EXTRACTION AND UTILIZATION OF ANTHOCYANIN PIGMENTS FROM JAMUN (*Syzygium cumini* Skeels.)

By

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(2013-12-121)

DEPARTMENT OF PROCESSING TECHNOLOGY
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VELLANIKKARA
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THESIS

Submitted in partial fulfillment of the requirement for the degree of

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DEPARTMENT OF PROCESSING TECHNOLOGY
COLLEGE OF HORTICULTURE
KERALA AGRICULTURAL UNIVERSITY
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2016

DECLARATION

I hereby declare that the thesis entitled **Extraction and utilization of anthocyanin pigments from jamun (*Syzygium cumini Skeels*.)** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associate ship, fellowship or other similar title of any other University or Society.

Vellanikkara

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DECLARATION

Certified that the thesis entitled "Extraction and utilization of anthocyanin pigments from jamun (*Syzygium cumini Skeels*.)" is a bonafide record of research work done independently by Mr. NARESH N. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associate ship to him.

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CERTIFICATE

We, the undersigned members of the advisory committee of **Mr. Naresh N.** (2013- 12- 121), a candidate for the degree of **Master of Science in Horticulture**, with major field in **Processing Technology**, agree that the thesis entitled **"Extraction and utilization of anthocyanin pigments from jamun (*Syzygium cumini Skeels*.)"** may be submitted by **Mr. Naresh N.** in partial fulfillment of the requirement for the degree.

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EXTERNAL EXAMINER

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Introduction

1. INTRODUCTION

Colour is the main feature of any food item as it enhances the appeal and acceptability of food. During processing, substantial amount of colour is lost and to

make any food commodity attractive to the consumers, synthetic or natural colours are added. Several types of dyes are available in the market as colouring agents to food commodities, but biocolourants are now gaining popularity and considerable significance because, synthetic dyes cause severe health problems. Biocolourants are prepared from renewable sources and majorities are of plant origin. The main food biocolourants are carotenoids, flavonoids, anthocyanidins, chlorophyll, betalain and crocin, which are extracted from several horticultural crops (Azeredo, 2009).

Jamun (*Syzygium cumini* Skeels) belonging to family Myrtaceae is a minor fruit of Indian origin. The harvesting span of jamun fruit is very short (30 to 40 days) during monsoon months. Jamun production in India is unorganized and scattered and there is huge loss of this fruit every year. Major chunk of fruits lost as littering waste beneath the trees. Compared to other popular fruits like sapota, papaya, banana and guava, jamun has higher level of antioxidant activity. The higher antioxidant activity in the fruit is attributed to the presence of antioxidant vitamins, tannin and anthocyanins. Jamun fruit is universally accepted to be very good for medicinal purposes especially for curing diabetes because of its effect on pancreas. Both, fruit and seed contain glycoside jamboline and ellagic acid which are reported to have the ability to check the conversion of starch into sugar in case of excess presence of blood sugar. There are some indications that this neglected fruit may be processed into various products for its gainful utilization by poor rural masses and health conscious metro population (Koley *et al*., 2011).

Anthocyanins (in Greek “anthos” means “flower” and “kyanos” means “blue”) are plant pigments visible to the human eye. They belong to the widespread class of phenolic compounds collectively named flavonoids. The most common anthocyanidins are cyanidin, delphidin, peonidin, petunidin and pelargonidin.

The most significant function of anthocyanins is their ability to impart colour to the plants or plant products in which they occur. They play a definite role in the

attraction of animals for pollination and seed dispersal and hence they are of considerable value in the co-evolution of these plant-animal interactions. The significant property of anthocyanins is their antioxidant activity, which plays a vital role in the prevention of neuronal and cardiovascular illnesses, cancer and diabetes, among others (Konczak and Zhang, 2004). There are several reports focused on the effect of anthocyanins in cancer treatments and human nutrition and their biological activity.

Anthocyanins find application in foods with pH value of 3.5 or less like fruit drinks, jam, jellies, ice creams, carbonated beverages etc. The isolated anthocyanins are highly instable and highly susceptible to degradation (Giusti and Wrolstad, 2003). Their stability is affected by several factors such as pH, storage temperature, chemical structure, concentration, light, oxygen, solvents and presence of enzymes, flavonoids, proteins and metallic ions. The anthocyanins chemical stabilisation is the main focus of recent studies due to their abundant and potential applications, their beneficial effects and their use as alternative to artificial colourants (Rein, 2005).

The main drawback of natural colours including anthocyanins is their poor stability during storage. Hence the present study is proposed to evaluate the feasibility of utilization of anthocyanin pigment from jamun fruits as a natural colourant in foods with the following objectives:

1. To standardize the process for extraction of anthocyanin pigments from jamun.
2. To study the feasibility of their utilization and storage stability in processed products.

Review of literature

2. REVIEW OF LITERATURE

Colour is the first notable characteristic of a food and often predetermines or "colours" our expectation. Since time immemorial human beings admired the beautiful natural colour of the plants and mineral and sought to enhance human

appearance through colour. In recent years there has been a revival of the use of dyes and colour of natural origin for colouring food, pharmaceutical and textile products. This increasing demand for material of natural origin is because of toxic nature of many of the synthetic dyes which is becoming widely recognized throughout the world (Hitesh *et al*., 2014).

Natural pigments are the highly coloured substances found in plants or animals. Biocolourants are prepared from renewable sources and majority is of plant origin. The main food biocolourants are carotenoids, flavanoids, anthocyanidins, chlorophyll, betalain and crocin, which are extracted from several horticultural plants. In addition to food colouring, biocolourants also act as antimicrobials, antioxidants and thereby prevent several diseases and disorders in human beings. Although, biocolourants have several potential benefits, tedious extraction procedures, low colour value, higher cost than synthetic dyes, instability during processing etc., hinder their popularity (Rymbai *et al.,* 2011).

2.1. Synthetic colours:

Most of the colours used today are artificial, and are made from coal tar dyes. Till 1990, there were no regulations on food colours. Any of over 80 dyes could be used to colour everything from cloth to candy. In 1996, the first comphrensive legislation was passed for seven colours, which were composed of known ingredients and showed no harmful effects. Synthetic colours permitted in India include Ponceau 4R, Carmoisine, Erythrosine, Sunset Yellow, Tartrazine, Indigo carmine, Brilliant Blue and Fast Green. Non permitted colours used in food are Auramine, Metanil yellow, lead chromite, Sudan Red and Malachite green (Bhat and Mathur, 1998).

Synthetic colours remain the most popualar type of food colourings, as they are brighter, more uniform, better characterized, and of higher tinctorial strength, encompass a wider range of hues, and are less expensive than colours derived from

nature. From the last twenty years, food sector is experiencing a trend back towards natural colourants.

2.2. Natural colours

The consumers are more concerned over possible health risks associated with synthetic food additives and there is strong demand for more natural products. Complimenting their colouring effect, many natural colours can provide nutritional and health benefits. The involvement of synthetic dyes in carcinogenesis makes natural dyes, which are harmless, more preferable for food and pharmaceutical industries (Downham and Collins, 1999).

Major class of pigments and their potential sources:

Pigments	Sources
Anthocyanins	Blue grape skin, blue berry, cherry plum, kokum fruit , hibiscus
Carotenoids	Annatto(seeds), paprika, alfalfa, carrot, saffron, marigold
Betalain (red, purple)	Beetroot
Flavanones and chalcones(orange)	Safflower florets
Chlorophyll (green)	Green plants, spinach , alfalfa
Miscellaneous	
Caramel(pale yellow to dark brown)	Modified sugar
Curcumin(yellow to orange)	Turmeric
Carminic acid(red)	Cochineal

(Rymbai *et al*., 2011)

2.2.1. Anthocyanins

Anthocyanins are a class of compounds belonging to phenolic substances widely distributed in vegetables, giving rise to the blue, purple, red and orange colour of flowers and fruits. The name has been derived from two Greek words ‘antho’ and ‘cyaniding’ meaning flower and dark blue respectively. Until now, more than 540 anthocyanin pigments have been identified in nature, with most of the structural variation coming from glycosidic substitution at the third and fifth positions and possible acylation of sugar residues with organic acids (Filimon, 2010).

The most common anthocyanidins are cyanidin (red-purple), delphinidin (blue-purple), malvidin (deep purple), peonidin (red), petunidin (purple) and pelargonidin (orange-red), and the distribution of these pigments in horticultural plants is not even. Some fruits contain a single type of anthocyanin (e.g. cyanidin in apple, cherry, fig, etc), some contain two major types (cyanidin and peonidin in cherry and cranberry); or some with several anthocyanins giving a variety of colours like red, purple, yellow and blue as in grape, raspberry or strawberry. Anthocyanins are used to colour a number of non beverage foods including gelatin desserts, fruit fillings and certain confectionaries (Chattopadhyay *et al*., 2008).

2.2.2. Jamun as source of anthocyanin pigment

Syzygium cumini Skeels, commonly known as jamun is a widely distributed forest tree in India and other tropical and subtropical regions of the world. The tree is rich in phytochemicals like glycoside jambolin, anthocyanins, tannins, terpenoids, gallic acid and various minerals. Jamun possess antineoplastic, radioprotective and chemopreventive effects which are useful in the prevention and treatment of cancer (Swami *et al*., 2012).

The fruits are purplish black in colour when ripe and have high anthocyanin content. Three types of anthocyanins have been identified in *S.cumini* as glycosides of delphidin and malvidin (Choudary and Mukhopadhyay, 2012). Compared to other popular fruits like sapota, papaya, banana and guava, jamun has higher level of

antioxidant activity. The higher antioxidant activity in the fruit is attributed to the presence of antioxidant vitamins, tannins and anthocyanins (Koley *et al*., 2011).

Anthocyanin pigments from *Syzygium cumini* fruit peels were characterized and evaluated for their antioxidant efficacy, and stability as extract and in formulation. Total anthocyanin content was 216 mg/100 ml of extract which is equivalent to 230 mg/100 g fruit on a dry weight basis (Veigas *et al*., 2007).

Ghojage *et al*., (2011) studied the physiochemical characters of 30 genotypes from seedling trees of jamun located in Gokak taluk of Belgaum district of Karnataka and Sawantwadi of Sindhudurg district of Maharashtra. KJS-18 registered the highest value of anthocyanin (1.393 ppm) while the lowest value was in KJS-1 (0.162 ppm).

2.2.3. Other fruits as source of anthocyanin pigment

Duran *et al.*, (2000) determined the monomeric anthocyanin content from banana bracts using UV-visible spectroscopy on cyanidin-3-rutinoside basis, the monomeric anthocyanin content was 32.3mg/100 g bracts.

Sharma *et al*., (2009) reported the anthocyanin content in jamun and guava as 138mg/100g and 172mg/100 g respectively.

According to Rizk *et al*., (2009) the anthocyanin content in red cabbage was 90.5 mg/100g on fresh weight basis by using HPLC.

Nayak *et al*., (2010) studied the isolation and characterisation of anthocyanins present in *Garcinia indica* Choisy (Kokum) and found that kokum contained a very high concentration of anthocyanins (2.4 g/100 g), compared to other natural sources.

Clemente and Galli (2010) extracted anthocyanins from residue of processed grapes. The extraction solution consisted of 70 mL of ethanol 70% and 30 mL of HCl 0.1% at pH 2.0. The results indicated that the anthocyanin content in the processed grapes residue was 26.20 mg/100g.

Lima *et al*., (2011) stated that maceration using ethanol acidified with HCl 1.5 mol/L (85:15) provides better pigment extraction and stability in jabuticaba with an anthocyanin content of 1.59 and 2.06 g/100 g of dry matter in the Paulista and Sabara varieties.

Physico-chemical properties of roselle calyces (*Hibiscus subdariffa*) as studied by Azza *et al*., (2011) indicated that moisture content, protein, fat, fiber and ash were 12.81 %, 7.51%, 0.46 %, 11.17 % and 11.24 %, respectively. The results showed that the roselle calyces powder contained ascorbic acid (140.13 mg/100g), total anthocyanins (622.91mg/100g) and total phenolics (37.42 mg/g dry weight).

Snyder *et al*., (2012) investigated total anthocyanin content and berry colour of six cultivars of primocane raspberries grown in dry climateand found that significantly higher concentration of anthocyanins was present in juice (20.86± 0.35 mg cyanidin-3-glucoside eq. /100 g), compared to pulp (13.96±0.35). Anthocyanin content of juice and pulp were significantly positively correlated with dark colour (L*).

Aishah *et al*., (2013) determined anthocyanins in freeze-dried *Hibiscus sabdariffa*, *Melastoma malabathricum* and *Ipomoea batatas* in various acidic pHs (pH 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5). Among the samples, *H.sabdariffa* recorded the highest monomeric anthocyanins (163.3 mg/L) followed by *M. malabathricum* (49.9 mg/L) and the lowest in *I.batatas* (13.8 mg/L).

The total anthocyanin content in both fruit skin and whole fruit of fully ripe strawberry was analysed by HPLC in 19 cultivars. The anthocyanin content varied greatly among cultivars, ranging from 22.04 to 87.16mg/100g in fruit skin and from 1.98 to 30.16mg/100g in whole fruit on fresh weight basis (Kim *et al*., 2013).

The content of anthocyanin derivatives were determined by Kiss *et al*., (2014) in black elderberry (*Sambucus nigra* L.), sweet cherry (*Prunus avium* L.), blackberry

(*Rubus fruticosus* L.), black currant (*Ribes nigrum* L.), and blackthorn (*Prunus spinosa* L.). The extraction efficiency was examined of several solvents including hot water, 2% phosphoric acid, ethanol and acetone. The highest anthocyanin content, determined with HPLC method, was found in the case of sweet cherry (222.7 mg/kg).

