Plants as well as plant parts are sources of natural colours like caretonoids, chlorophyll, anthocyanins and betalains which are proved to be alternatives to synthetic colorants. The feasibility of incorporating natural colour, anthocyanin, extracted from pomegranate peel, in three processed food products *viz*., squash, jelly and candy each of liquid, semisolid and solid nature was tested in the present experiment.

Lime squash, jackfruit jelly and ashgourd candy were prepared each with 1%, 2% and 3% anthocyanin pigment along with control (no pigment) and were subjected to evaluation of chemical, nutritional and sensory quality parameters for selecting the most acceptable concentration of pigment.

Acidity and total sugar were not influenced by pigment incorporation. Squash without pigment incorporation had the highest TSS content (42^o brix). When the anthocyanin pigment was incorporated as a food colourant, the TSS content of squash was seen reduced. Ascorbic acid content was same for 1% pigment incorporated squash and colourless squash. As the concentration of anthocyanin pigment incorporation was increased, ascorbic acid content of the product was reduced. Ascorbic acid content was 332.8mg $100g^{-1}$ in 2% pigment incorporated squash and the ascorbic acid was reduced when the pigment concentration was enhanced to 3%. The incorporation of more extract from pomegranate peel had resulted in reduced ascorbic acid content. This may be because of the low vitamin C content recorded in pomegranate peel (Barros *et al.,* 2014). As the incorporation of anthocyanin pigment increased, the anthocyanin content in squash also gradually increased. Squash with 2% anthocyanin pigment had a comparatively higher anthocyanin content (7.42 mg 100 g^{-1}) and 3% anthocyanin pigment incorporated squash had highest anthocyanin content (10.10 mg 100g^{-1}). But all the pigment incorporated squash had the same antioxidant activity irrespective of pigment concentration, which was very high compared to colourless squash. Antioxidant activity of 1% pigment incorporated squash was 90.08%, it was 89.44% for 2% incorporated squash and the activity was 87.80 % for 3% anthocyanin incorporated squash. Colour is also thought to be related to antioxidant capacity (Comert *et al*, 2020). The overall concentration of bioactive substances, such as ascorbic acid, tocopherols, carotenoids, and phenolic compounds, is represented by the total antioxidant capacity of fruits and vegetables. According to Pennington and Fisher (2009), some of these antioxidant molecules are known to be pigmented and have a distinctive colour. These pigmented bioactive compounds, anthocyanins, being one of the main phenolic groups in fruits and vegetables (Melo *et al*., 2009) come into prominence when accounting for their antioxidant capacity. The color of fruits and vegetables is consistent with their antioxidant content and consequently with their antioxidant capacity. The pomegranate peel had a high amount of phenolic compounds and high levels of antioxidants, and this peel was used to enrich a commercially-available juice .The antioxidant activity of the pomegranate peel was thirty times greater than peels of apple and grape (Barros *et al*., 2014).

Any product rich in nutritional quality can be recommended as a quality product. But sensory quality has a key influence on how consumers perceive the quality of a product. Consumers are therefore interested not only in nutritional value of food, but also in its content of natural substances that improve the sensory appeal of the food viz., color, flavor etc. When the pigment incorporated squash were scored with colourless squash for sensory parameters, all the parameters except texture were seen significantly influenced. But the sensory evaluation showed no difference between the commercially-prepared guava juices with and without incorporating dried ethanol extract pomegranate peels (Barros *et al.,* 2014). The difference in sensory scoring may be due to the concentration of pigment incorporation tried for enrichment. Though the darker colour is generally accepted for any product by the consumers, 3% concentration was not at all acceptable to the panel, which was proven by the least sensory scores for appearance (6.50), colour (6.77), flavour(6.22), taste (6.04) and hence with the least overall acceptability (6.49). Squash with no pigment addition had a higher score for appearance (7.59), colour (6.90), flavour (7.63), taste (8.09) and hence with the highest overall acceptability (7.56) indicating that the natural colour of lime was most appealing to the panel and addition of purple colour in lime was not acceptable to the panel members (Fig 2). As the objective was to find out the best concentration of natural colour pigment, 2% concentration, which had resulted in squash with high mean scores for colour (7.86), appearance (8.22) and overall acceptability (7.52) was as selected as the best concentration.

Production cost of one litre squash with 1%, 2%, 3% anthocyanin incorporation was Rs.60.90/-, Rs.73.30/-, and Rs.85.70/- respectively whereas production cost of 1 litre colourless squash was Rs.48.50/-, indicating the economic feasibility.

The second product was jelly prepared from byproduct of jackfruit, representing semisolid nature. Anthocyanin pigment was incorporated at 1%,2%,3% along with control and the products were evaluated for chemical, nutritional and sensory quality parameters for selecting the most acceptable concentration of pigment.

1% anthocyanin incorporated jelly had the highest TSS (66^{o} Brix), acidity (1.19%) and reducing sugar (20.89%). Ascorbic acid content was highest (118.45mg $100g^{-1}$) in jelly with no anthocyanin incorporation. When extract from pomegranate peel was incorporated, ascorbic acid content of the jelly was reduced and as the pigment concentration was increased, ascorbic acid content was reduced gradually. As the concentration of pigment enhanced from 1 to 3%, anthocyanin content of the jelly was seen increased.

Moisture content was highest in jellies with no added colour pigment. But in another study by Salihu *et al*., (2023), the enrichment of biscuits with blueberry powder significantly increased the moisture content. In the present study, the extract was in the viscous formulation and it might have absorbed the extra moisture content in the jelly, resulting in reduced moisture content in all the coloured jellies. With the increase in pigment concentration, moisture content of the jelly was increased.

Antioxidant activity of the pigment incorporated jellies was higher compared to colourless jelly. All the pigment incorporated jellies had similar and high antioxidant activity, with 43.45, 39.78 and 41.0 % for 1, 2 and 3% pigment incorporation respectively. Kanemozhi *et al*., (2020) have formulated good quality jelly incorporated with pomegranate peel.The antioxidant activity of anthocyanins in food is well known. The coloured pigments of anthocyanin from berries, blackcurrants, and other types of red to blue coloured fruits are strong antioxidants. A direct relationship was observed between the pomegranate peel incorporated food product and increased antioxidant activity (Barros *et al*., 2014). The lowest concentration tested by them (0.3%), did not show an antioxidant activity that was significantly different from the control (0%). But in the present experiment, the concentrations tried were 1, 2 and 3%.

A prerequisite for a product launch is usually thought to be sensory analysis (Arazi and Kilcast, 2001). The addition of the extracts in the three concentrations studied did not cause a reduction in product acceptance in relation to the attribute of flavor and taste. This was in accordance with the findings of Barros et al. (2014) in enriched guava juice. Appearance, colour, texture and overall acceptability were affected by pigment incorporation (Fig 3). The jelly with no added colour had least sensory scores for appearance, colour, texture and overall acceptability indicating the possibility of colouring the jelly made from non edible parts of jackfruit for enhanced acceptance. With increase in pigment concentration, acceptance was also increased. Jelly with 2 and 3% pigment incorporation had same acceptability. Considering the superior biochemical and sensory quality parameters, 3% was selected as best pigment concentration for incorporation in jelly.

Cost of production of one kilogram pigment incorporated jelly at 1%,2% and 3% was estimated as Rs.72.40/-, Rs 89.80/-, Rs.97.20/ respectively whereas production cost of one kilogram colourless jelly was Rs.65.00/- proving the economic feasibility of colour incorporated jelly preparation.

The third product selected representing a solid nature was ashgourd candy, which was prepared with 1%,2% and 3% anthocyanin pigment along with control.

All the biochemical parameters of candy except reducing sugar were influenced by incorporation of anthocyanin pigment. Ascorbic acid content was least (39.28mg

100g^{-1})for colourless candy and candy incorporated with 1% pigment (45.23 mg 100g^{-1}), indicating that concentration of 1% was too low to improve the ascorbic acid content in candy. When the concentration was increased to 2 and 3%, ascorbic acid was increased to 65 mg 100g^{-1}. This situation was different from two other products.

Anthocyanin content, antioxidant activity and moisture content were lowest for colourless candy. When anthocyanin pigment was incorporated into candy, all these parameters in candy were improved. As the pigment concentration was increased, the content was also increased in candy. Addition of colour pigment in viscous formulation has resulted in enhanced moisture content in coloured candies. The use of anthocyanins as food colorants not only confers improvements in product appearance but also prevents autoxidation and lipid peroxidation in biological systems (Shipp and Abdel-Aal, 2010). However, studies about their potential health benefits are further necessary. The incorporation of jamun fruit peel in the preparation of processed products was found to increase the availability of bioactive compounds in such enriched food products (Santiago *et al*., 2016). As in other two products, the antioxidant properties of candies were improved with addition of anthocyanin pigment.

Sensory scores for flavour, taste and texture were not influenced by pigment incorporation, indicating that the addition of pomegranate peel pigment will not adversely affect the flavour, taste and texture of candy. But the colour and appearance of candies with no pigment were highest. This indicates that pigment incorporation was not appealing to the panel with respect to parameters like colour and appearance. Computation of overall acceptability also showed the superiority of colourless candy with highest overall acceptability score (8.30). Among the coloured candies, candy with 2% pigment recorded the highest overall acceptability of 8.00 (Fig 4).

In short, candy with 2% anthocyanin pigment had highest ascorbic acid content (65mg 100g^{-1}) and antioxidant activity (59.09%) lowest acidity (0.26%) with highest score for appearance (7.80), colour (7.90) and overall acceptability (8.0) among coloured candies; hence selected as the optimum concentration of anthocyanin pigment for incorporation in candy.

Cost of production of one kilogram pigment incorporated candy at 1%, 2% and 3% was estimated as Rs.118.90/-, Rs.131.30/-and Rs.138.70/- respectively whereas production cost of candy colourless candy was Rs.106.50/-.

In general, incorporation of anthocyanin pigment resulted in products enriched with anthocyanin content and antioxidant activity. As the concentration increased from 1 to 3% there was significant increase in anthocyanin concentration. But antioxidant activity was same and higher for all the pigment incorporated products compared to colourless products. As consumer acceptance is the most important parameter to be considered, the concentration of pigment was selected for each commodity. The use of natural ingredient makes any food product more acceptable (Sharma, *et al.* 2005). This is correct in case of food colourants too. Kamatar (2013) stated 8 specific reasons for adding colours to any food stuff, viz., enhanced appearance, greater stability and less wastage in storage, product identity, original appearance, product homogeneity, intensifying natural color, and the capacity to color otherwise uncolored food. Hence addition of anthocyanin pigment can be recommended, as it enhanced the antioxidant activity. Antioxidants for use in food must be safe, cheap, easy to incorporate stable and capable in holding on during processing, no odour, taste or colour of their own, effective at low concentrations, and have a good solubility (Shahidi and Ambigaipalan, 2015). The pomegranate peel added as food colourant acted as an efficient antioxidant in all the three food formulation satisfying all the above requirements.

5.2. Evaluation of pigment stability in products

Any functional ingredient, which is incorporated in food formulation, should improve the quality of food and should be stable till it reaches the consumer. Instability is one of the major limitations or disadvantages in application of natural food colours. Hence stability of the pigment was tested in lime squash, jackfruit jelly and ashgourd candy, which were prepared by incorporating anthocyanin pigment at the selected optimum concentration and stored in glass containers under ambient and refrigerated (4-7^{o}c) condition for a period of three months.

All the quality parameters of all the three colour incorporated products (2% colour incorporated squash, 3% colour incorporated jelly and 2% pigment incorporated candy) were changed during the storage period. Biochemical and nutritional parameters like TSS, reducing sugar, total sugar, ascorbic acid, anthocyanin and antioxidant activity of the products were decreased whereas acidity was increased during storage period. Since most food products are defined by a relative stability over time, influenced by both internal and external structural factors, which can modify their fundamental properties through some degradation, alteration, chemical, or microbiological processes, the food quality can undergo significant changes during storage. Nature and quality of food, storage conditions, environmental factors viz., temperature and light, nature of packaging etc. affect the shelf life and quality of food products to great extent. The alteration consists of modification, in a negative sense, of the initial properties of the food product, so that the respective product registers losses of chemical and nutritional value. There are several parameters *viz*., water activity, pH which are optimal considered to control the food quality (Bonciu *et al*., 2022). Along with biochemical parameters, nutritional parameters viz., ascorbic acid, anthocyanin and antioxidant activity also showed a gradual reduction. Ascorbic acid content of lime squash was reduced from 52.01 to 43.78 mg $100g^{-1}$, 63.01 to 44.40 mg$100g^{-1}$ in jackfruit jelly and it was reduced from 47.90 - 41.04 mg $100g^{-1}$ in ashgourd candy (Fig 5).

Foods with antioxidant qualities have gained popularity in recent years due to the well-known antioxidant activity of their anthocyanins. By incorporating the anthocyanin extract of pomegranate peel, antioxidant activity of the products was increased. But antioxidant activity of lime squash was reduced from 87.64 to 87.22%, from 58.51 to 39.07% in jackfruit jelly and from 77.60 to 56.77% in ashgourd candy during three months of storage (Fig 6), showing a percent reduction of 0.48%, 33.23% and 26.84 % in squash, jelly and candy respectively.

Though the biochemical properties and antioxidant activity were considerably reduced, the stability of anthocyanin pigment was considerably high in all the three products. One of the main restrictions on using these natural colors is their instability. (Enaru *et al*., 2021). Anthocyanin content of lime squash was reduced from 7.17 to 6.38 mg $100g^{-1}$ during three months of storage ; from 5.38 to 5.04 mg $100g^{-1}$ in jackfruit jelly and in ashgourd candy, anthocyanin content was reduced from 7.64 to 7.37 mg $100g^{-1}$(Fig 7) . The products showed a reduction of 11.02%, 6.32% and 3.53% in lime squash, jackfruit jelly and ashgourd candy respectively indicating a reasonable stability of anthocyanin pigment during storage. Loss of 10% and 36% was reported in anthocyanin content of jamun peel powder after the second month and fifth month of storage due to occurrence of undesirable chemical reactions such as oxidation and polymerization of anthocyanins (Santiago *et al*., 2016).

The pigment incorporated products stored under refrigerated condition had superior quality compared to those stored under ambient condition. Anthocyanins are valuable biocompounds with colour that are becoming more and more extracted globally, but their poor environmental stability limits their practical applications (Tena *et al*., 2020). In industrial food processing, temperature is a crucial factor that affects the food matrix, especially heat-sensitive substances like anthocyanins. Anthocyanin pigments have been widely used as natural food colorants. However, the color and stability of these pigments are influenced by pH, light and temperature (Oancea, 2021). Compared to refined extracts, crude extracts provide more thermally stable anthocyanins.In the present experiment, anthocyanain has been extracted by traditional method of maceration, which may be the reason for reduced reduction of anthocyanin content in tested products.

Sensory scores for appearance and colour of pigment incorporated products were affected during storing period. Sensory scores were reduced significantly and reduction was less under refrigerated storage. This loss did not affect the product's quality as a colored product.

The present study could formulate three different processed products incorporated with anthocyanin pigment as natural colourant. The optimum concentration of anthocyanin pigment was standardized. All the processed product were rich in antioxidant activity as natural food colourant, proving the potential of pomegranate peel as a natural food colourant in processing industry. This peel color extract could also be used as a functional ingredient in the development of food products due its antioxidant properties.