2.3. Extraction methods of anthocyanin pigments:

Du and Francis (1973) extracted *Roselle* calyxes powder in a tested solvent and kept at 4^0 C overnight. The mixture was filtered through a filter paper (Whatman No. 1) and the residue on the filter paper was re extracted four times with suitable quantity of the solvent and water till the filtrate became almost colourless.

Lachman *et al.,* (2003) extracted anthocyanins from *Musa acuminata* bract with 10 ml acidified methanol. The mixture was centrifuged at 10,000 rpm for 10 min and supernatant was taken for analysis.

Maran *et al*., (2015) extracted natural pigment and colours from pulp of jamun fruit under different extraction conditions such as extraction temperature (40-60 ˚C), time (20-100 min) and solid-liquid ratio (1:10-1: 15 g/ml) by aqueous extraction method. Optimum extraction conditions for maximizing the extraction yield of total anthocyanin (10.58 mg/100 g) and colours (10618.3 mg/l) were found to be: extraction temperature of 44 °C, extraction time of 93 min and solid-liquid ratio of 1:15 g/ml.

2.4. Effect of solvents on recovery of anthocyanins:

Kammerer *et al.,* (2005) extracted anthocyanins from Cabernet Mitos grape pomace by using various solvents and studied the recovery percent. The recovery rate ranged from 96 to 100% when methanol was used, from 86 to 96% for ethanol and from 78 to 88% when pigments were eluted with 2 propanol.

Hua *et al*., (2013) extracted anthocyanins from the fruit residue of *Vaccinium uliginosum* Linn. during juice production. A maximum partition coefficient of 10.67 and a recovery of 96.09% for anthocyanins could be obtained using an extraction system consisting of 30% (w/w) ethanol and 19% ammonium sulfate.

2.5. Colour (Visual and colourimetric method):

Anthocyanin and colours from the pulp of jamun was extracted under different extraction conditions such as temperature (40-60^{0}C), time (20-100 min) and solid liquid ratio (1:10-1:15 g/ml) by aqueous extraction method. The anthocyanin content was 10.58mg/100g and colour (10618.3 mg/l) at temperature of 44^0 C; time 93 minutes and solid liquid ratio 1:15 g/ml (Maran *et al*., 2015).

2.6. Factors influencing pigment stability

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 and sulfur dioxide.

2.6.1. pH:

Effect of different pH buffers and temperatures on colour and stability of anthocyanins in the pericarp of litchi (*Litchi chinensis*) cv. Huizhi was studied by XueQun *et al*., (2001). The colour of the anthocyanins changed after dilution in different pH buffers. The absorption value at 510 nm decreased with increase in pH. The colour of the anthocyanin extract changed to red brown at pH 1.47 and 3.03, brown at pH 4.69, dark brown at pH 7.01 after storage at room temperature for 20 days. The colour of anthocyanins faded, turned brown and became unstable as pH increased; however, it became stable at low temperatures.

Boliver and Luis (2004) studied stability of anthocyanins from red sweet potato and purple corn and compared it with purple carrot and red grapes. For acylated anthocyanins from red sweet potato, the highest absorbance (0.8) was observed at pH 0.9. At pH 3 it was 0.7. Fifty percent reduction in absorbance was observed at pH 4.

The effect of pH on the stability of anthocyanin pigments of grapes (*Vitis vinifera*) in the aqueous extract was studied and compared with the stability of anthocyanin pigments in 4 varieties of grapes (Ghezel, Rishbaba, Tabarzeh and Tobrehghareh) . Increasing the pH of samples from 2 to 6 accelerated the pigment destruction in the aqueous extract (Heidari *et al.,* 2006).

The effect of pH on thermal stability of anthocyanins was studied at six different pHs (2.5-7.0) in citrate phosphate buffer solutions and significant decrease in anthocyanin stability was observed at pH above 5.0 (Kirca *et al*., 2007).

Hunjaroen and Chantanawarangoon (2008) studied the stability of anthocyanins in mulberry juice at pH 2.5-8.0. They reported that the stability of anthocyanins decreased when pH increased and this led to the decrease in half life. Among pH evaluated, anthocyanins were most stable at pH 2.5 followed by 4.0, 6.0 and 8.0, respectively.

Quin *et al*., (2010) studied the effect of pH on mulberrry anthocyanins. It contained 60% cyaniding-3-rutinoside and 38% cyaniding-3-glucoside. To study effect of pH, 10µg/ml of mulberry anthocyanin was dissolved in 6 different buffer solutions ranging from pH 1 to 11. Mulberry anthocyanins, in acidic range of pH, showed the absorption maxima at 520 nm. The highest absorbance of 0.2 was obtained at pH 1. The IC 50 was obtained at pH 3 where the absorbance was 0.1 and at pH 5 it almost declined to zero.

Gauche *et al.* (2010) studied the effect of various pH on Cabernet Sauvignon grapes skin anthocyanins. Highest absorbance of 1.26 was obtained at pH 1 which drastically reduced to 50% at pH 3. At pH 3 the relative absorbance was 0.53 and at 3.5 it reduced to 0.29. At pH 4.5, it declined to 0.12.

Ibrahim *et al*., (2011) studied the stability of *Brassica oleracea* anthocyanins in aqueous solutions at various pH levels between 1.0 and 14.0 for a period of 10 days. Colours were expressed by the CIELAB coordinates, colour tone, colour intensity and colour lightness. Powdered anthocyanin extracts were more stable compared to juice anthocyanin at most pH values, showing no changes in colour intensity and colour tone and little change in colour lightness.

According to Roobha *et al*., (2011) increase in pH was found to spoil the anthocyanin extract from *Musa acuminata* bract and the anthocyanin extract was more stable at pH 5.1 and 6.0.

The effect of pH on changes in anthocyanin concentrations in chokeberry drinks during storage was analysed by dissolving chokeberry juice concentrate in a buffer at pH 3.0 and 5.0 with anthocyanin concentration of 25 mg. The application of a lower pH in model solutions made it possible to significantly enhance the stability of anthocyanins in all samples (Tomczak, 2012).

2.6.2. Temperature

Gous (1989) reported that crude black sorghum anthocyanin extract was relatively stable to temperature and light, with no change in absorbance when subjected to 70°C for up to 36 h and only 9% reduction in absorbance, when subjected to 1000 μ -einstein (equivalent to half-sunlight intensity) at 24°C for 48 hours.

The effect of temperature on the stability of shalgam anthocyanins stored at 4, 25, and 40°C for 90 days was investigated by Turker *et al*., (2004). Acylated

anthocyanins were more stable when compared to nonacylated ones at all storage temperatures. The highest anthocyanin retention was observed at 4°C storage temperature with a half-life between 231 and 239 days.

Nuzhet *et al.* (2004) reported that acylated anthocyanins from black carrot are significantly more stable than nonacylated anthocyanins at all storage temperatures. However, the extracts were stable when stored at 0°C.

The effect of storage temperature on the stability of anthocyanin pigments of grapes (*Vitis vinifera*) in the aqueous extract of 4 varieties of grapes (Ghezel, Rishbaba, Tabarzeh and Tobrehghareh) was studied. The results with 'Tobrehghareh' and 'Rishbaba' varieties of grape showed that increasing the storage temperature of aqueous extracts from 5 to 35°C greatly accelerated the pigment destruction (Heidari *et al*., 2006).

The effect of high temperature on anthocyanin composition was examined using *Vitis vinifera* L. cv. Cabernet Sauvignon. High temperature (maximum 35^0C) reduced the total anthocyanin content to less than half of that in the control berries with maximum 25^0C (Mori *et al*., 2007).

The effect of storage time and temperature on colour and pigment during the storage of the blackberry nectars at room temperature (+20°C) and refrigerator temperature (+4°C) for 7 months was investigated. Colour and pigment analyses were done monthly. The effects of storage temperatures and storage time on L, a, hue angle, polymeric colour, colour density, tannin contribution and total anthocyanin were found to be significant at $p<0.01$ level. The decrease in the amount of total anthocyanin which gives original colour to nectar was 95% in nectars stored at room temperature and 77% in nectars stored at refrigerator temperature (Yuksel and Koca, 2008).

Hunjaroen and Chantanawarangoon (2008) investigated the stability of anthocyanins in mulberry juice at temperature 70-90°C. Increasing temperature resulted in increase in the first-order reaction rate constant and a decrease in half-life periods. At pH 2.5, half-life periods (t1/2) of anthocyanins at 70, 80 and 90°C were 13.7, 8.4 and 4.6 hours, respectively.

Effect of storage on colour stability of reduced calorie sour cherry jam stored at room temperature (20°C) and refrigerator temperatures (4°C) for 8 months was studied by Koca and Ustun (2009). Results showed that during storage period, changes in L, b, total anthocyanin, colour density, pH and total acidity were significant ($p<0.05$). The effects of storage temperature on total anthocyanin, pH, total acidity and 'a' value were also significant ($p<0.05$). Average total anthocyanin decrease of the samples stored at room temperature and refrigerator temperature were 15.74 and 6.91%, respectively. Total anthocyanin content decreased by 28.21% after 8 months of storage.

Roobha *et al*., (2011) reported that increasing the temperature was found to spoil the anthocyanin extract from *Musa acuminata* bract and the anthocyanin extract was more stable at temperature between 20° C and 30° C.

The effect of temperature and colour stability of anthocyanins extracted from red tamarind was studied by Mayavel *et al*., (2012) for a period of 30 days at different temperature viz -8°C, 4°C, 20°C, 30°C and 40°C. Among different storage temperatures the pigment stored at -8°C showed the lowest reduction in anthocyanin content (7%) where as the pigment stored at 40°C recorded highest reduction of 56.85% after 30 days storage.

Stability of the colour of anthocyanin from barberry (*Berberis vulgaris*) was investigated by Sharifi and Hassani (2012). Extract was stored in chromatic bottles for three months. The maximum and minimum amounts of destruction of

anthocyanins were observed at 40°C and -18°C respectively. The most stable colour during the storage period was the one stored at -18 ºC.

2.6.3. Light

The effect of light on the stability of anthocyanin pigments of grapes (*Vitis vinifera*) in the aqueous extract was studied and compared with the stability of anthocyanin pigments in 4 varieties of grapes (Ghezel, Rishbaba, Tabarzeh and Tobrehghareh). In the aqueous extracts, light increased the destruction of pigments (Heidari *et al*., 2006).

Roobha *et al*., (2011) reported that exposure to light would spoil the anthocyanin extract from *Musa acuminata* bract.

Effects of different mild preservation technologies on onion flavonoids were evaluated in minimally processed onions, which were packed either in closed plastic cups or under vacuum conditions, taking into account the effect of light exposure. In general, after storage in the dark, a slight increase in flavonols was observed, whereas a clear decrease in the relatively low amounts of anthocyanins was evident. However, the best performance was obtained when the more transparent polystyrene cups were stored under light. Both types of flavonoids increased, with an enhanced increase of total flavonols by 58%, and an increase in total anthocyanins by 39% (Pérez-Gregorio *et al*., 2011).

The effect of oxygen and light availability on changes in anthocyanin concentrations in chokeberry drinks during storage was analysed by Tomczak, (2012). Solutions were stored for 6 weeks under aerobic or relatively anaerobic conditions, with access or no access to light, at a temperature of 10°C. The highest stability of pigments was found in samples with no access to light under relatively anaerobic conditions. The highest degradation of anthocyanins was recorded in

samples with accessibility to light and oxygen, in which pigment losses amounted to 90%.

Colour stability of red tamarind extract at different light intensities viz sun light, 285 lux, 510 lux and in dark was studied. Among different light intensities evaluated the pigment stored in dark recorded lowest reduction (36%) where as anthocyanins exposed to sunlight recorded highest reduction in content (93.48%) after 30 days (Mayavel *et al*., 2012).

Storage stability of the dried plum anthocyanin powder with 30°B maltodextrin as carrier in squash based model solution showed more effect of temperature at 35°C than in 0°C. There was less change in dark light condition than in day and UV light. The change in colour was rapid in the first 1 month than in the later period of storage. By the use of plum pomace and with the above optimized conditions, crude anthocyanin pigments can be produced (Devi and Joshi, 2012).

The effect of medicinal plant phenolic extracts on colour evolution in anthocyanin extracts during storage under different light conditions was investigated by Ristovski *et al*., (2014). Spectrophotometric colour characteristics and CIE Lab parameters have shown that the addition of phenolic extracts from medicinal plants had positive impact on colour stability of the anthocyanin extracts. Phenolic extract obtained from immortelle possessed a higher capacity to slow down changes of anthocyanin colour in comparison to the other two. The most stable in colour were the anthocyanin extracts stored at low temperature in dark, while under the daylight the changes were the most expressive.

2.6.4. Storage conditions:

Islam *et al*., (2014) extracted fruit juices of pineapple and orange in different ratios. Juices were pasteurized at 97°C for 5 minutes and stored for 35 days in PET bottles at refrigerated temperature. The pH of the different types of juice was found in

the range of 4.33 to 4.53. pH was found to increase progressively during storage period.

Biochemical analysis of three types of jackfruit pulp stored at about -20°C was carried out at 0, 30, 60, 90, 180 and 270 days of storage by Mortuza *et al*., (2014). The results show that titrable acidity of all the varieties decreased and pH values increased. Carotene and vitamin C content decreased gradually for all the varieties up to 270 days of storage period. TSS content increased slightly during storage. Total, reducing and non-reducing sugar contents were almost stable throughout the storage period.

2.7. Effect of additives on colour stability during storage:

Anthocyanin pigments are unstable during the storage in processed products. So in order to make the pigment stable many additives are added to improve the stability.

2. 7.1. Acids

According to Shivani *et al*., (2008) acidity and anthocyanin content of jamun jam and chutney decreased significantly with the advancement in storage period.

Clemente and Galli (2010) studied the stability of anthocyanins extracted from residue of processed grapes by adding caffeic acid at 0.5:1 w/v; 0.8:1 w/v; and 1:1 w/v concentrations and he reported that anthocyanins extract reached the greatest stability at 0.5:1 w/v concentration, with 82.47% colour retention and a half-life period of 15 days.