Though the quality parameters were affected during storing period, the reduction was less under refrigerated condition. Sources rich in anthocyanins are very interesting options as functional food colours (Motohashi and Sakagami, 2009). As pomegranate peel is rich in bioactive compounds, value can be added to the final product, as these antioxidant compounds are known to protect health and improve the life quality of the consumers. It will be possible to rationally create stable functional items in the future that maintain these bioactive molecules and their functions to a large degree by ensuring certain thermal parameters are met throughout the processing and storage of anthocyanin-rich food (Enaru *et al*., 2021).

Figure 2. Sensory quality parameters of anthocyanin pigment added squash

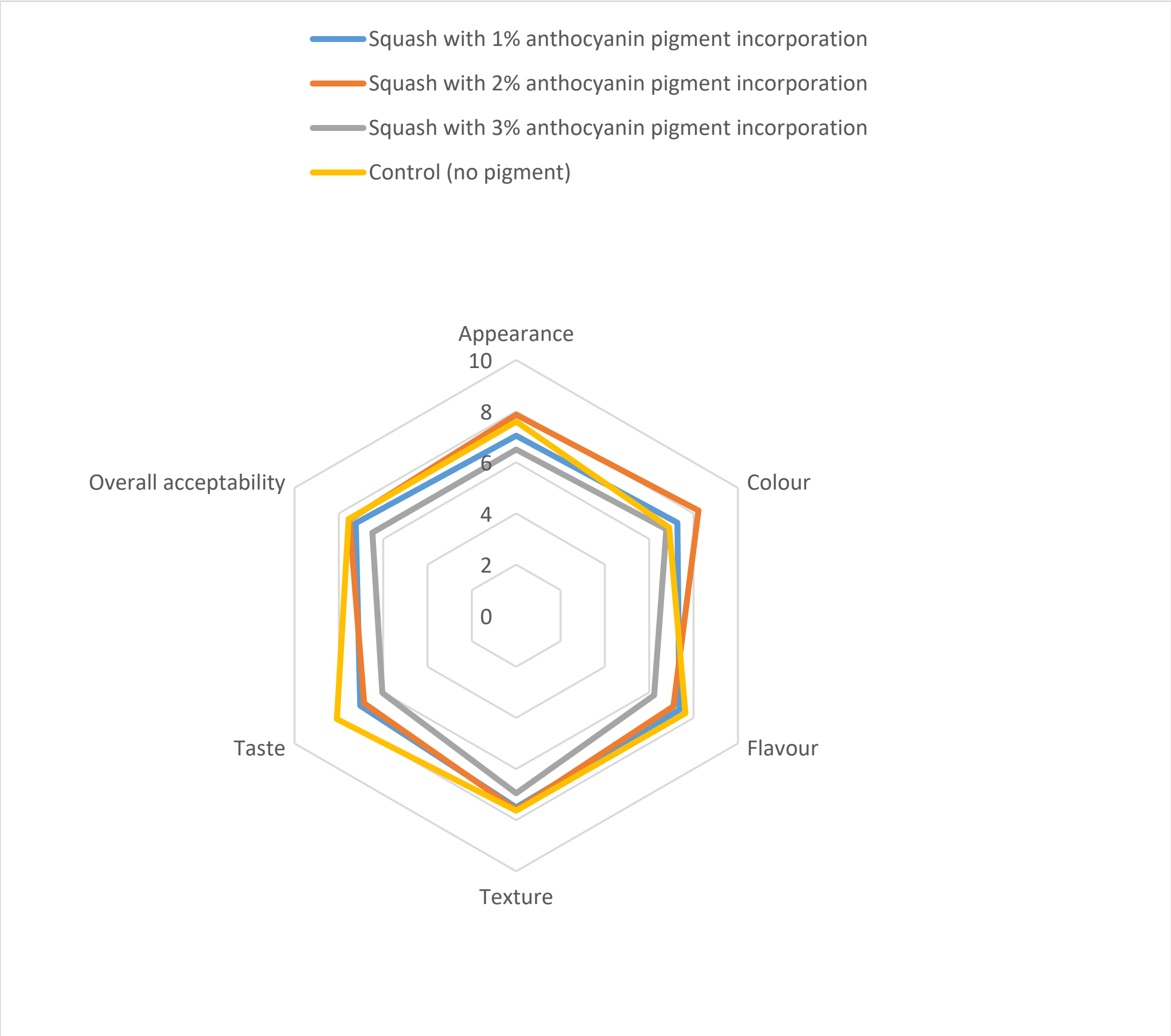


Figure 3. Sensory quality parameters of anthocyanin pigment added jelly

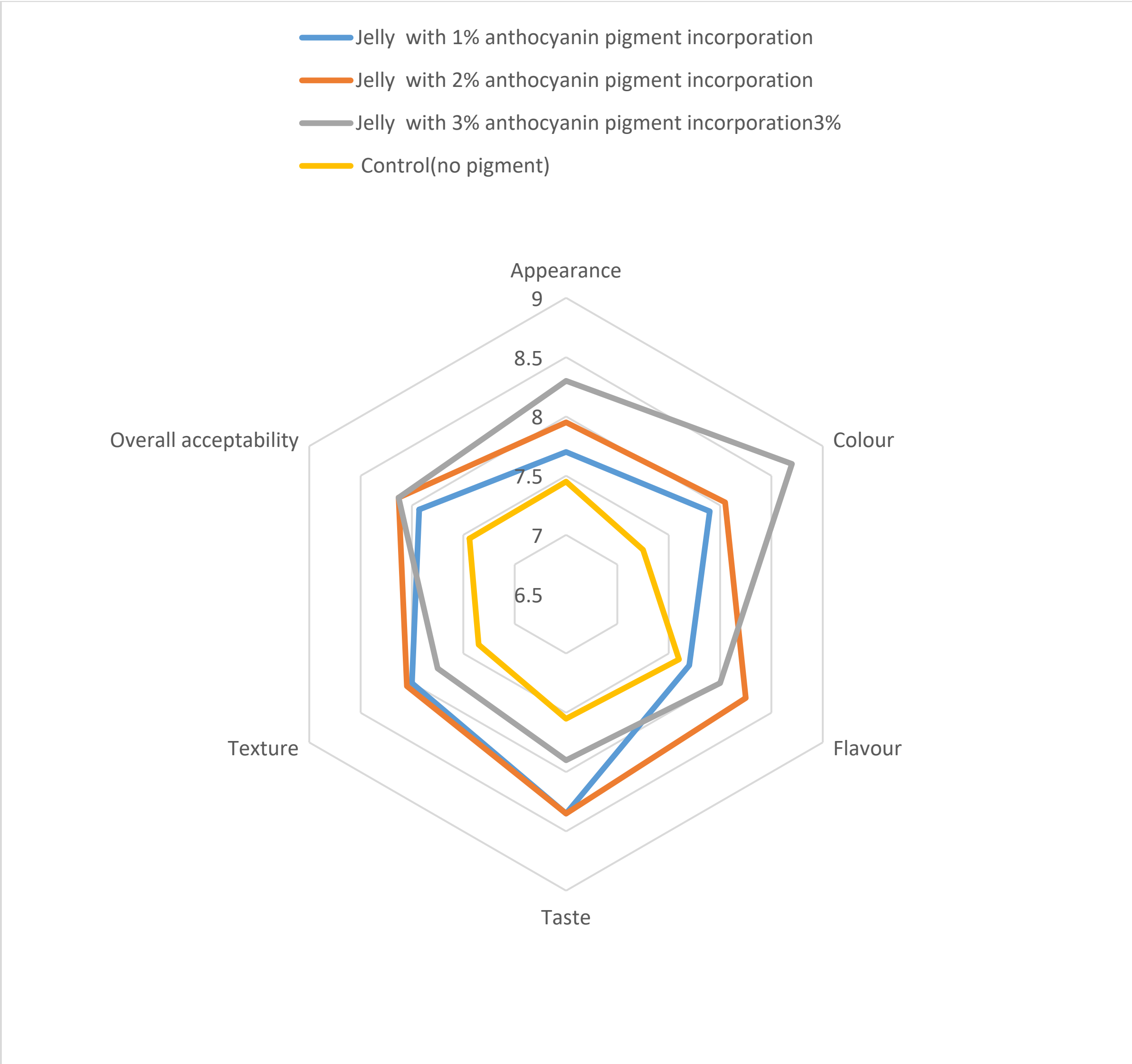

Jelly with 1% anthocyanin pigment incorporation
Jelly with 2% anthocyanin pigment incorporation
Jelly with 3% anthocyanin pigment incorporation3%
Control(no pigment)
Appearance
9
8.5
8
7.5
7
6.5
Colour
Flavour
Taste
Texture
Overall acceptability

Figure 4. Sensory quality parameters of anthocyanin pigment added candy

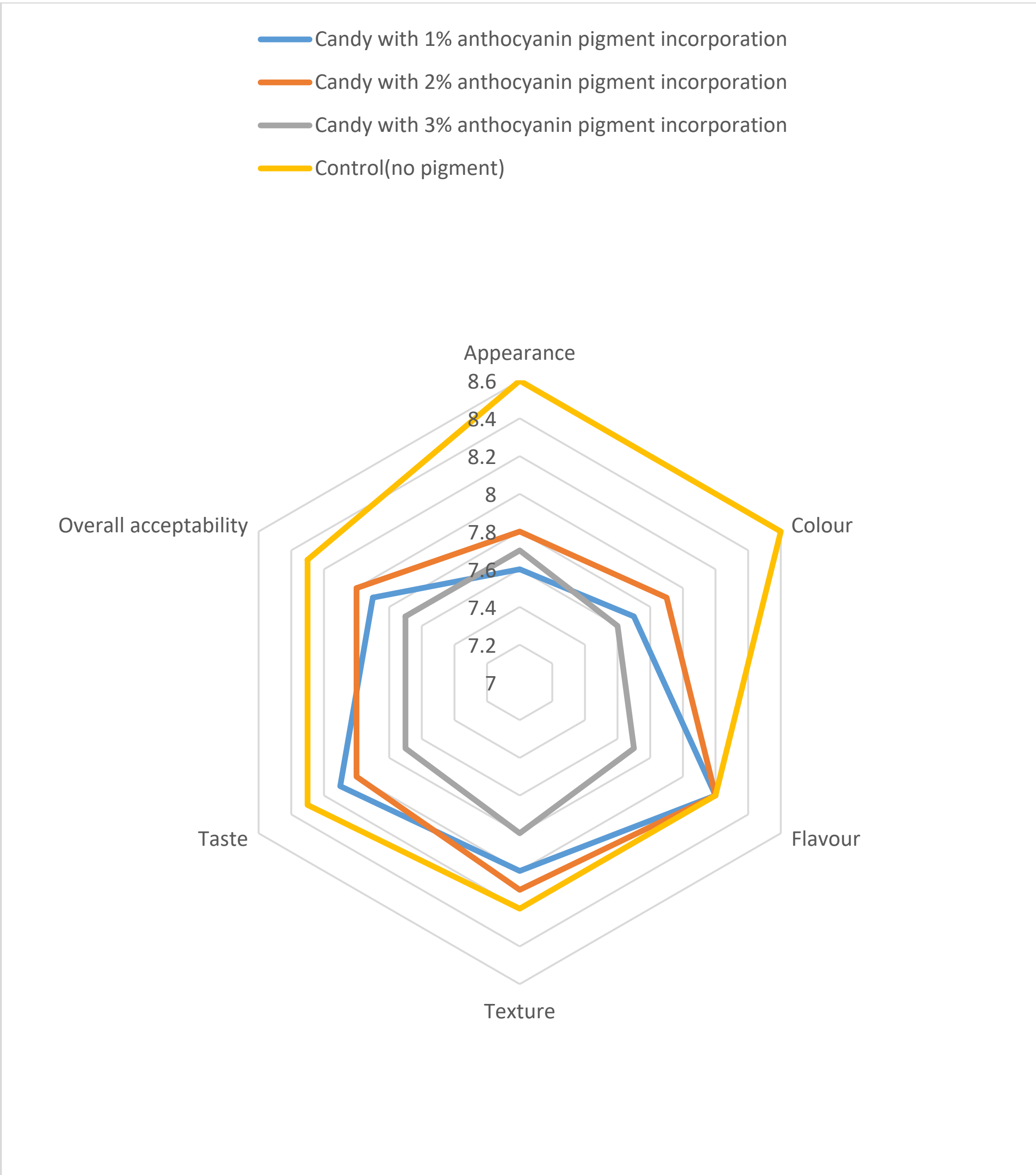


Figure 5. Effect of storage on ascorbic acid content of squash, jelly and candy (mg $100g^{-1}$)

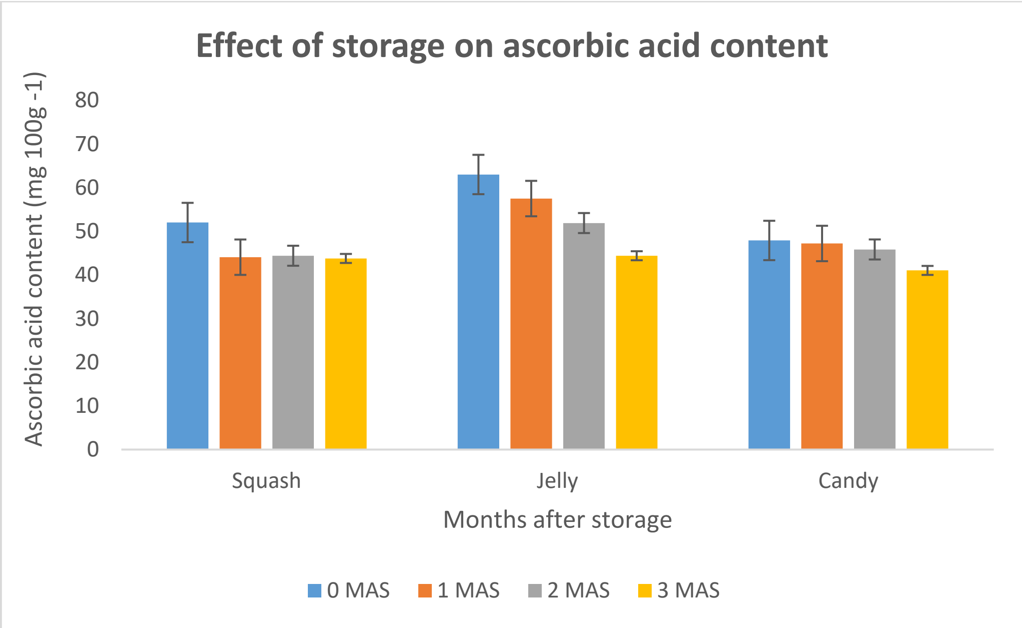


Figure 6. Effect of storage on total antioxidant activity(%) of squash, jelly and candy