According to Durge *et al*., (2013) addition of 1% citric acid in coloured rice flour increased the retention of anthocyanin up to 18.2% which could reduce the requirement of pre-extrusion colouring by almost 25%.

Fresh strawberry was immersed in various concentrations of citric acid and calcium lactate alone or in combination for 5 min and kept at low temperature (5°C) overnight before freezing or frozen directly. Pretreatment with citric acid dip obviously enhanced the retention of ascorbic acid and total anthocyanin content and lowered the browning index while calcium lactate maintained the texture by reducing the drip loss and increasing the firmness after thawing. The precooling treatment did not show any enhanced affect on the quality indices of frozen berries. Compared to other treatments, the use of 0.4% citric acid - 1% Ca lactate combination dip achieved better quality attributes, including reduction in drip loss and enhancement of firmness, retention of ascorbic acid and anthocyanin content in addition to improving the colour attributes (Abd-Elhady, 2014)

2.7.2. Vitamin C (Ascorbic acid)

Kalt *et al*., (1999) reported that ascorbate content and antioxidant capacity were negatively correlated (R = - 0.80) since ascorbate levels were low in the fruit where antioxidant capacity was high.

Anthocyanin and polyphenolic compounds present in acai (*Euterpe oleracea* Mart.) were determined and colour stability of acai anthocyanins against hydrogen peroxide (0 and 30 mmol/L) over a range of temperatures (10−30 °C) was also determined and compared to common anthocyanin sources. Additionally, stability was evaluated in the presence of ascorbic acid and naturally occurring polyphenolic cofactors. Cyanidin 3glucoside (1040 mg/L) was the predominant anthocyanin in acai and correlated to antioxidant content. In the presence of ascorbic acid, acylated anthocyanin sources generally had increased colour stability (Insfran, 2004).

Stability of anthocyanins for ascorbic acid (AA) from acerola extracts was determined and compared to those from acai, which have no detectable AA. The addition of three different levels of AA to the solution of acai anthocyanins resulted in a 110-fold increase in the degradation rate (kobs) at the highest fortification level

(276 mg/ml). The higher the level of AA addition to acai anthocyanin solutions, the greater was the colour fading, indicated by increase of L* and decrease of a* and C* values (Rosso and Mercadante, 2007).

Stability of a beverage formulated with acerola fruit juice and green coconut water with added caffeine was studied by Lima *et al*., (2009) during six months of storage at room temperature (27°C). The vitamin C content decreased significantly throughout storage, from 399.5 to 189.6 mg/100 mL, although it has remained relatively high.

Anthocyanin pigment was extracted from 3 different berries (*Morus nigra* L., *Morus alba* var. *nigra*, and *Fragaria* L.). After soaking and wetting in ethanol (1% acidified), the extracted anthocyanin pigments were exposed to 3 different concentrations of ascorbic acid (AA) (10%, 25%, and 50%) and H_2O_2 (9.31, 18.61, and 27.92 mmol/L). Six groups of anthocyanin solutions were refrigerated and kept in darkness for 63 days, and every 3 weeks anthocyanin absorbance was recorded at 526 nm. AA absorbance decreased relative to the blank in all the treated samples. These results indicated the destructive effect of AA on anthocyanins. In the samples treated with H_2O_2 anthocyanin degradation increased and the intensity of colour decreased as the concentration of H_2O_2 increased (Nikkhah *et al*., 2010).

Colour and stability properties of jambolan anthocyanins, both natural and copigmented forms, were investigated in beverage model by Jung *et al*., (2011). Natural anthocyanins of jambolan revealed low colour intensity due to glycosylation structure of the anthocyanins as diglucoside. The intermolecular copigmentation of anthocyanins with sinapic acid, caffeic acid, ferulic acid, and rosemary polyphenolic extract could enhance the colour intensity, which was observed through spectrometric parameters. In addition to sinapic acid, caffeic acid, and rosemary polyphenolics also increased the stability of the anthocyanin colour during exposure to white fluorescent

light and storage at refrigeration and room temperatures, whereas on high thermal treatments, this phenomenon was not observed.

The retention of total anthocyanins (TAR), ascorbic acid (AAR) and total vitamin C (Vit C), and the colour changes of fresh-cut strawberries after washing disinfection with peracetic acid (PAA) at different concentrations (0-100 mg L-1), times (10-120 s) and temperatures (4-40°C) of two strawberry cultivars (Camarosa and Selva) were determined by Velde *et al* ., (2013). TAR (%) and AAR (%) were principally affected by PAA concentration and processing time in both cultivars and there was an approximately 90% Vit C retention at any condition in the experimental domain for Camarosa cultivar. However, in the case of Selva cultivar, total vitamin C retention and colour changes were affected by the processing variables.

The colour and chemical stabilities of six anthocyanins, including cyanidin 3glucoside, highly purified and present in semipurified extracts from grape pomace, purple corn and black rice, were determined in combination with ascorbic acid in solutions at differing pH values (3.0 and 4.0) and temperatures (6–40°C) and lyophilized powders at different relative humidities (43–98% RH). Colour and chemical changes were analyzed using CIELAB measurements and HPLC, respectively. In liquids, stability was inversely related to increasing pH and temperature; for powders, stability was inversely related to RH. The mutual destruction of anthocyanins and ascorbic acid in solution was confirmed (West and Mauer, 2013).

Fruit juices at pH 3.7 were prepared with different combinations of ascorbic acid, rutin (quercetin 3-rutinoside) and concentrated anthocyanin extract of plums (cv. Black Gold). The anthocyanins in the concentrated extract were cyanidin 3-glucoside and cyanidin 3-rutinoside, in a proportion of 76% and 24% respectively. The fruit juices were stored for 17 weeks in darkness at 20 °C. The colour stability was improved by the presence of rutin and strongly damaged by the ascorbic acid.

Fortification of anthocyanin fruit juices with ascorbic acid originated the degradation of most of anthocyanins. However, anthocyanins improved ascorbic acid stability during storage. The copigmentation of anthocyanin and rutin showed a beneficial effect on colour stability during the 5 weeks of storage (Herrero and Frutos, 2014).

2.7.3. Other additives:

Colour intensity, hue, total phenolics and ethanol produced were monitered in grape must treated with Rapidase pectolytic enzyme on the colour of Shiraz red wine by Clare (2002). Treated wines showed 22 per cent higher colour intensity compared to control wine.

Lima *et al*., (2009) studied the stability of a beverage formulated with acerola fruit juice and green coconut water with added caffeine. The anthocyanins initially present (0.025 mg 100/ mL) were completely lost during six months of storage at a mean rate of 4 μg/100 mL/month.

Howard *et al*., (2013) studied the effects of three pH levels (2.8, 3.2, and 3.6) and four β-cyclodextrin (BCD) concentrations (0, 0.5, 1, and 3%) alone and in combination on the stability of chokeberry juice anthocyanins before and after pasteurization and over 8 months of storage at 4 and 25°C. Lowering the pH from 3.6 to 2.8 in the absence of BCD provided marginal protection against anthocyanin losses during processing and storage. Addition of 3% BCD at the natural chokeberry pH of 3.6 resulted in excellent protection of anthocyanins, with 81 and 95% retentions after 8 months of storage at 25 and 4°C, respectively.

Effect of additives on rate of colour degradation was conducted on 7 paprika, 3 chili pepper and 2 chili powder products stored in ziploc bags for up to 6 months at 4 different storage conditions, namely: 35°C/80% RH, room temperature ~ 22°C/45% RH, refrigerated temperature at 7°C and frozen temperature at -8°C. Representative samples were collected at time 0, 2, 4, 6, 8, 12, 16, 20 and 24 weeks from each

storage condition and analyzed for moisture and water activity (Aw), extractable colour (ASTA) and surface colour (Hunter L, a, b). Results showed that samples with ethoxyquin demonstrated a significantly lower extractable colour loss than samples without ethoxyquin. Further, samples that had been irradiated demonstrated a significantly higher surface colour loss than samples that had not been irradiated (Addala *et al*., 2015).

2.7.4. Changes in chemical constituents during storage:

2.7.4.1. Anthocyanin content

The physicochemical changes in jamun (*S. cumini*) fruit products like, ready-to-serve-beverage (RTS), squash, syrup and jam during storage were studied by Kannan and Thirumaran (2004). Total soluble solids and reducing sugars increased whereas the total sugars and acidity decreased slightly. The total phenolics (tannins) decreased throughout the storage period. The anthocyanin content rapidly degraded during 6 months of storage. The maximum retention of anthocyanin was in jam followed by syrup, squash and RTS beverage. The jamun products stored in colourless glass bottles were accepted even after 6 months of storage at ambient conditions. The appearance, colour, flavour, texture, taste and overall acceptability were found to be good.

Changes in colour and phenolic composition in sweet red wines made from Merlot, Syrah and Tempranillo grapes was studied by Marquez *et al*., (2014) in order to assess the influence of bottle storage over a period of 12 months. Wine colour parameters, sensory analysis and concentration of monomeric anthocyanins, pyranoanthocyanins, flavan 3-ol derivatives and flavonols were measured. Hue increased and red colours decreased with the storage time, particularly over the first 3 months. The concentrations of low molecular weight flavan-3-ol derivatives decreased with time due to the effect of their conversion into tannins of high molecular weight. In addition, the glycosylated flavonols decreased through

hydrolysis. Overall, the concentration of phenolic compounds decreased markedly with storage time, whereas the antioxidant activity in the wines remained constant throughout. A panel of expert tasters judged the colour, aroma and flavour of all initial and final wines to be acceptable.

2.7.4.2. Acidity

Kalsi and Dhawan (2001) reported a significant decrease in acid content of guava powder in all varieties during storage. Dabhade and Khedkar (1980) also reported similar results for mango powder packed in glass bottles. Firoz *et al.* (2003) observed that the total acid content of pulse based papaya powder decreased upto second month of storage.

Bitter gourd ready-to-serve (RTS) beverage was prepared by optimizing the levels of fruit juice, sugar and citric acid and also keeping quality of RTS beverage was studied by Satkar *et al*., (2013). They reported that there was decrease in acidity and total sugar content and increase in TSS and reducing sugars during storage of RTS beverage at ambient (27±5ºC) and refrigerated (5±1ºC) temperature.

Kumar and Singh (2013) reported that the TSS, acidity and optical density of banana and kinnow RTS beverage increased with increase in the level of banana juice ratio at different storage condition. The pH decreased with increase in the level of kinnow juice and pH values of the samples composition B70:K30, B60:K40 and B50:K50 after 90 days of storage were observed as 1.60, 1.41 and 1.20, respectively at refrigerated condition.

2.7.4.3. Ascorbic acid

Kumar *et al*. (2005) and Singh *et al*. (2005) reported that the ascorbic acid content of aonla fruits decreased continuously during storage under ambient conditions. The candy prepared from fresh aonla fruits showed maximum ascorbic

acid content (113 mg/100g) compared to those prepared from 3 days stored fruits (103 mg/100g).

The ascorbic acid content of bael pulp decreased gradually during the storage period (Chand *et al*., 2007). Sanusi *et al*., (2008) reported that the ascorbic acid loss was more than 10% in marmalades and jams after storage for 12 months at ambient room temperature.

Patil *et al*., (2011) reported that ascorbic acid content registered decreasing trend in nectar developed from blend of rose apple and jamun during three months of storage.

Kumar and Singh (2013) reported that the ascorbic acid content of the banana and kinnow based RTS samples decreased during storage period. The minimum ascorbic acid content in the sample of juice ratio of the fruits B70:K30, B60:K40 and B50:K50 after 90 days of storage were observed as 1.50, 2.00 and 2.60, respectively at refrigerated condition.

Rehman *et al*., (2014) extracted fresh juice from fresh ripe fruits of mango and peaches. The extracted filtered juices were pasteurized at 72°C for 15 minutes following by condensation using evaporation. The product was stored at refrigeration temperature and evaluated for chemical and organoleptic analysis at zero to thirty days after every ten days interval. Results showed that the loss of ascorbic acid was minimum (11.42%) in peach. The maximum loss of ascorbic acid was recorded in mango (16.05%) at the end of storage period of 30 days. In case of malic acid it had increased slightly.

2.7.4.4. Other chemical constituents

Effect of packaging and storage time on dehydrated pineapple was evaluated in four packaging materials: transparent polyethylene, polyethylene with an aluminium layer, vinylidene polychloride transparent under vacuum and vinylidene

polychloride with an aluminium layer under vacuum. Dehydrated pineapple was stored at room temperature (27±2°C) and under natural light. Significant differences were observed in moisture content, titratable acidity, soluble solid ratio, hardness and colour coordinates for the storage variable time and significant differences on moisture content, carotenoids, water activity, hardness and colour coordinates a* and b* for the packaging (Ramos *et al.,* 2008).

According to Patil *et al*., (2011) decrease in acidity corresponding to increase in pH was observed in nectar developed from blend of rose apple and jamun during three months of storage.

2.8. Sensory evaluation:

The colour, appearance, flavour, taste and overall acceptability of jamun jam and chutney decreased significantly with the increase in storage period, however, their overall rating remained above the acceptable level even after three months storage (Shivani *et al*., 2008).

According to Sonia *et al* ., (2008) jamun RTS drink prepared with 20 per cent pulp, 14 per cent TSS and 0.24 per cent acidity was found most acceptable (7.88), while its nectar prepared with 30 per cent pulp, 15 per cent TSS and 0.25 per cent acidity was found most acceptable (7.79).

Sensory analyses of the beverage formulated with acerola fruit juice and green coconut water with added caffeine was done by Lima *et al*., (2009). The product was acceptable during the 6 months of storage, presenting sensory scores (colour, taste and overall acceptance) from 6.5 to 5.5.

Sensory analysis of natural coloured as well as synthetic coloured ice cream and yoghurt revealed the superiority of natural colour in all the characters like colour, flavour, taste and consistency (Sreevidya, 2010).

Nectar was prepared by blending rose apple and jamun in three different proportions of 75:25, 50:50 and 25:75 (rose apple: jamun). Nectar containing 20 per cent blended juice (50: 50 per cent juice of rose apple and jamun, respectively), 20 per cent TSS and 0.5 per cent acidity was found to be more acceptable with good organoleptic scores (Patil *et al*., 2011).

Satkar *et al*., (2013) reported that the score for overall acceptability of bitter gourd RTS beverage decreased during storage. The RTS drink which was stored at refrigerated temperature was found to be more acceptable after 3 months of storage.

Sensory evaluation of four mango cultivars which are commonly found in Saurashtra region was carried out by Shinde *et al*., (2013). Mango pulp was extracted and stored up to 240 days and sensory evaluation was conducted. The cultivar Kesar recorded maximum score for taste and colour, whereas the cultivar Alphanso recorded maximum score for flavour, texture and overall acceptance.

A study was conducted to develop value added product, jamun fruit shrikhand by blending jamun fruit, a neglected / under-utilized fruit by Lakshmi *et al*., (2013). Among the different blends of jackfruit-jamun fruit pulp, 60:40 blends was adjudged best. Various sensory attributes of best adjudged product revealed that the developed product was found highly acceptable.

Materials and Methods

3. MATERIALS AND METHODS

The research work on "Extraction and utilization of anthocyanin pigments from jamun (*Syzygium cumini* Skeels.)" was conducted at the Department of Processing Technology, College of Horticulture, Vellanikkara during 2013-2015.

The study was divided into three different experiments

Experiment I : Standardization of method for extraction of anthocyanin pigment

Experiment II: Storage stability of anthocyanin pigment

Experiment III: Evaluation of pigment stability in processed food products

3.1. Raw materials:

Ripe jamun fruits were collected from the trees maintained in the Central Nursery at KAU Main campus, Vellanikkara.

3.1.1. Standardization of method for extraction of anthocyanin pigment:

Ripe jamun fruits were taken washed thoroughly, seeds removed, pulp separated and homogenized in a mortar and pestle.

Four extraction methods:

3.1.1.1. Hot water extraction:

Fifty grammes of the homogenate was placed in the beaker containing water equal to thrice the quantity of homogenate and then it was boiled for about 5-6 hrs at 60-70 ^{0}C for complete extraction of the pigment. After extraction, the pigment was filtered with Whatman filter paper and stored in the glass bottles at refrigerated

condition by covering with aluminium foil for protection from light (Harbone, 1978). The process was replicated four times.

3.1.1.2. Acidified aqueous extraction (0.5% citric acid):

Fifty grammes of the homogenate was placed in the beaker containing water equal to thrice the quantity of homogenate and 0.5% citric acid and then it was boiled for about 5-6 hrs at 60-70 ^{0}C for complete extraction of the pigment. After extraction, the pigment was filtered with Whatman filter paper and stored in glass bottles at refrigerated condition by covering with aluminium foil for protection from light (Harbone, 1978). The process was replicated four times.

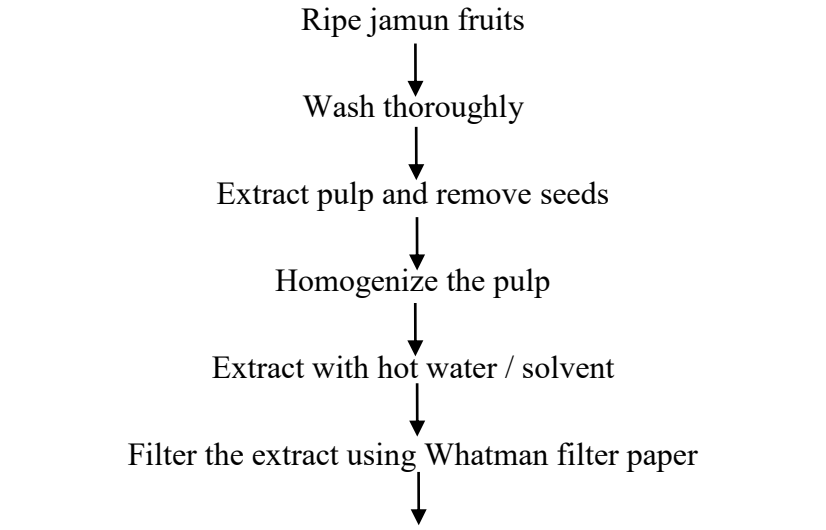
3.1.1.3. Solvent extraction (20% Ethanol):

The extraction was done according to method suggested by Abdulrahman *et al.,* (2004). Fifty grammes of the homogenate was filled in the glass bottle containing solvent (20% ethanol) equal to thrice the quantity of homogenate and it was kept for extraction by percolation method for about 72 hours in dark condition at room temperature by covering with aluminium foil and by occasional shaking. After extraction, the pigment was filtered with Whatman filter paper and stored in glass bottles at refrigerated condition. The process was replicated four times.

3.1.1.4. Acidified solvent extraction (20% Ethanol + 0.5% Citric acid):

The extraction was done according to method suggested by Abdulrahman *et al.,* (2004). Fifty grammes of the homogenate was filled in the glass bottle containing solvent (20% ethanol + 0.5% citric acid) equal to thrice the quantity of homogenate and it was kept for extraction by percolation method for about 72 hours in dark condition at room temperature by covering with aluminium foil and by occasional shaking. After extraction, the pigment was filtered with Whatman filter paper and stored in glass bottles at refrigerated condition. The process was replicated four times (Plate 1 and 2). All the extracts were made up to 250ml volume in a standard flask.

Flow chart for extraction of anthocyanin pigment (Plate 3)



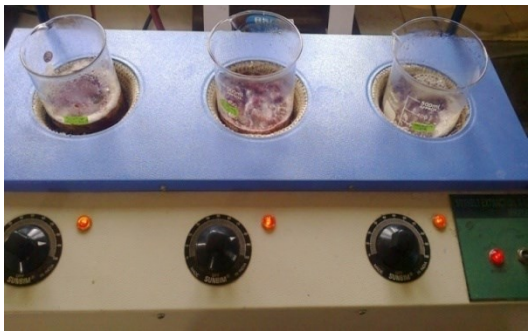
3.2. Observations:

3.2.1. Anthocyanin content:

Anthocyanin content estimation was done based on the method given by Iland *et al*., (1996). Malvidin-3-glucoside was used as the standard. Transferred 0.2ml of the extract to a test tube, added 3.8 ml of 1.0 M HCl to it and incubated for 22 hours at room temperature by covering with parafilm. This step is critical in allowing full expression of the colour. Measured the absorbance of the acidified diluted extract at 520 nm using a 1.0 M HCl blank in a UV spectrophotometer.

$$\text{Anthocyanin content} = \frac{A_{520} \text{ X D.F X final volume (ml) X berry wt (g) X 1000}}{\text{d X100 X homogenate wt (g)}}$$

Plate 1. Extraction methods of anthocyanin pigment



Hot water

Acidified aqueous extraction

Solvent extraction (ethanol 20%)

Acidified solvent extraction (ethanol 20% + 0.5% citric

Plate 2. Fresh extracts of anthocyanin pigment

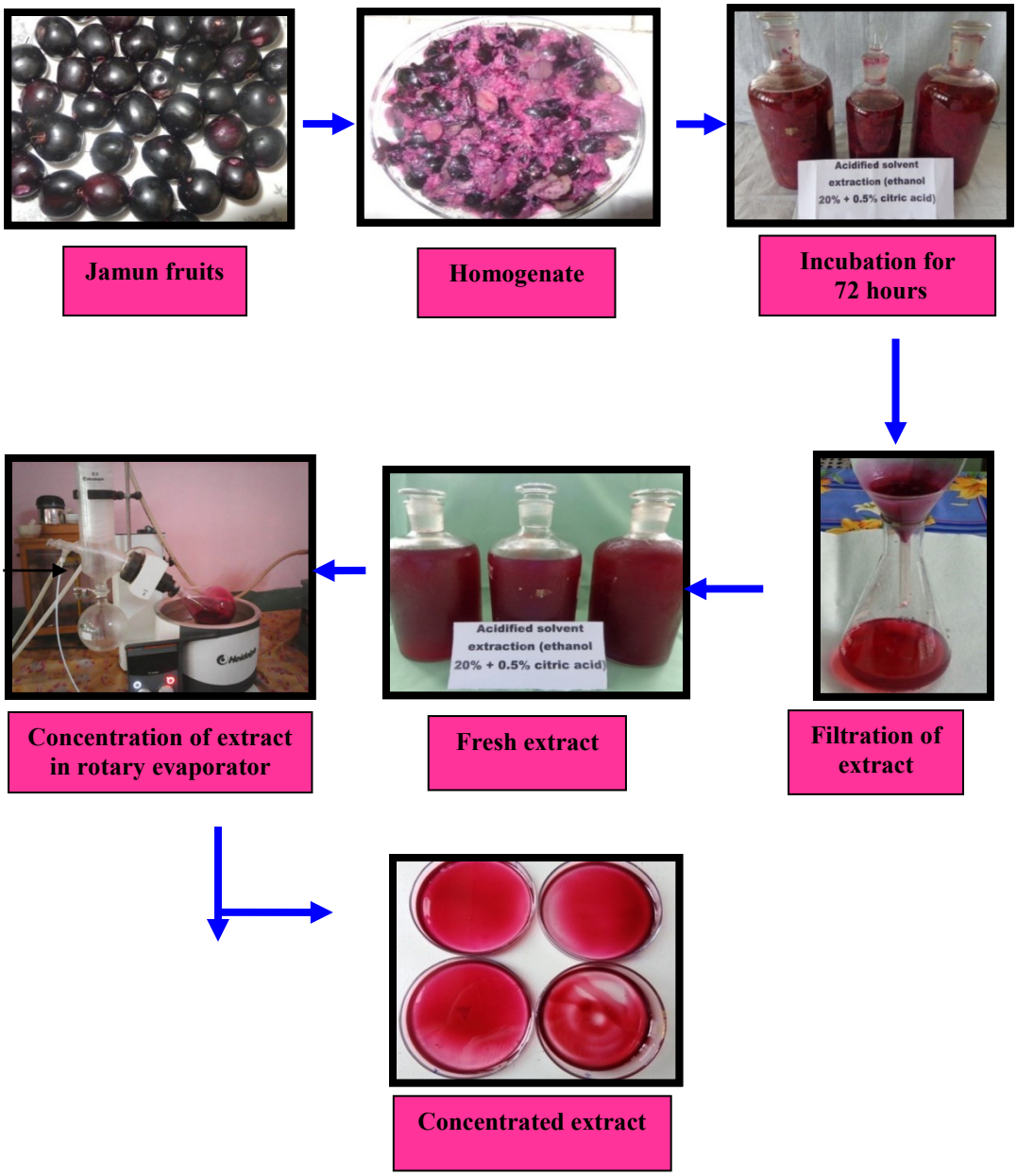
Hot water extraction

Acidified aqueous extraction (0.5% citric acid)

Solvent extraction

Acidified solvent extraction (ethanol 20% + 0.5% citric

Plate 3. Flow chart for extraction of anthocyanin pigment from jamun



Where,

D.F = Dilution factor

d = absorbance of a one per cent w/v solution of malvidin-3-glucoside

3.2.2. Recovery percent:

Took an empty petriplate and recorded the weight W_1, pipetted out 10 ml of the extract and dried in hot air oven till it got dried completely. Recorded the weight of dried sample W_2.

$$\text{Recovery per cent} = \frac{(W_2\text{-}W_1) \text{ X final volume X } 100}{\text{Volume of sample taken (ml) X homogenate weight (g)}}$$

3.2.3. Colour (Visual and colorimetric method):

Colour measurement was done manually by using colour chart.

Colour hue: Colour hue was determined as the ratio of absorbance at 420 nm and 520 nm (Harbertson and Spayd, 2006).

Colour intensity: Colour intensity was determined by adding the values obtained at 420 nm and 520 nm.

Among the four extraction methods compared in the first experiment acidified solvent extraction method (20% ethanol + 0.5% citric acid) was found to be superior in anthocyanin content, colour hue, colour intensity and recovery per cent. Hence it was selected as the best extraction method for further studies.

3.3. Storage stability of anthocyanin pigment

Anthocyanin pigment was extracted by acidified solvent extraction (20% Ethanol + 0.5% citric acid)

3.3.1. Thermal stability of the anthocyanin pigment:

Thermal stability of jamun anthocyanins was determined according to method suggested by Mok and Hettiarachchy (1991). In this method, 10 ml of the extract was placed in screw capped test tubes and heated in a thermostatically controlled water bath at 70, 80 and 90^0 C for 30, 45 and 60 minutes. The tubes were cooled down immediately in an ice bath and total anthocyanins were determined at 30, 45 and 60 minutes by the method given by Iland *et al*., (1996).

3.3.2. Effect of pH:

The effect of pH variation on the stability of jamun anthocyanin was studied at different pH values viz., 2.5, 3.0, 4.0, 5.0, 6.0 and 7.0. The procedures were followed as described by Von Elbe *et al.,* (1974). In this method, one ml of jamun anthocyanin extract was mixed with 10 ml of prepared buffers of the desired pH value in screw capped test tubes. The tubes were wrapped with aluminum foil to provide full darkness and kept at room temperature (25 ±2°C). Degradation of anthocyanins was studied by measurement of anthocyanin content of the samples according to the method given by Iland *et al*., (1996) at 520 nm at 1 hr, 1 day and then subsequently after 2, 3, 4, 5, 6, 13 and 20 days in the different buffered anthocyanin extracts.