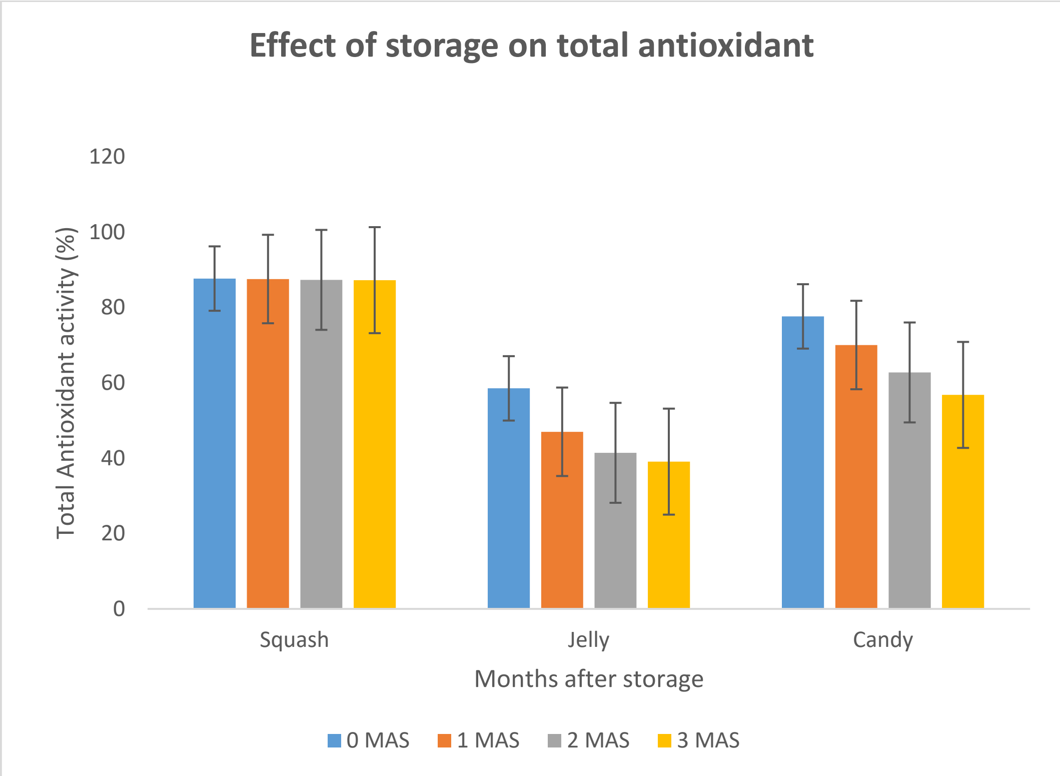
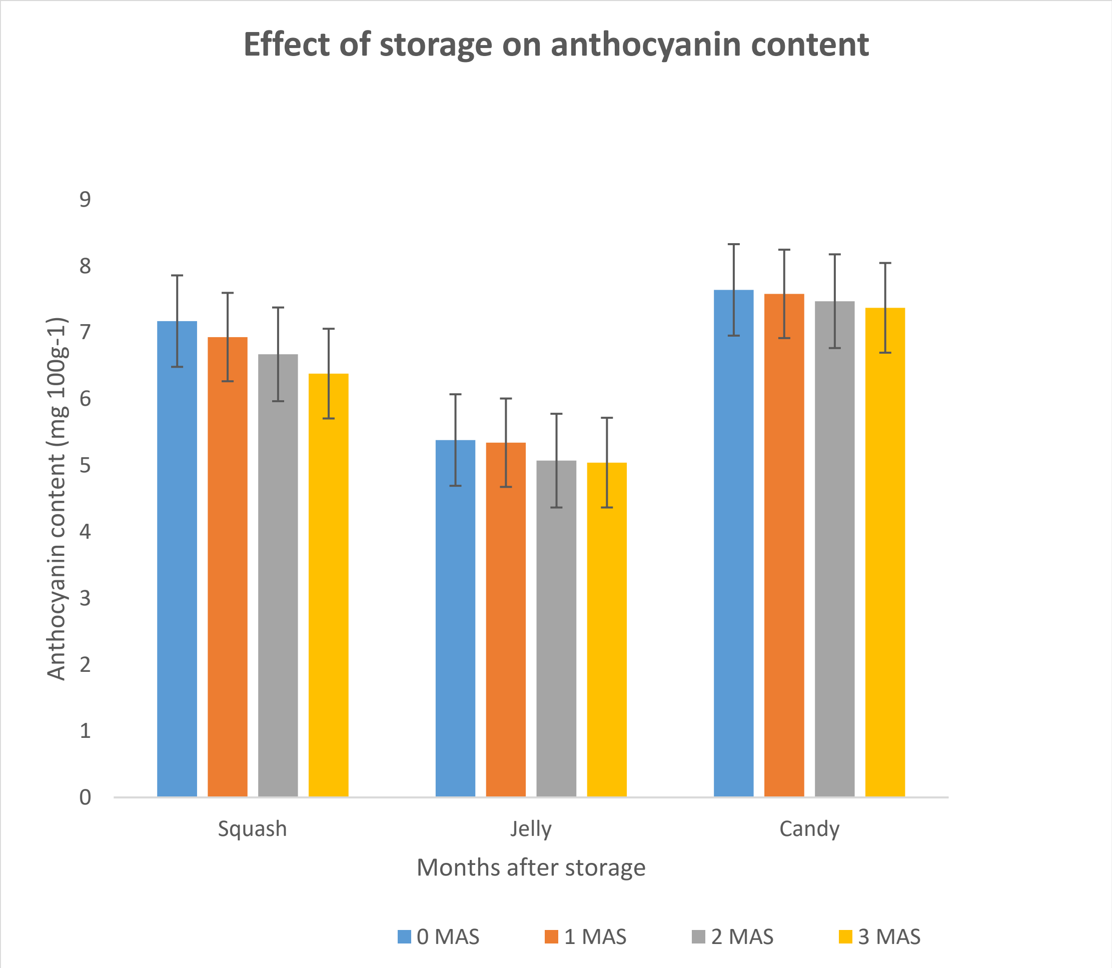


Figure 7. Effect of storage on anthocyanin content of squash, jelly and candy (mg $100g^{-1}$)



Effect of storage on anthocyanin content
Anthocyanin content (mg 100g-1)
9
8
7
6
5
4
3
2
1
0
Squash
Jelly
Candy
Months after storage
0 MAS
1 MAS
2 MAS
3 MAS

6. SUMMARY

The present study titled “Utilization of pomegranate (*Punica granatum L.*) peel as a natural food colourant” was carried out in the Department of Postharvest Management, College of Agriculture,Vellayani during the period 2021-2023, with the objective of standardizing food formulations using pomegranate peel as a natural colourant.

The study was carried out as two different independent experiments.

6.1. Development of processed products with anthocyanin pigment.
6.2. Evaluation of pigment stability in products

Major findings of the experiment are summarized below

6.1 DEVELOPMENT OF PROCESSED PRODUCTS WITH ANTHOCYANIN PIGMENT.

Anthocyanin pigment was extracted from peel of pomegranate fruits which was collected as a by product from processing unit as per the standardized protocol (Mini *et al*., 2021 and Gayathri, 2023). The pomegranate peels were cut into approximately 2 cm^3 size, stored at 4^0C in dark for 24hrs and dried in a cabinet drier at $50\underline{+}5^0$ C for 24 hrs. The crushed dehydrated peel pieces were macerated using acidified ethanol with 1% HCl in 2:1 liquid to solid ratio for 48 hrs under room temperature (30-35^0C) & 75-80% relative humidity. The filtered infusion mixture was evaporated under water bath at 60^0C for complete removal of solvent and collection of anthocyanin extract, for incorporation into food products.

Three processed food products *viz*., squash, jelly and candy each of liquid, semisolid and solid nature were selected for incorporation of anthocyanin pigment as natural food colourant.

Lime squash was prepared with 1%, 2% and 3% anthocyanin pigment along with control (no pigment) as per FSSAI specification and were subjected to evaluation of quality parameters for selecting the most acceptable concentration of pigment.