3.3.3. Effect of light and storage conditions on pigment stability:

The effect of light and storage conditions on pigment stability was evaluated in two different containers viz. transparent glass bottles and amber coloured glass bottles at refrigerated and ambient conditions. Ten ml of extract was stored in each bottle and kept under ambient and refrigerated conditions for three months and colour

retention/anthocyanin content at monthly intervals was recorded by the method given by Iland *et al*., (1996).

3.4. Evaluation of pigment stability in processed food products

3.4.1. Guava RTS beverage

The effect of antioxidant (ascorbic acid), pH and storage temperature on pigment stability was evaluated in guava RTS beverage by following the treatments.

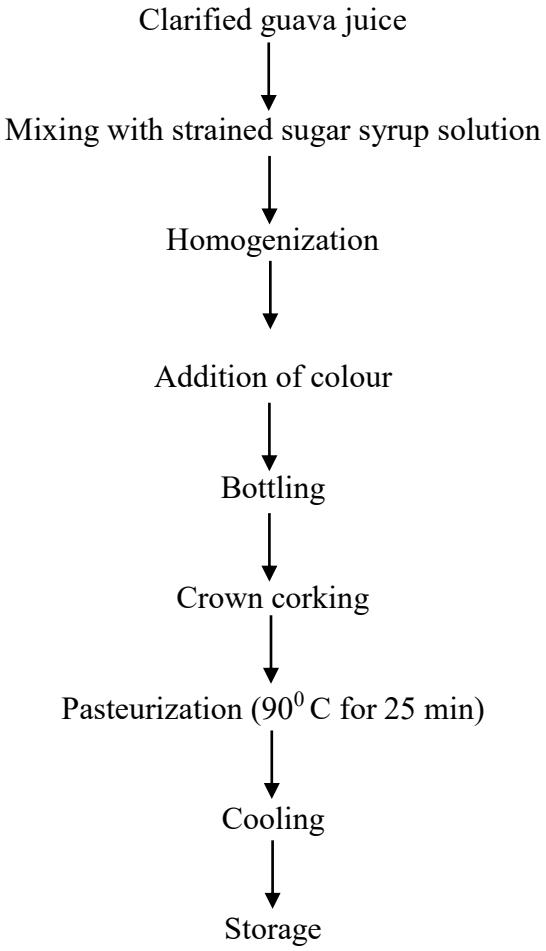
Treatments

1. Anthocyanin pigment(AC) + 0.3% citric acid(CA) + 0.01% ascorbic acid(AA)
2. AC + 0.4% CA + 0.01% AA
3. AC + 0.5% CA + 0.01% AA
4. AC + 0.3% CA + 0.02% AA
5. AC + 0.4% CA + 0.02% AA
6. AC + 0.5% CA + 0.02% AA
7. AC + 0.5% CA
8. Synthetic colour alone
9. Anthocyanin pigment (AC) alone (control)

The RTS beverage was prepared by standard procedure according to FSSAI specification (TSS 15^0 Brix and 15% juice, 0.3% acidity) and stored for three months under refrigerated and ambient conditions and observations recorded at fortnightly intervals.

The quantity of anthocyanin pigment was standardized for RTS beverage as 0.68g/200ml or 3.4g/litre. Synthetic colour (raspberry red) was added at a rate of 0.05g/litre.

Preparation of guava RTS beverage



3.4.2. Mixed fruit jam:

Feasibility of utilization of the pigment in mixed fruit jam as well as colour stability during storage was studied.

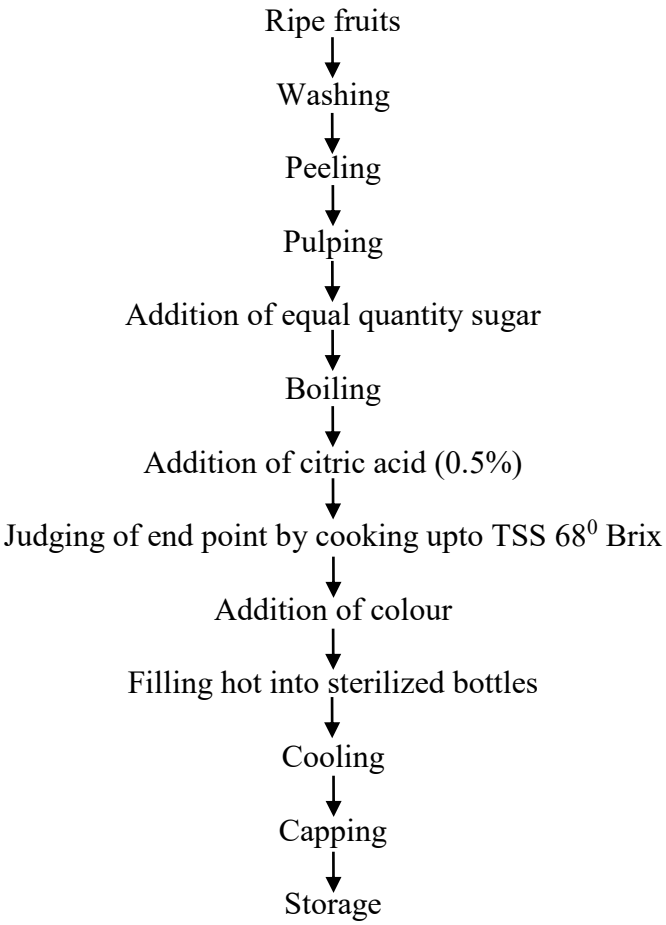
The product was prepared by standard procedure and according to FSSAI specification (TSS 68^0 Brix and 45% fruit) and stored under ambient and refrigerated conditions for three months. Pulp from pineapple and guava were used for preparation of mixed fruit jam.

The quantity of anthocyanin pigment was standardized for mixed fruit jam as 1.8g /200g or 9 g/kg. Synthetic colour (raspberry red) was added at a rate of 0.1g/kg (Srivastava and Kumar, 2002).

Treatments

1. Control [Product + anthocyanin pigment (AC) alone)]
2. Product + synthetic colour
3. Control (product without added colour)

Preparation of mixed fruit jam



3.4.3. Observations:

Observations were recorded on the following biochemical constituents during storage in RTS beverage and mixed fruit jam at monthly intervals.

3.4.3.1. Ascorbic acid:

Ten ml of fruit juice sample was taken and made upto 100 ml with 4 per cent oxalic acid and filtered. Pipetted 5 ml of filtrate into a conical flask and added 10 ml 4 per cent oxalic acid and titrated with the standard dye 6-dichlorophenol indophenol dye to a pink end point and expressed as mg/100g sample (AOAC, 1980).

3.4.3.2. Acidity:

Acidity of the fruit juice was determined by titration with standard sodium hydroxide solution (0.1N NaOH), and expressed as percentage (Ranganna, 1997).

Procedure: Ten ml of fruit juice was taken and diluted it to 100ml with distilled water. Titrated the known volume of diluent against standard NaOH solution using phenopthalein as indicator. Expressed the acidity in percentage.

3.4.3.3. Initial colour and colour retention:

Colour retention was recorded by using Royal Horticultural Society colour chart.

3.4.3.4. pH:

The pH was determined using digital type pH meter.

3.4.3.5. Sensory evaluation:

Sensory evaluation was carried out using the 9 point hedonic scale score card by a panel of selected 15 judges.

3.5. Statistical analysis

The observations were recorded and tabulated and the data were analyzed statistically as Completely Randomized Design (CRD). Two factor analysis was done for parameters pH, acidity and ascorbic acid of RTS beverage. The scores of sensory evaluation were analyzed by Kendall's Coefficient of concordance.

Results

4. RESULTS

The results of the present study entitled "Extraction and utilization of anthocyanin pigments from jamun (*Syzygium cumini* Skeels.)" are presented in this chapter under the following sections.

4.1. Standardization of method for extraction of anthocyanin pigment
4.2. Storage stability of anthocyanin pigment
4.3. Evaluation of pigment stability in processed food products

4.1. Standardization of method for extraction of anthocyanin pigment

Jamun fruits were collected from the KAU Main Campus and used for extraction of anthocyanin pigment.

Extraction of anthocyanin pigment was done by four different methods viz. Hot water extraction (T_1) acidified aqueous extraction (T_2), solvent extraction (T_3) and acidified solvent extraction (T_4). The results obtained are presented below.

4.1.1. Anthocyanin content

The anthocyanin content ranged from 20.1mg/100g to 61.07mg/100g (Table 1). It was maximum in T_4 (61.07mg/100g) followed by T_3 (33.50mg/100g) and it was minimum in T_1 (20.1mg/100g).

4.1.2. Colour hue

The colour hue was maximum in T_4 (1.30) followed by T_3 (1.15) and it was minimum in T_2 (0.74).

4.1.3. Colour intensity

The colour intensity was highest in T_4 (1.13) followed by T_3 (1.02) and it was least in T_2 (0.70).

Table 1. Effect of extraction methods on colour and *anthocyanin* content

Treatments	Anthocyanin content (mg/100g)	Colour hue	Colour intensity	Visual colour	Recovery per cent
T_1	20.10	0.74	0.76	Vivid Red (45A)	7.50
T_2	21.50	0.86	0.70	Vivid Red (45B)	7.50
T_3	33.50	1.15	1.02	Deep Yellowish Pink (44D)	12.50
T_4	61.07	1.30	1.13	Vivid Reddish Orange (44B)	13.75
CV%	7.04	5.92	10.13		5.09
CD (0.05)	3.69	0.09	0.14		32.07

T_1 - Hot water extraction

T_2 - Acidified aqueous extraction

T_3 - Solvent extraction

T_4 - Acidified solvent extraction

4.1.4. Colour (visual)

The visual colour of T_1 was Vivid Red (45A), T_2 was Vivid Red (45B), T_3 was Deep Yellowish Pink (44D) and T_4 Vivid Reddish Orange (44B).

4.1.5. Recovery per cent

Highest recovery per cent was recorded in T_4 (13.75%) followed by T_3 (12.5%). T_1 and T_2 recorded the least recovery per cent (7.5%).

Highest anthocyanin content, recovery per cent, colour hue and colour intensity was recorded when acidified solvent extraction was done with 20% ethanol and 0.5% citric acid (T_4) Hence the pigment extracted by this method was used for further storage studies.

4.2. Storage stability of anthocyanin pigment

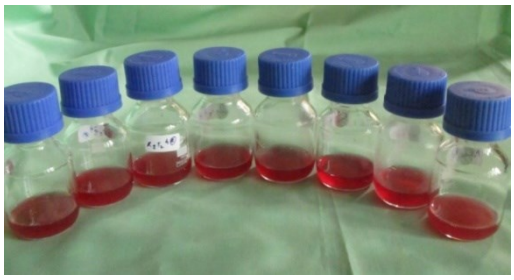
Storage stability of anthocyanin pigment extracted by acidified solvent extraction method was studied under this experiment. The stability of the pigment to high temperature, pH, light and storage conditions was evaluated (Plate 4).

4.2.1. Effect of light and storage temperature on anthocyanin pigment

Anthocyanin pigment was stored in two different types of containers viz., amber coloured and transparent glass bottles under refrigerated and ambient conditions.

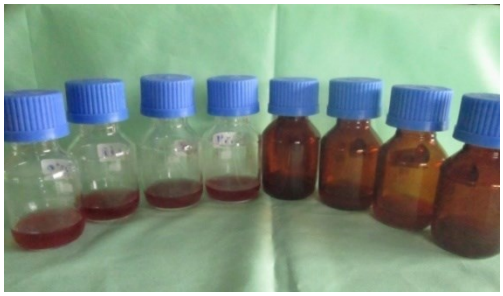
During storage, the degradation of anthocyanin pigment was faster in the bottles stored at ambient conditions compared to that stored under refrigerated condition. There was darkening of the pigment in the bottles stored under ambient conditions due to oxidation which was not seen in refrigerated bottles. Storage in amber coloured bottles under refrigerated conditions was found to be the best for anthocyanin pigment.

Plate 4. Storage study of anthocyanin pigment

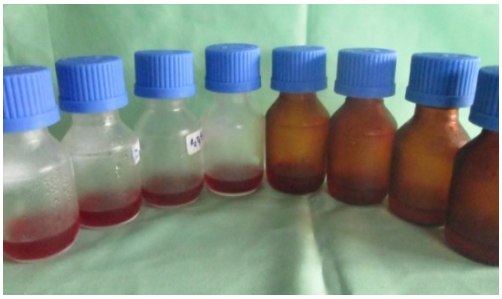


Anthocyanin pigment in transparent glass bottles

Anthocyanin pigment in amber coloured glass



Anthocyanin pigment at ambient condition 3



Anthocyanin pigment at refrigerated condition 3

Stability study of anthocyanin pigment at

The initial anthocyanin content of the extract was 61.25mg/100g. After one month it decreased to 58.53, 57.72, 42.30 and 40.95mg/100g in T_1, T_2, T_3 and T_4 respectively. Maximum decrease in anthocyanin pigment was observed in T_4 both at second (27.26 mg/100g) and third month (15.55 mg/100g) of storage. Degradation was faster for pigment stored in transparent glass bottles as compared to amber coloured bottles.

Throughout the storage period least decrease in anthocyanin content was observed when it was stored in amber coloured bottles under refrigerated conditions (T_1). In this treatment a decrease in anthocyanin content from an initial value of 61.25mg/100g to a final value of 44.84mg/100g three months after storage was observed (Table 2a).

Minimum change in anthocyanin content was observed in T_1 and T_2 throughout the storage period where in storage was done under refrigerated condition. The per cent change observed in T_1 was 4.42, 6.38 and 26.79 at one, two and three months after storage. T_2 also recorded comparatively lesser changes in anthocyanin content and it was 5.75, 10.04 and 28.34 at one, two and three months after storage.

Maximum change was recorded when pigment was stored in transparent glass bottles at ambient conditions (T_4) during storage. The per cent changes in T_4 were 33.14, 55.48 and 74.61 at one, two and three months after storage (Table 2b).