1% anthocyanin pigment incorporated squash had lowest acidity with highest antioxidant activity (90.48%) and ascorbic acid content (416 mg $100g^{-1}$). The 3% anthocyanin pigment incorporated squash had highest anthocyanin content (10.10 mg $100g^{-1}$) and higher antioxidant activity (89.44%). But 3% concentration was not at all acceptable to the panel, which was proven by the least sensory scores for appearance (6.50), colour (6.77), flavour(6.22), taste (6.04) and hence with the least overall acceptability (6.49). Squash with 2% anthocyanin pigment had highest antioxidant activity (89.44%) and a comparatively higher anthocyanin content (7.42 mg 100 g^{-1}) with maximum overall acceptability score of (7.52) among the coloured squash.It had higher mean scores for appearance (7.86) and colour (8.22); hence 2% was selected as the best concentration of anthocyanin pigment for incorporation in lime squash.

Cost of production of one litre squash with 1%,2%,3% anthocyanin incorporation was Rs.60.90/-,Rs.73.30/-,Rs.85.70/- respectively whereas production cost of 1 litre colourless squash was Rs.48.50/-indicating the economic feasibility.

Jelly was prepared using non edible portions of jackfruit and anthocyanin pigment was incorporated at 1%,2%,3% along with control as per the FSSAI specification and were subjected to evaluation of quality parameters for selecting the most acceptable concentration of pigment.

1% anthocyanin incorporated jelly had highest TSS (66° Brix), acidity (1.19%), reducing sugar (20.89%) and highest antioxidant activity (43.45%); but had least overall acceptability among coloured jelly. 2% anthocyanin incorporated jelly had higher antioxidant activity (39.78%) and overall acceptability (8.13). Jelly with 3% anthocyanin had lowest acidity(0.89%), highest anthocyanin content(5.52mg/$100g^{-1}$) and antioxidant activity(41.00%) with highest mean score of appearance(8.30) and colour(8.70) and overall acceptability score (8.13).Hence 3% was selected as best pigment concentration for incorporation in jelly.

Antioxidant activity of the pigment incorporated jellies was higher compared to colourless jelly. The jelly with no added colour had least sensory scores for appearance, colour, texture and overall acceptability indicating the possibility of colouring the jelly. With increase in pigment concentration, acceptance was increased. Jelly with 2 and 3% pigment incorporation had same acceptability. Considering the superior biochemical and sensory quality parameters, 3% was selected as best pigment concentration for incorporation in jelly.

Cost of production of one kilogram pigment incorporated jelly at 1%,2% and 3% was estimated as Rs.72.40/-, Rs 89.80/-, Rs.97.20/ respectively whereas production cost of one kilogram colourless jelly was Rs.65.00/-.proving the economic feasibility of colour incorporated jelly preparation.

Ashgourd candy was prepared with 1%,2% and 3% anthocyanin pigment along with control as per the FSSAI specification and were subjected to quality evaluation for selecting the most acceptable concentration of pigment.

The candy with 3% anthocyanin pigment had highest TSS(76.50^{0}Brix),total sugar (78.12 %$^{)}$,ascorbic acid (65mg 100g^{-1}),anthocyanin content (6.24 mg 100g-^{1}) and antioxidant activity (79.68%), but had the lowest sensory acceptability (7.70).Candy incorporated with 1% pigment had least anthocyanin content(3.93 mg 100g-^{1}) and highest total sugar(69.44%). Candy with 2% anthocyanin pigment had highest ascorbic acid content (65mg 100g^{-1}) and antioxidant activity (59.09%) and lowest acidity (0.26%).

Anthocyanin content and antioxidant activity were lowest for colourless candy. When pigment was incorporated into candy, all these parameters were improved. Addition of pomegranate peel pigment did not adversely affect the flavour, taste and texture of candy, but the colour and appearance of candies with no pigment were highest, indicating that pigment incorporation was not appealing to the panel with respect to parameters like colour and appearance. Computation of overall acceptability also showed the superiority of colourless candy with highest overall acceptability score (8.30). Among the coloured candies, candy with 2% pigment recorded the highest overall acceptability of 8.00; hence 2% was selected as the optimum concentration of anthocyanin pigment for incorporation in candy.

Cost of production of one kilogram pigment incorporated candy at 1%,2% and 3% was estimated as Rs.118.90/-, Rs 131.30/-, Rs.138.70/- respectively whereas production cost of candy colourless candy was Rs.106.50/-.

In general, incorporation of anthocyanin pigment resulted in products enriched with anthocyanin content and antioxidant activity. As the concentration increased from 1 to 3% there was significant increase in anthocyanin concentration. But antioxidant activity was same and higher for all the pigment incorporated products compared to colourless products.

6.2. EVALUATION OF PIGMENT STABILITY IN PRODUCTS

Lime squash, jackfruit jelly and ashgourd candy were prepared by incorporating anthocyanin pigment at the optimum concentration and subjected to a storage study for assessing the pigment stability. Stability of anthocyanin pigment was assessed by storing pigment incorporated products in glass containers under ambient and refrigerated (4-7^{o}c) condition for a period of three months.

All the quality parameters of all the three colour incorporated products (2% colour incorporated squash, 3% colour incorporated jelly and 2% pigment incorporated candy) were changed during the storage period. Biochemical and nutritional parameters like TSS, reducing sugar, total sugar, ascorbic acid, anthocyanin and antioxidant activity of the products were decreased whereas acidity was increased during storage.

Ascorbic acid, anthocyanin and antioxidant activity showed a gradual reduction. Ascorbic acid content of lime squash was reduced from 52.01 to 43.78 mg 100g^{-1}, 63.01 to 44.40 mg100g^{-1} in jackfruit jelly and it was reduced from 47.90 - 41.04 mg 100g^{-1} in ashgourd candy.

Antioxidant activity of lime squash was reduced from 87.64 to 87.22%, from 58.51 to 39.07% in jackfruit jelly and from 77.60 to 56.77% in ashgourd candy during three months of storage, showing a percent reduction of 0.48%, 33.23% and 26.84 % in squash, jelly and candy respectively.

Anthocyanin content of lime squash was reduced from 7.17 to 6.38 mg 100g^{-1} during three months of storage; from 5.38 to 5.04 mg 100g^{-1} in jackfruit jelly and in ashgourd candy, anthocyanin content was reduced from 7.64 to 7.37 mg $100g^{-1}$. The products showed a reduction of 11.02%, 6.32% and 3.53% in lime squash, jackfruit jelly and ashgourd candy respectively indicating a reasonable stability of anthocyanin pigment during storage.

The pigment incorporated products stored under refrigerated condition had superior quality compared to those stored under ambient condition.

Sensory scores for appearance and colour of pigment incorporated products were affected during storing period. Sensory scores were reduced significantly and reduction was less under refrigerated storage. Despite these losses, the product maintained its quality as a coloured product.

The present study could formulate three different processed products incorporated with anthocyanin pigment as natural colourant. The optimum concentration of anthocyanin pigment was standardized. All the processed product were rich in antioxidant activity as natural food colourant, proving the potential of pomegranate peel as a natural food colourant in processing industry. This peel color extract could also be used as a functional ingredient in the development of food products due its antioxidant properties. Though the quality parameters were affected during storing period, the reduction was less under refrigerated condition.

Future line of work

- Enhancement of pigment stability by modification of storage condition and extraction techniques
- Production of high value functional foods with retention of biochemical and nutritional parameters.
- Screening of other by products of processing industry as natural colorant.

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Cost of production of 1litre squash													
No	Ingredients	Squash with 1% pigment incorporation			Squash with 2% pigment incorporation			Squash with 3% pigment incorporation			Control (without pigment)		
		Quantity	Rate (kg)	Cost (Rs)	Quantity	Rate (kg)	Cost (Rs)	Quantity	Rate (kg)	Cost (Rs)	Quantity	Rate (kg)	Cost (Rs)
1	Lemon Fruit juice	250g	90	22.50	250g	90.00	22.50	250g	90	22.50	250g	90	22.50
2	Sugar	400g	42	17.00	400g	42.00	17.00	400g	42	17.00	400g	42	17.00
3	Preservatives	1g	500	1.00	1g	500	1.00	1g	500	1.00	1g	500	1.00
4	Anthocyanin pigment*	10g	130	12.40	20g	130	24.80	30g	130	37.20	nil	nil	nil
5	Storage bottles	2		6.00	2		6.00	2		6.00	2		6.00
6	Miscellaneous (labour, etc)			2.00			2.00			2.00			2.00
		Total cost		63.40	Total cost		75.80	Total cost		88.20	Total cost		51.00

Annexure cost of production 1

Cost of production of 1Kg jelly													
No	Ingredients	Jelly with 1% pigment incorporation			Jelly with 2% pigment incorporation			Jelly with 3% pigment incorporation			Control (without pigment)		
		Quantity	Rate (kg)	Cost (Rs)	Quantity	Rate (kg)	Cost (Rs)	Quantity	Rate (kg)	Cost (Rs)	Quantity	Rate (kg)	Cost (Rs)
1	Jackfruit pectin extract(by product utilization)	1000g	nil	nil	1000g	nil	nil	1000g	nil	nil	1000g	nil	nil
2	Sugar	1000g	42	42.00	1000g	42	42.00	1000g	42	42.00	1000g	42	42.00
3	Citric acid	5g	440	3.00	5g	440	3.00	5g	440	3.00	5g	440	3.00
4	Anthocyanin pigment*	10g	130	12.40	20g	130	24.80	30g	130	37.20	nil	nil	nil
5	Storage bottles	2		15.00	2		15.00	2		15.00	2		30.00
6	Miscellaneous (labour, Packaging cost etc)			5.00			5.00			5.00			5.00
		Total cost		92.40	Total cost		104.80	Total cost		115.20	Total cost		80.00

*cost of production of 100ml anthocyanin pigment as per the standardized protocol =Rs.124

Annexure Cost of production 2

*cost of production of 100ml anthocyanin pigment as per the standardized protocol =Rs.124

Annexure Cost of production 3

Cost of production of 1Kg candy													
No	Ingredients	Candy with 1% anthocyanin pigment			Candy with 2% anthocyanin pigment			Candy with 3% anthocyanin pigment			Control (without pigment)		
		Quantity	Rate (kg)	Cost (Rs)	Quantity	Rate (kg)	Cost (Rs)	Quantity	Rate (kg)	Cost (Rs)	Quantity	Rate (kg)	Cost (Rs)
1	Ashgourd	1000g	55	55.00	1000g	55	55.00	1000g	55	55.00	1000g	55	55.00
2	Calcium carbonate	40g	300	12.00	40g	300	12.00	40g	300	12.00	40g	300	12.00
3	Sugar	400g	42	17.00	400g	42	17.00	400g	42	17.00	400g	42	17.00
4	Citric acid	2g	440	1.50	2g	440	1.50	2g	440	1.50	2g	440	1.50
5	Preservatives	1g	500	1.00	1g	500	1.00	1g	500	1.00	1g	500	1.00
6	Anthocyanin pigment*	10g	130	12.40	20g	130	24.80	30g	130	37.20	nil	nil	nil
7	Storage bottle	2		30.00	2		30.00	2		30.00	2		30.00
8	Miscellaneous(labour charge,Packaging charge etc)			5.00			5.00			5.00			5.00

		Total cost	133.90	Total cost	146.30	Total cost	153.70	Total cost	121.50

*cost of production of 100ml anthocyanin pigment as per the standardized protocol =Rs.124

UTILIZATION OF POMEGRANATE (*Punica granatum* L.) PEEL AS A NATURAL FOOD COLOURANT

by

DEENA
BIJOY
(2021-12- 040)

ABSTRACT

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MASTER OF SCIENCE IN HORTICULTURE

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ABSTRACT

The present study titled "Utilization of pomegranate (*Punica granatum* L.) peel as a natural food colourant" was carried out in the Department of Postharvest Management,College of Agriculture,Vellayani during the period 2021-2023, with the objective of standardizing food formulations using pomegranate peel as a natural colourant.

The study was carried out as two experiments with anthocyanin pigment extracted by acidified ethanolic maceration of dehydrated peels of pomegaranate (*Punica granatum* L.) fruits which were collected as a byproduct from processing unit.

Three processed food products viz., lime squash, jackfruit jelly and ashgourd candy, each of liquid, semisolid and solid nature were prepared with incorporation of anthocyanin pigment at 1%, 2% and 3% concentration along with control in the first part of the experiment and were subjected to evaluation of chemical, nutritional and sensory quality parameters for selecting the most acceptable pigment concentration.

Lime squash with 2% anthocyanin pigment had highest antioxidant activity (89.44%) and higher anthocyanin content (7.42 mg100g^{-1}) with highest mean scores for appearance (7.86), colour (8.22) and overall acceptability (7.52); hence, 2% was selected as the optimum concentration of anthocyanin pigment for incorporation in lime squash.

Jackfruit jelly incorporated with 3% anthocyanin pigment had lowest acidity(0.89%), highest anthocyanin content (5.52 mg100g^{-1}) and a comparatively higher antioxidant activity (41.00%) with maximum mean score of appearance (8.30), colour(8.70) and overall acceptability score (8.13) and hence 3% was selected as the optimum concentration of anthocyanin pigment for incorporation in jelly.

The optimum concentration of anthocyanin pigment for candy preparation was found to be 2%, as it had high antioxidant activity (59.09%), higher anthocyanin

(6.24 mg $100g^{-1}$) and highest ascorbic acid content (65 mg 100g-1) as well as best appearance (7.80), color (7.90), and overall acceptability (8.0) score among the colored candies. Anthocyanin content and antioxidant activity were lowest for colourless candy and pigment incorporation did not adversely affect the flavour, taste and texture, but colour and appearance of candies with no pigment were highest. Computation of overall acceptability also showed the superiority of colourless candy with highest overall acceptability score (8.30). Among the coloured candies, candy with 2% pigment recorded the highest overall acceptability of 8.00; hence 2% was selected as the optimum concentration of anthocyanin pigment for incorporation in candy.

Incorporation of anthocyanin pigment resulted in production of products rich in anthocyanin contents and antioxidant activity. As the concentration increased from 1-3%, anthocyanin content was increased and antioxidant activity was same for all the pigment incorporated products.

The three products were prepared by incorporating the respective optimum concentration of anthocyanin and pigment stability in products was assessed by storing the products in glass containers under ambient and low temperature condition for a period of 3 months in the second part of the study.

Ascorbic acid, anthocyanin content and antioxidant activity were higher under low temperature compared to ambient storage in all the three pigment incorporated products. The quality parameters of all the products were affected by storage period. A reduction of 11.02%, 6.32% and 3.53% anthocyanin content was recorded in lime squash, jackfruit jelly and ashgourd candy respectively, indicating a reasonably higher pigment stability during storage.

The present study could formulate three different antioxidant rich processed products incorporated with natural anthocyanin from pomegranate peel, with comparatively better pigment stability for a period of three months, proving the potential of pomegranate peel as a natural colourant in processing industry.

<u>സംഗ്രഹം</u>

വെള്ളായണി കാർഷിക കോളേജിലെ പോസ്റ്റ് ഹാർവെസ്റ്റ് മാനേജ്മെന്റ് വിഭാഗത്തിൽ 2021-2023 കാലയളവിൽ മാതളത്തിന്റെ തൊലി ഒരു പ്രകൃതിദത്ത ഭക്ഷ്യ നിറമായി ഉപയോഗിക്കുന്നതിന്റെ സാധ്യത എന്ന വിഷയത്തിൽ ഒരു പഠനം നടത്തുകയുണ്ടായി.