4.2.2. Effect of temperature on anthocyanin pigment

The effect of high temperature on the anthocyanin content was studied at the temperatures 70, 80 and 90^0 C. Anthocyanin content was measured at 30 minutes, 45 minutes and one hour of incubation at each temperature. Results are presented in Table 3.

Results showed a significant decrease in anthocyanin content with increase in temperature and duration of heating. Initial anthocyanin content was 61.25mg/100g

Table 2a. Effect of light and storage temperature on anthocyanin pigment

Treatments	Anthocyanin content (mg/100g)			
	Initial	**1 MAS**	**2 MAS**	**3 MAS**
T_1	61.25	58.53	57.33	44.83
T_2	61.25	57.72	55.10	43.88
T_3	61.25	42.30	33.05	18.95
T_4	61.25	40.95	27.26	15.55
CV%		5.19	8.10	6.26
CD (0.05)		3.98	5.39	2.97
Table 2b: Progressive change in anthocyanin content during storage				
T_1	61.25	4.42^{b}	6.38^{c}	26.79^{c}
T_2	61.25	5.75^{b}	10.04^{c}	28.34^{c}
T_3	61.25	30.93^{a}	46.04^{b}	69.06^{b}
T_4	61.25	33.14^{a}	55.48^{a}	74.61^{a}
CV%		22.76	19.37	6.33
CD (0.05)		6.51	8.80	4.85

T_1 - Amber coloured glass bottles (refrigerated condition)
T_2 - Transparent glass bottles (refrigerated condition)
T_3 - Amber coloured glass bottles (ambient condition)
T_4 - Transparent glass bottles (ambient condition)
MAS- Months after Storage.

Table 3. Effect of temperature on anthocyanin content

Temperatures	Anthocyanin content (mg/100g)			
	Initial value	**30min**	**45min**	**1hr**
70^0 C	61.25	55.96	54.73	53.07
80^0 C	61.25	54.74	53.66	52.34
90^0 C	61.25	53.57	50.87	50.24
CV%		0.52	0.50	0.43
CD (0.05)		0.39	0.37	0.31

and it was 55.96, 54.74 and 53.57 mg/100g at 70, 80 and 90^0 C after 30 minutes of heating. The anthocyanin content further decreased to 54.73, 53.66 and 50.87 mg/100g at 70, 80 and 90^0 C respectively after 45 minutes of heating. The anthocyanin content recorded after one hour of heating was 53.07, 52.34 and 50.24 mg/100g at 70, 80 and 90^0 C respectively.

4.2.3. Effect of pH on anthocyanin pigment

The effect of pH on stability of anthocyanin pigment was investigated under this experiment. The stability of pigment at six different pHs (2.5, 3, 4, 5, 6 and 7) was evaluated at room temperature. Anthocyanin content was recorded after one hour of incubation at each pH and subsequently at 1, 2, 3, 4, 5, 6, 13 and 20 days in the different buffered anthocyanin extracts.

At the pH of 2.5 and 3 the pigment was of reddish tinge and it changed to a yellowish tinge as the pH increased from 3 to 7.

A decrease in anthocyanin content with a corresponding increase in pH was observed. Highest values for anthocyanin pigment was observed at pH 2.5 (61.1mg/100g) followed by pH 3 (60.03mg/100g) and least at pH 7 (47.16mg/100g) one hour after incubation (Table 4a). The anthocyanin content decreased to 48.81, 46.75, 46.41, 45.63, 44.76, and 34.28 mg/100g at pH 2.5, 3, 4, 5, 6, and 7 respectively 20 days after incubation at room temperature. At pH above 6 there was drastic reduction in anthocyanin content throughout the study period.

Minimum change in anthocyanin content was observed in T_1 and T_2 during the three months of storage. The per cent change observed in pH 2.5 (T_1) was 0.24, 3.26, 8.78, 12.27, 11.83, 12.27, 14.96, 18.88 and 20.29 respectively at 1 hour, 1, 2, 3, 4, 5, 6, 13 and 20 days after incubation.

Table 4a. Effect of pH on anthocyanin content

Treatments/pH	Anthocyanin content (mg/100g)									
	Initial	**1hr**	**1st day**	**2nd day**	**3rd day**	**4th day**	**5th day**	**6th day**	**13th day**	**20th day**
T_1 (2.5)	61.25	61.10	59.25	55.86	54.00	53.73	53.73	52.08	49.75	48.81
T_2 (3.0)	61.25	60.03	52.86	54.00	52.25	51.73	53.20	49.43	48.98	46.75
T_3 (4.0)	61.25	55.00	52.35	52.66	51.73	51.11	49.58	49.43	49.58	46.41
T_4 (5.0)	61.25	51.73	49.85	51.11	50.21	49.43	48.90	48.48	48.90	45.63
T_5 (6.0)	61.25	51.11	47.38	47.36	50.03	48.73	51.11	46.38	48.55	44.76
T_6 (7.0)	61.25	47.16	35.98	35.46	40.55	34.30	34.55	35.43	33.00	34.28
CV%		0.50	0.78	0.98	0.89	0.61	0.79	0.93	0.85	1.08
CD (0.05)		0.48	0.69	0.86	0.79	0.52	0.68	0.77	0.70	0.85

Maximum change was recorded in pH 7 (T_6) during storage. The per cent change was 22.99, 41.25, 42.09, 44, 33.79, 43.59, 42.14, 46.12, and 44.03 respectively at 1 hour, 1, 2, 3, 4, 5, 6, 13 and 20 days after incubation (Table 4b).

4.3. Evaluation of pigment stability in processed food products

The effect of ascorbic acid used as antioxidant, pH and storage temperature on stability of anthocyanin pigment was evaluated under this experiment. Guava RTS beverage was prepared from clarified fruit juice. Anthocyanin pigment extract was added to the RTS beverage at the rate of 0.68g/200ml. The product coloured with anthocyanin pigment served as the control (Plate 5 and 6).

The influence of pH on anthocyanin pigment was evaluated by comparing three levels of citric acid (0.3, 0.4 and 0.5%). The effect of the ascorbic acid at 0.01 and 0.02% on the stability of anthocyanin pigment was studied. The products prepared with different levels of additives were stored under ambient and refrigerated condition.

4.3.1. Effect of storage conditions on pH of RTS beverage

The pH of RTS beverages were recorded initially and subsequently at fortnightly intervals during the storage period of three months. pH of the RTS beverage followed a decreasing trend with advancement in duration of storage (Table 5). At 15 days after storage there was no significant difference between treatments in pH.

Minimum changes in pH were observed in RTS beverage prepared with 0.3% citric acid and 0.01% ascorbic acid and stored at refrigerated condition (T_{10}) throughout the period of study. The per cent change in pH observed in T_{10} was 1.69, 2.08, 5.37, 7.96, 7.96 and 8.78 respectively at 15, 30, 45, 60, 75 and 90 DAS.

Table 4b. Progressive change in anthocyanin content (%)

Treatments/pH	Per cent decrease in anthocyanin content									
	Initial	**1hr**	**1st day**	**2nd day**	**3rd day**	**4th day**	**5th day**	**6th day**	**13th day**	**20th day**
T_1 (2.5)	61.25	0.24^{f}	3.26^{e}	8.78^{f}	12.27^{f}	11.83^{d}	12.27^{e}	14.96^{e}	18.77^{d}	20.29^{e}
T_2 (3.0)	61.25	1.98^{e}	13.68^{d}	11.83^{e}	15.53^{e}	14.69^{c}	13.14^{e}	19.29^{d}	20.02^{cd}	23.67^{d}
T_3 (4.0)	61.25	10.20^{d}	14.536^{d}	14.01^{d}	16.54^{d}	15.53^{c}	19.04^{d}	19.29^{d}	19.04^{bc}	24.21^{cd}
T_4 (5.0)	61.25	15.53^{c}	18.61^{c}	16.54^{c}	19.29^{c}	18.01^{b}	20.16^{c}	20.84^{c}	20.16^{bc}	25.49^{c}
T_5 (6.0)	61.25	16.54^{b}	22.63^{b}	22.66^{b}	20.43^{b}	18.31^{b}	16.54^{b}	24.27^{b}	20.73^{b}	26.91^{b}
T_6 (7.0)	61.25	22.99^{a}	41.25^{a}	42.09^{a}	44.00^{a}	33.79^{a}	43.59^{a}	42.14^{a}	46.12^{a}	44.02^{a}
CV%		3.97	3.34	4.10	2.25	3.87	3.01	3.03	2.68	2.86
CD (0.05)		0.79	1.12	1.41	0.85	1.28	1.11	1.26	1.15	1.39

Plate 5. Guava RTS beverage prepared with synthetic/natural colour

Guava RTS beverage without added colour

Guava RTS beverage with anthocyanin pigment

Guava RTS beverage with synthetic colour

Plate 6. Stability of anthocyanin pigment in guava RTS beverage

Guava RTS beverage 1 MAS at ambient condition

Guava RTS beverage 3 MAS at ambient condition

Guava RTS beverage 1 MAS at refrigerated condition

Guava RTS beverage 3 MAS at refrigerated condition

Table 5. Progressive changes in pH of RTS beverage during storage

Treatments	Per cent decrease in pH of RTS beverage						
	Initial	**15 DAS**	**30 DAS**	**45 DAS**	**60 DAS**	**75 DAS**	**90 DAS**
T_1	3.36	3.96 (1.87)	5.15^{cdef}	5.75^{hi}	7.14^{j}	11.70^{i}	14.28^{f}
T_2	3.20	3.64 (1.83)	5.20^{cdef}	9.89^{de}	11.77^{defg}	11.97^{i}	12.5^{fg}
T_3	3.10	5.37 (2.29)	8.60^{bc}	9.35^{def}	9.67^{ghi}	12.36^{hi}	12.25^{fg}
T_4	3.50	3.42 (1.79)	7.90^{bcd}	12.85^{bc}	16.38^{b}	15.33^{defg}	17.14^{de}
T_5	3.11	5.68 (2.37)	4.50^{cdef}	6.64^{fghi}	7.82^{ij}	8.78^{j}	9.95^{gh}
T_6	3.30	4.54 (2.11)	7.07^{bcde}	8.08^{defgh}	9.59^{ghij}	13.13^{fghi}	27.90^{b}
T_7	3.65	1.91 (1.24)	7.48^{bcde}	10.68^{cd}	12.69^{def}	12.96^{ghi}	14.61^{ef}
T_8	4.30	4.72 (2.16)	25.73^{a}	28.06^{a}	26.13^{a}	27.90^{a}	33.13^{a}
T_9	3.55	1.69 (1.33)	7.9b^{cd}	13.14^{bc}	14.08^{bcd}	15.77^{cdef}	18.96^{cd}
T_{10}	3.35	1.69 (1.28)	2.08^{f}	5.37^{hi}	7.96^{ij}	7.96^{j}	8.78^{h}
T_{11}	3.40	3.43 (1.78)	3.62^{def}	5.88^{hi}	8.13^{hij}	13.23^{fghi}	17.35^{d}
T_{12}	3.11	6.10 (2.45)	7.28^{bcde}	7.50^{efgh}	11.57^{efg}	16.39^{bcde}	20.15^{c}
T_{13}	3.32	3.81 (1.85)	6.02^{cdef}	8.83^{defg}	13.25^{cde}	16.66^{bcde}	18.27^{cd}
T_{14}	3.60	2.96 (1.58)	10.64^{b}	13.79^{b}	16.20^{b}	18.05^{bcd}	19.16^{cd}
T_{15}	3.30	3.83 (1.91)	10.80^{b}	10.80^{cd}	15.65^{bc}	18.18^{bc}	20.70^{c}
T_{16}	2.90	5.86 (2.37)	8.27^{bc}	13.79^{b}	15.17^{bc}	18.62^{b}	18.96^{cd}
T_{17}	4.25	4.31 (2.06)	5.88^{cdef}	6.43g^{hi}	10.58^{fgh}	14.90^{efgh}	18.82c^{d}
T_{18}	3.66	3.18 (1.67)	3.46^{ef}	4.18^{i}	11.83^{defg}	12.84^{ghi}	13.93^{f}
CV%		54.25 (29.94)	34.55	16.59	11.93	11.22	8.79
CD (0.05)		N S	4.37	2.76	2.47	2.75	2.56

DAS- Days after storage

Maximum change in pH was recorded in RTS beverage prepared without additives citric acid and ascorbic acid and stored under ambient conditions (T_8).

4.3.2. Effect of storage conditions on acidity of RTS beverage

Acidity of the RTS beverage increased with advancement in storage period. At 15 days after storage there was no significant change between the treatments in acidity of RTS beverage. Results are given in Table 6.

Maximum change in acidity was observed in product coloured with synthetic colour (T_8) during three months of storage. The per cent change in acidity observed in T_8 was 15.81, 27.77, 74.07, 92.59, 98.14 and 105.55 respectively at 15, 30, 45, 60, 75 and 90 DAS. Minimum change was recorded in product coloured with anthocyanin pigment and 0.5% citric acid and 0.01% ascorbic acid (T_{12}). The per cent change in acidity observed in T_{12} was 6.66, 10, 14.44, 17.77, 16.66, and 21.11 respectively at 15, 30, 45, 60, 75 and 90 DAS.

4.3.3. Effect of storage conditions on ascorbic acid content of RTS beverage

Ascorbic acid content of the RTS beverage was found to decrease continuously during storage under both conditions (Table 7). At 15 days after storage there was no significant change between the treatments in ascorbic acid content.

A minimum change in ascorbic acid was observed in RTS beverage prepared with 0.4% citric acid and 0.02% ascorbic acid and stored at refrigerated condition (T_{14}) throughout the period of study. The per cent change in ascorbic acid observed in T_{14} was 2.05, 6.58, 8.64, 10.69, 11.11 and 11.93 respectively at 15, 30, 45, 60, 75 and 90 DAS.

Maximum change in ascorbic acid was recorded in RTS beverage prepared with 0.3% citric acid and 0.01% ascorbic acid and stored at ambient condition (T_1).

Table 6. Progressive changes in acidity of RTS beverage during storage

Treatments	Per cent increase in acidity of RTS beverage						
	Initial	**15 DAS**	**30 DAS**	**45 DAS**	**60 DAS**	**75 DAS**	**90 DAS**
T_1	0.30	1.11 (1.12)	19.44^{cd}	15.27^{a}	25.55^{abcd}	34.44^{de}	35.55^{cde}
T_2	0.30	1.11 (1.78)	18.88^{cd}	20.00^{abc}	27.77^{bcd}	36.66^{ef}	40.00^{def}
T_3	0.32	14.58 (3.84)	17.70^{bcd}	35.41^{hi}	37.50^{efgh}	47.91^{h}	48.95^{ghi}
T_4	0.25	10.66 (3.23)	24.00^{de}	41.33^{i}	44.00^{h}	61.33^{j}	73.33^{j}
T_5	0.31	6.45 (2.58)	17.20^{bcd}	26.88^{cdef}	33.33^{defg}	36.55^{ef}	39.78^{def}
T_6	0.34	9.80 (3.17)	18.62^{cd}	20.58^{abcd}	27.45^{bcd}	31.37^{de}	29.41^{abc}
T_7	0.33	12.12 (3.53)	19.19^{cd}	27.27^{def}	33.33^{defg}	41.41^{fg}	41.01^{efg}
T_8	0.25	8.00 (2.85)	27.77^{e}	74.07^{j}	92.59^{i}	98.14^{k}	105.55^{k}
T_9	0.25	9.33 (3.06)	14.66^{abc}	28.00^{efg}	33.33^{defg}	45.33^{gh}	54.66^{hi}
T_{10}	0.24	9.72 (3.18)	7.77^{a}	16.66^{ab}	25.00^{abc}	29.16^{cd}	37.50^{cde}
T_{11}	0.27	9.87 (3.20)	17.28^{bcd}	20.98^{abcde}	29.62^{cde}	33.33^{de}	46.91^{fgh}
T_{12}	0.30	6.66 (2.39)	10.00^{ab}	14.44^{a}	17.77^{a}	16.66^{a}	21.11^{a}
T_{13}	0.23	8.69 (2.97)	21.01^{cde}	28.98^{fgh}	30.43^{cdef}	31.88^{de}	49.27^{ghi}
T_{14}	0.28	9.52 (3.02)	20.23^{cde}	23.80^{bcdef}	26.19^{bcd}	25.00b^{c}	31.78^{bc}
T_{15}	0.31	9.67 (3.16)	13.97^{abc}	19.35^{ab}	20.43^{ab}	23.65^{b}	26.88^{ab}
T_{16}	0.27	9.87 (3.20)	17.28^{bcd}	16.04^{a}	20.98^{ab}	29.62^{cd}	30.86^{bc}
T_{17}	0.18	14.81 (3.72)	28.00^{e}	34.66^{ghi}	40.00^{gh}	54.66^{i}	57.33^{i}
T_{18}	0.20	11.66 (3.47)	24.50^{de}	30.00^{fg}h	38.33^{fgh}	45.00^{gh}	55.00^{hi}
CV%		53.93 (27.36)	25.31	15.90	14.34	8.28	11.27
CD (0.05)		N S	7.86	7.26	7.96	5.50	8.55

DAS- Days after storage

Table 7. Progressive changes in ascorbic acid content of RTS beverage during storage

Treatments	Per cent decrease in ascorbic acid of RTS beverage						
	Initial	**15 DAS**	**30 DAS**	**45 DAS**	**60 DAS**	**75 DAS**	**90 DAS**
T_1	49.00	2.17 (1.56)	6.28^{bc}	26.93^{a}	35.68^{a}	37.19^{a}	31.63^{a}
T_2	48.60	1.92 (1.52)	2.60^{cde}	10.15^{efg}	16.32^{de}	18.03^{ef}	23.52^{b}
T_3	45.60	2.04 (1.72)	4.97^{bcd}	18.85^{b}	22.51^{b}	23.61^{bc}	23.97^{b}
T_4	46.22	2.63 (1.68)	4.80^{bcd}	6.96^{fgh}	16.34^{de}	21.75^{cd}	22.83^{bc}
T_5	44.30	2.18 (1.59)	2.93^{bcde}	10.83^{ef}	10.45^{f}	13.84^{gh}	15.72^{gh}
T_6	40.12	1.12 (1.20)	1.96^{de}	2.37^{i}	19.82^{bcd}	20.23^{de}	20.23^{cde}
T_7	36.22	1.52 (1.35)	2.44^{e}	12.57^{de}	12.11^{f}	13.95^{gh}	14.41^{hi}
T_8	46.88	4.01 (2.08)	12.54^{a}	16.80^{bc}	23.20^{b}	23.20^{bc}	24.27^{b}
T_9	42.20	2.84 (1.73)	0.868^{e}	5.60^{hi}	10.34^{f}	11.53^{hi}	18.47^{defg}
T_{10}	55.20	2.04 (1.48)	3.40^{bcde}	5.44^{hi}	6.46^{g}	7.48^{j}	14.92^{i}
T_{11}	48.35	2.10 (1.50)	6.23b^{c}	16.58^{bc}	18.64^{cd}	21.40^{cd}	21.40^{bcd}
T_{12}	48.33	2.06 (1.49)	4.13^{bcde}	19.99^{b}	17.58^{cd}	22.40^{cd}	23.09^{bc}
T_{13}	40.33	1.64 (1.41)	2.47^{cde}	6.19^{ghi}	9.08^{fg}	16.10^{fg}	16.93^{fgh}
T_{14}	40.50	2.05 (1.56)	6.58^{b}	8.64^{efgh}	10.69^{f}	11.11^{i}	11.93^{i}
T_{15}	36.50	3.19 (1.81)	4.10^{bcde}	12.32^{de}	12.78^{ef}	15.06^{g}	16.89^{fgh}
T_{16}	36.60	3.46 (1.89)	5.28^{bcd}	14.84^{cd}	16.21^{de}	18.48^{ef}	18.94^{def}
T_{17}	40.20	2.98 (1.76)	11.27^{a}	18.32^{bc}	20.81^{bc}	25.78^{b}	28.68^{a}
T_{18}	40.20	2.98 (1.76)	5.47^{bcd}	7.13^{fgh}	11.69^{f}	14.59^{g}	17.91^{efg}
CV%		90.542 37.91	47.96	19.64	14.08	8.65	9.44
CD (0.05)		N S	3.90	3.98	3.769	2.675	3.15

The per cent change in ascorbic acid observed in T_1 was 2.17, 6.28, 26.93, 35.68, 37.19 and 31.63 respectively at 15, 30, 45, 60, 75 and 90 DAS.

The differential response of the treatments over the stepped up storage periods was noticed as regards to major parameters viz., pH, acidity and ascorbic acid.

The steep decrease in ascorbic acid for all the treatments as evidenced from the progressive per cent change was more pronounced in the upward storage period.

4.3.4. Effect of storage conditions on colour of RTS beverage

RTS beverage stored under refrigerated conditions retained colour throughout the storage period as compared to ambient condition where there was a greater loss of the colour.

Colour of the RTS beverage was light yellowish pink (19B) initially in all the treatments except T_8 and T_{17} which was Light Purplish Pink (55C) [Table 8].

After 15 days of storage the colour changed to light yellow green (2C) in T_1, T_2, T_3, T_4, T_5 and T_6, light orange yellow (16B) in T_7 and T_9 and in T_8 it remained same in ambient condition. In refrigerated condition colour was same in all the treatments except T_{16} and T_{18} it was moderate yellowish pink (13D). The colour followed similar trend throughout the storage period.

The colour was pale yellow (8D) ninety days after storage in treatments T_1, T_2, T_3, T_4, T_5 and T_6, pale yellow (11D) in T_7 and T_9 and in T_8 it was same in ambient condition. In refrigerated condition colour was same in all the treatments except T_{16} and T_{18} where it was moderate yellowish pink (13D) at the end of storage period.

4.3.5. Effect of storage on sensory attributes of guava RTS beverage

Guava RTS beverage was organoleptically evaluated by selected panel of judges. Sensory scores followed a declining trend during storage period.

Table 8. Effect of storage conditions on colour of RTS beverage

Treatments	Colour						
	Initial	**15 DAS**	**30 DAS**	**45 DAS**	**60 DAS**	**75 DAS**	**90 DAS**
T_1	Light yellowish pink (19B)	Light yellow green (2C)	Light yellow green (2C)	Light yellow green (2C)	Light yellow green (2C)	Pale yellow (8D)	Pale yellow (8D)
T_2	Light yellowish pink (19B)	Light yellow green (2C)	Light yellow green (2C)	Light yellow green (2C)	Light yellow green (2C)	Pale yellow (8D)	Pale yellow (8D)
T_3	Light yellowish pink (19B)	Light yellow green (2C)	Light yellow green (2C)	Light yellow green (2C)	Light yellow green (2C)	Pale yellow (8D)	Pale yellow (8D)
T_4	Light yellowish pink (19B)	Light yellow green (2C)	Light yellow green (2C)	Light yellow green (2C)	Light yellow green (2C)	Pale yellow (8D)	Pale yellow (8D)
T_5	Light yellowish pink (19B)	Light yellow green (2C)	Light yellow green (2C)	Light yellow green (2C)	Light yellow green (2C)	Pale yellow (8D)	Pale yellow (8D)
T_6	Light yellowish pink (19B)	Light yellow green (2C)	Light yellow green (2C)	Light yellow green (2C)	Light yellow green (2C)	Pale yellow (8D)	Pale yellow (8D)
T_7	Light yellowish pink (19B)	Light orange yellow (16B)	Light orange yellow (16B)	Pale orange (16D)	Pale orange (16D)	Pale yellow (11 D)	Pale yellow (11 D)
T_8	Light Purplish Pink (55C)	Light Purplish Pink (55C)	Light Purplish Pink (55C)	Light Purplish Pink (55C)	Light Purplish Pink (55C)	Light Purplish Pink (55C)	Light Purplish Pink (55C)

Table 8 contd…

T_9	Light yellowish pink (19B)	Light orange yellow (16B)	Light orange yellow (16B)	Pale orange (16D)	Pale orange (16D)	Pale yellow (11 D)	Pale yellow (11 D)
T_{10}	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)
T_{11}	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)
T_{12}	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)
T_{13}	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)
T_{14}	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)
T_{15}	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)	Light yellowish pink (19B)
T_{16}	Light yellowish pink (19B)	Moderate yellowish pink (13 D)	Moderate yellowish pink (13 D)	Light orange yellow (16B)	Pale orange yellow (16D)	Moderate yellowish pink (13 D)	Moderate yellowish pink (13 D)
T_{17}	Light Purplish Pink (55C)	Light Purplish Pink (55C)	Light Purplish Pink (55C)	Light Purplish Pink (55C)	Light Purplish Pink (55C)	Light Purplish Pink (55C)	Light Purplish Pink (55C)
T_{18}	Light yellowish pink (19B)	Moderate yellowish pink (13 D)	Moderate yellowish pink (13 D)	Light orange yellow (16B)	Pale orange yellow (16D)	Moderate yellowish pink (13 D)	Moderate yellowish pink (13 D)

DAS- Days after storage

Initially highest mean scores for appearance (7.90), colour (8.00), taste (8.40) and overall acceptability (8.00) were recorded for RTS beverage prepared with 0.3% citric acid and 0.01% ascorbic acid and stored at refrigerated condition (T_{10}) and flavor (7.80) and texture (7.60) in RTS beverage prepared with 0.4% citric acid + 0.01% ascorbic acid and stored at ambient condition (T_2) Table 9a.

Three months after storage highest mean scores for appearance (7.50), colour (7.75), flavor (7.50) and texture (7.37) taste (7.25) and overall acceptability (7.50) were recorded for RTS beverage prepared with 0.3% citric acid and 0.01% ascorbic acid and stored at refrigerated condition (T_{10}) Table 9b.

The products stored under refrigerated conditions recorded highest scores in organoleptic evaluation as compared to those kept at ambient conditions.

4.3.6. Storage stability of pigment in mixed fruit jam

Pigment stability in mixed fruit jam was studied under this experiment. The three treatments included were mixed fruit jam without added colour (T_1), synthetic colour (T_2) and anthocyanin pigment (T_3). The product prepared was stored under ambient and refrigerated conditions for a period of three months (Plate 7 and 8).

Observations were recorded on pH, initial colour and colour retention at monthly intervals during storage. Organoleptic evaluation of the products was carried out during storage.