സംസ്കരണ യൂണിറ്റിൽ നിന്ന് ഉപോൽപ്പന്നമായി ശേഖരിച്ച മാതളത്തിന്റെ തൊലിയിൽ നിന്ന് ഈതൈൽ ആൽക്കഹോൾ ഉപയോഗിച്ചുള്ള മാസറേഷൻ (maceration) എന്ന പ്രക്രിയ വഴി വേർതിരിച്ചെടുത്ത ആന്തോസയനിൻ നിറം ഉപയോഗിച്ചുള്ള പഠനം, രണ്ട് ഘട്ടങ്ങളായാണ് നടത്തിയത്.

മാതളത്തിന്റെ തൊലിയിൽ നിന്ന് വേർതിരിച്ചെടുത്ത നിറം 1% , 2%, 3% എന്നീ അളവിൽ ചേർത്ത് ദ്രാവക, അർദ്ധ ഖര, ഖര സ്വഭവത്തിലുള്ള യഥാക്രമം നാരങ്ങാ സ്ക്വാഷ്, ചക്ക ജെല്ലി, കുമ്പളങ്ങാ ക്യാൻഡി എന്നിങ്ങനെ മൂന്ന് സംസ്കരിച്ച ഭക്ഷ്യ ഉൽപ്പന്നങ്ങൾ തയ്യാറാക്കുകയും അവയെ ഏറ്റവും സ്വീകാര്യമായ നിറത്തിന്റെ അളവ് തിരഞ്ഞെടുക്കുന്നതിനുള്ള ഗുണനിലവാര പരിശോധനയ്ക്ക് വിധേയമാക്കുകയും ചെയ്തു.

2% നിറം ചേർത്ത് തയ്യാറാക്കിയ നാരങ്ങാ സ്ക്വാഷിൽ ഉയർന്ന അളവിൽ ആന്റിഓക്സിഡന്റുകൾ, (88.44%), ആന്തോസയനിൻ (7.42mg $100g^{-1}$) എന്നിവയും ഉയർന്ന സ്വീകാര്യതയും (7.52) ഉള്ളതിനാൽ നാരങ്ങാ സ്ക്വാഷിൽ

സംയോജിപ്പിക്കുന്നതിന് 2% സ്വീകാര്യമായ അളവായി തിരഞ്ഞെടുത്തു.

ഏറ്റവും കുറഞ്ഞ പുളിപ്പ് (0.89%), ആന്തോസയനിൻ (5.52mg 100g-1) താരതമ്യേന ഉയർന്ന ആന്റിഓക്സിഡന്റുകൾ (41.00%), മൊത്തത്തിലുള്ള സ്വീകാര്യത (8.13) എന്നിവ കണക്കിലെടുത്ത് ജെല്ലിയിൽ ചേർക്കുന്നതിനായി ഏറ്റവും യോജിച്ച അളവായി 3% തിരഞ്ഞെടുത്തു.

ഉയർന്ന ആന്റിഓക്സിഡന്റുകൾ (59.09%), ആന്തോസയനിൻ (6.24 mg 100g-1), അസ്കോർബിക് ആസിഡ് (65 mg 100g-1) എന്നിവ ഉള്ളതിനാൽ ക്യാൻഡി തയ്യാറാക്കുന്നതിനായി യോജിച്ച അളവ് 2% ആണെന്ന് കണ്ടെത്തി. നിറം ചേർക്കുന്നതുവഴി ക്യാൻഡികളുടെ സ്വാദ്, രുചി, ഘടന എന്നിവ പ്രതികൂലമായി ബാധിച്ചില്ല, എന്നാൽ നിറം ചേർക്കാത്ത ക്യാൻഡികളുടെ നിറവും രൂപവും ഏറേ സ്വീകാര്യമായിരുന്നു.

മാതളത്തിന്റെ തൊലിയിൽ നിന്ന് വേർതിരിച്ചെടുത്ത ആന്തോസയനിൻ നിറം ചേർക്കുന്നതുവഴി ഉൽപ്പന്നങ്ങളിലെ ആന്തോസയനിനും ആന്റിഓക്സിഡന്റുകളും വർദ്ധിക്കുന്നതായി കണ്ടു.

നാരങ്ങാ സ്കാഷ്, ചക്ക ജെല്ലി, കുമ്പളങ്ങാ ക്യാൻഡി എന്നീ ഉൽപ്പന്നങ്ങൾ ഒന്നാം ഭാഗത്തിൽ കണ്ടെത്തിയ ആന്തോസയനിൻ നിറത്തിന്റെ അളവിൽ (2%-നാരങ്ങാ സ്കാഷ്, കുമ്പളങ്ങാ ക്യാൻഡി, 3%- ചക്ക ജെല്ലി) ചേർത്ത് ഉണ്ടാക്കുകയും അവ ഗ്ലാസ് കുപ്പികളിൽ നിറച്ച് സാധാരണ താപനിലയിലും താഴ്ന്ന താപനിലയിലും മൂന്ന് മാസം സംഭരിച്ച് വച്ച് അവയുടെ ഗുണ നിലവാരം പരിശോധിക്കുകയും ചെയ്തു.

അസ്കോർബിക്ആസിഡ്,ആന്തോസയനിൻ,ആന്റിഓക്സി ഡന്റുകൾ എന്നിവയുടെ അളവ് താഴ്ന്ന ഉഷ്മാവിൽ സൂക്ഷിച്ച മൂന്ന് ഉൽപ്പന്നങ്ങളിലും കൂടുതലായി കണ്ടു. നിറം ചേർത്ത ഉൽപ്പന്നങ്ങളുടെ ഗുണങ്ങളെല്ലാം തന്നെ സംഭരണ കാലയളവിൽ കുറയുകയുണ്ടായി. നിറം ചേർത്ത് തയ്യാറാക്കിയ നാരങ്ങാ സ്കാഷ്, C, കുമ്പളങ്ങാ ക്യാൻഡി എന്നീ ഉൽപ്പന്നങ്ങളുടെ ആന്തോസയനിന്റെ അളവ് യഥാക്രമം 11.02%, 6.32%, 3.53% എന്ന അളവിൽ മാത്രമാണ് മൂന്ന് മാസത്തെ സംഭരണ സമയത്ത് കുറയുകയുണ്ടായത്. ഇത് മൂന്ന് മാസത്തെ സംഭരണ സമയത്തെ താരതമ്യേന ഉയർന്ന നിറത്തിന്റെ സ്ഥിരതയെ സൂചിപ്പിക്കുന്നു.

മാതളത്തിന്റെ തൊലിയിൽ നിന്ന് വേർതിരിച്ചെടുക്കുന്ന ആന്തോസയനിൻ നിറം കൃത്യമായ അളവിൽ ചേർത്ത് സംസ്കരിച്ച ഭക്ഷ്യോൽപ്പന്നങ്ങൾ ഉണ്ടാക്കാനും അവ മൂന്ന് മാസത്തോളം താഴ്ന്ന ഊഷ്മാവിൽ സംഭരിച്ചു വയ്ക്കാനും കഴിയുമെന്ന് ഈ പഠനം തെളിയിക്കുന്നു. കൂടാതെ സംസ്കരണ രംഗത്തെ ഉപോൽപ്പന്നങ്ങൾ ഉൽപ്പന്ന നിർമാണത്തിലുപയോഗിക്കുന്നതുവഴി ഗുണമേന്മയുള്ള ഉൽപ്പന്നങ്ങൾ ഉണ്ടാക്കാം എന്നും ഈ പഠനം തെളിയിക്കുന്നു.