4.3.6.1. Effect of storage condition on pH of mixed fruit jam

pH of mixed fruit jam registered a decreasing trend with increase in storage period (Table 10) One month after storage minimum change in pH was observed in T_1 (2.38%) followed by T_2 (2.55%) and maximum change (4.74%) recorded in T_3. Thereafter at second and third months after storage minimum changes was recorded

Table 9a. Mean Sensory ranks of RTS beverage (Initial)

Treatments	Appearance	Colour	Flavour	Consistency	Odour	Taste	Overall acceptability
T_1	7.80	7.60	7.50	7.20	7.50	8.10	7.60
T_2	7.60	7.30	7.80	7.60	7.70	7.80	7.80
T_3	7.20	7.40	7.40	7.40	7.80	7.80	7.70
T_4	6.90	7.00	7.30	7.50	7.30	7.40	7.30
T_5	7.40	7.20	7.20	7.40	7.40	7.40	7.70
T_6	7.40	7.20	6.90	7.20	7.30	7.30	7.50
T_7	7.40	7.20	7.20	6.90	6.90	6.70	7.10
T_8	6.80	7.20	6.40	6.50	6.70	6.20	6.30
T_9	7.40	6.80	6.80	6.7	6.50	6.00	6.20
T_{10}	7.90	8.00	7.70	7.40	7.70	8.40	8.00
T_{11}	7.60	7.60	7.60	7.40	7.50	7.80	7.60
T_{12}	7.40	7.30	7.60	7.40	7.70	7.60	7.60
T_{13}	7.60	7.40	7.70	7.30	7.30	7.70	7.70
T_{14}	7.40	7.40	7.10	7.30	7.20	7.30	7.40
T_{15}	7.30	7.30	7.10	7.10	7.00	7.10	7.00
T_{16}	7.70	7.60	6.80	6.90	7.00	6.90	7.00
T_{17}	7.30	7.20	6.60	6.50	6.60	6.30	6.60
T_{18}	7.40	6.70	6.80	6.70	6.80	6.10	6.40
Kendal's W test	0.47	0.38	0.21	0.27	0.40	0.29	0.28

Table 9b. Mean Sensory ranks of RTS beverage (3 MAS)

Treatments	Appearance	Colour	Flavour	Consistency	Odour	Taste	Overall acceptability
T_1	6.75	6.75	6.75	6.60	6.50	6.50	6.70
T_2	6.75	6.50	6.60	6.50	6.40	6.40	6.75
T_3	6.50	6.50	6.75	6.62	6.60	6.70	6.75
T_4	7.00	7.00	7.25	7.00	6.80	6.87	6.70
T_5	6.75	6.75	7.00	6.50	6.50	6.50	7.00
T_6	6.75	6.75	7.00	6.60	6.50	6.50	6.80
T_7	6.75	6.75	6.25	6.25	6.25	6.25	6.70
T_8	6.50	6.50	6.90	6.60	6.60	6.70	6.50
T_9	6.50	6.25	6.50	6.25	6.12	6.12	6.50
T_{10}	7.50	7.75	7.50	7.37	7.25	7.25	7.50
T_{11}	7.40	7.25	6.75	7.00	7.10	6.90	7.00
T_{12}	7.25	6.75	6.50	6.60	6.75	6.60	6.75
T_{13}	7.50	7.00	6.50	6.25	6.50	6.25	7.25
T_{14}	7.25	6.75	6.75	6.40	6.75	6.50	7.50
T_{15}	6.75	7.00	6.62	6.60	6.60	6.60	7.00
T_{16}	7.25	7.00	7.00	6.75	6.75	6.60	7.25
T_{17}	7.25	7.00	7.00	6.50	6.60	6.50	7.25
T_{18}	7.00	7.00	7.25	6.90	6.80	6.80	7.20
Kendal's W test	0.082	0.167	0.228	0.044	0.051	0.023	0.034

Table 10. Progressive changes in pH of mixed fruit jam during storage

Treatments	Change in pH of mixed fruit jam (%)			
	Initial	**1 MAS**	**2MAS**	**3MAS**
T_1	4.19	2.38^{c}	3.16^{d}	6.32^{c}
T_2	4.30	2.55^{c}	8.77^{bc}	12.03^{ab}
T_3	4.06	4.74^{a}	10.46^{ab}	14.65^{a}
T_4	4.18	2.63^{bc}	7.11^{c}	9.980^{b}
T_5	4.25	3.52^{b}	$8.52b^{c}$	11.47^{b}
T_6	4.13	4.66^{a}	12.53^{a}	14.95^{a}
CV%		18.18	23.40	18.39
CD (0.05)		0.92	2.93	3.16

MAS- Months after storage

R**efrigerated condition**

T_1 - Control (product without added colour)

T_2 - Product + synthetic colour

T_3 - Product + anthocyanin pigment (AC)

Ambient condition

T_4 - Control (product without added colour)

T_5 - Product + synthetic colour

T_6 - Product + anthocyanin pigment (AC)

Plate 7. Mixed fruit jam prepared with synthetic/natural colour

Mixed fruit jam without added colour

Mixed fruit jam with synthetic colour

Mixed fruit jam with anthocyanin pigment

Plate 8. Stability of anthocyanin pigment in mixed fruit jam

Mixed fruit jam stored under refrigerated condition

Mixed fruit jam stored under ambient condition

Mixed fruit jam stored under refrigerated condition (3

Mixed fruit jam stored under ambient condition

in control under refrigerated and ambient condition (T_1 and T_4) and a maximum change was seen in T_6 (14.95%).

The differential response of the treatments over the stepped up storage periods was noticed as regards to pH of mixed fruit jam. The steep decrease in pH for all the treatments as evidenced from the progressive per cent change was more pronounced in the upward storage period.

4.3.6.2. Effect of storage on colour of mixed fruit jam:

There was no significant change in the colour of the mixed fruit jam. A slight darkening of the colour was seen in the bottles stored at ambient condition.

The colour of the mixed fruit jam initially was light yellow (14D) in T_1 and T_4, vivid red (52A) in T_2 and T_5 and moderate reddish orange (41C) in T_3 and T_6 (Table 11).

One month after storage colour changed to light orange yellow (22 B) in T_1 and light orange yellow (22 D) in T_4 and it remained same in all other treatments.

The colour was light orange yellow (22 D) in T_1 and T_4, it was moderate reddish orange (41D) in T_3 and moderate reddish orange (42D) in T_6 and remained same in all other treatments at the end of storage period.

4.3.6.3. Effect of storage on sensory attributes of mixed fruit jam

Mixed fruit jam was organoleptically evaluated by selected panel of judges. Sensory scores followed a declining trend during storage period (Table 12, 13 and 14) Colour is one of the important factors for selection of product by consumers.

One month after storage, highest mean scores for appearance (7.96) and colour (7.96) were recorded in product coloured with synthetic colour (T_2). Highest scores for flavour (7.76) texture (7.23), odour (7.46) and overall acceptability (7.56)

Table 11. Effect of storage conditions on colour of mixed fruit jam

Colour				
Treatments	**Initial**	**1 MAS**	**2MAS**	**3MAS**
T_1	Light yellow(14D)	Light orange yellow(22 B)	Light orange yellow(22 C)	Light orange yellow (22 D)
T_2	Vivid red (52A)	Vivid red (52A)	Vivid red (52A)	Vivid red (52A)
T_3	Moderate reddish orange (41 C)	Moderate reddish orange (41 C)	Moderate reddish orange (41 D)	Moderate reddish orange (41D)
T_4	Light yellow(14D)	Light orange yellow(22 D)	Light orange yellow(22 D)	Light orange yellow (22 D)
T_5	Vivid red (52A)	Vivid red (52A)	Vivid red (52A)	Vivid red (52A)
T_6	Moderate reddish orange (41 C)	Moderate reddish orange (41 C)	Moderate reddish orange (42D)	Moderate reddish orange (42D)

MAS- Months after storage

Refrigerated condition

T_1 - Control (product without added colour)

T_2 - Product + synthetic colour

T_3 - Product + anthocyanin pigment (AC)

Ambient condition

T_4 - Control (product without added colour)

T_5 - Product + synthetic colour

T_6 - Product + anthocyanin pigment (AC)

Table 12. Mean sensory scores of mixed fruit jam (1MAS)								
Treatments	**Appearance**	**Colour**	**Flavour**	**Texture**	**Odour**	**Taste**	**After taste**	**Overall acceptability**
T_1	7.13	7.36	7.76	7.23	7.46	7.66	7.46	7.56
T_2	7.96	7.96	7.23	7.06	7.33	7.66	7.20	7.20
T_3	6.90	7.16	7.30	6.86	6.93	7.26	7.26	7.16
T_4	7.50	7.06	7.36	6.93	7.13	7.93	7.53	7.50
T_5	7.66	7.70	7.26	7.23	7.30	7.26	7.46	7.13
T_6	7.06	7.00	7.23	7.10	7.46	7.26	7.60	7.16
Kendal's W test	0.24	0.18	0.05	0.11	0.06	0.11	0.05	0.10
Table 13. Mean sensory scores of mixed fruit jam (2MAS)								
T_1	7.13	6.73	6.86	6.66	6.86	7.06	6.86	7.20
T_2	7.86	8.00	7.53	7.60	7.06	7.86	7.06	7.33
T_3	7.00	7.06	7.53	7.13	7.13	7.33	6.73	7.26
T_4	7.16	7.13	7.10	7.16	7.30	7.33	7.26	7.30
T_5	7.43	7.93	7.46	7.23	7.13	7.20	7.23	7.16
T_6	7.06	6.96	6.76	7.06	6.93	7.53	7.20	7.46
Kendal's W test	0.14	0.33	0.17	0.09	0.04	0.03	0.10	0.07
Table 14. Mean sensory scores of mixed fruit jam (3MAS)								
T_1	6.60	6.56	6.86	6.66	7.30	7.06	6.86	7.20
T_2	6.26	7.93	7.53	7.60	7.06	7.86	6.60	7.33
T_3	6.40	6.40	7.53	7.13	7.13	7.33	6.26	7.26
T_4	6.56	6.36	6.56	6.56	6.86	6.60	6.40	6.60
T_5	6.56	6.56	6.56	6.56	7.13	6.26	7.23	6.26
T_6	6.40	6.96	6.40	6.40	6.93	6.40	7.20	6.40
Kendal's W test	0.05	0.39	0.32	0.32	0.04	0.27	0.18	0.28

were recorded in jam without added colour and stored under refrigerated condition and for taste (7.93) in jam without added colour and stored under ambient condition.

Three months after storage highest mean scores for colour (7.93), flavour (7.53), texture (7.6), taste (7.86) and overall acceptability (7.33) was recorded in product coloured with synthetic colour (T_2).

The products stored under refrigerated conditions recorded highest scores in organoleptic evaluation as compared to those kept at ambient condition.

Discussion

5. Discussion

Syzygium cumini Skeels, commonly known as Jamun, is a widely distributed minor fruit tree in India and other tropical and sub tropical regions of the world. The tree has a great economic importance since most of the parts like bark, leaves, seed and fruits are used as alternative medicine to treat various diseases. It is used in well known traditional medicines to control the blood sugar level in the patients suffering from diabetes. The tree is rich in phytochemicals like glycoside jambolin, anthocyanins, tannins, terpenoids, gallic acid and various minerals. The fruits are purplish black in colour when ripe and have high anthocyanin content (Chaudhary and Mukhopadhyay, 2012).

Consumer concern over the safety of synthetic food colourants has increased the demand for natural food colourants. Especially, there is a growing demand for natural red food colourants as alternatives to the most commonly used synthetic red colourant, FD&C Red #40 (Giusti and Wrolstad, 2003). Natural red colourants permitted in foods include anthocyanins, betanin, cochineal (carmine and carminic acid) and carotenoids (paprika, canthaxanthin) (Askar, 1993; Francis, 1994).

Anthocyanins are the best-known natural red colourants used in food due to their bright and attractive colours, non-toxicity, and water solubility, which allows their incorporation into aqueous food systems. In addition to colourant properties, interest in anthocyanins has intensified because of their possible role in reducing the risk of coronary heart disease, cancer and stroke (Wrolstad, 2004).

The colour and stability of anthocyanins are affected by several factors such as the chemical structure, concentration, pH, temperature, light, presence of co pigments, metal ions, enzymes, oxygen, ascorbic acid, sugars and their degradation products, and sulphur dioxide (Shipp and Abdel-Aal, 2010; Cavalcanti *et al*., 2011).

Methods of extraction of anthocyanin pigment, stability of the pigment to pH, light, temperature, storage conditions and in processed products was studied under this project. The results obtained are discussed here under.

5.1. Standardization of method for extraction of anthocyanin pigment

The efficacy of four methods of extraction of anthocyanin pigment was studied under this experiment.

Extraction methods:

5.1.1 Hot water extraction

Anthocyanin, the pigment present in jamun is water soluble. Low anthocyanin content was obtained from hot water extraction method. The yield of anthocyanin content was 20.1mg/100g with a recovery per cent of 7.5. Some of the pigments are highly unstable at high temperature and hence the lower yield of anthocyanin content in hot water extraction.

Tsimidou and Tsatsaroni (1993) reported degradation of carotenoid pigment crocin when extracted using water. Bechtold *et al*., (2006) extracted natural dye from berries and vegetables with boiling water. Diouf *et al*., (2009) reported the extraction of *Pica marina* by refluxing with water. Petersson *et al*., (2010) reported the degradation of anthocyanins from red onion when extracted by pressurized hot water extraction.

5.1.2. Acidified aqueous extraction (0.5% citric acid)

In acidified aqueous extraction method, 0.5% citric acid was added to water and used for the extraction. There was an increase in anthocyanin content up to 21.50mg/100g with a recovery per cent of 7.5. The addition of citric acid might have increased the permeability of the anthocyanin pigment. Similar results were reported

by Debicks- Perpisal *et al*., (1983) in annatto. According to Anu (2009), in Malay apple when 4% citric acid was used for the extraction, better yield was obtained.

5.1.3. Solvent extraction (20% ethanol)

On comparison with the above methods, solvent extraction using 20% ethanol yielded higher anthocyanin content. There was an increase in anthocyanin content up to 33.50mg/100g with a recovery per cent of 12.5.

The yield of anthocyanin from fruit residues of *Vaccinium uliginosum* Linn. was more when 50% (w/w) ethanol solution was used instead of water, indicating that ethanol could facilitate the dissolution of anthocyanins from cells (Hua *et al*., 2013).

Monrad *et al*., (2010) obtained low yield of anthocyanin from grape pomace when hydroethanolic solvents were used for extraction.

5.1.4. Acidified solvent extraction (20% ethanol + 0.5% citric acid)

Among all the above methods acidified solvent extraction method using 20% ethanol + 0.5% citric acid recorded the highest anthocyanin content (61.07mg/100g) and recovery per cent (13.75%) Fig 1 and 2. The increase in anthocyanin content in this method compared to use of solvent alone might be due to increase in the permeability of the anthocyanin pigment due to addition of citric acid.

Spagna *et al.,* (2003) also obtained higher yield of anthocyanin when fresh grape (cv. Ancellotta) was extracted with ethanol acidified with tartaric acid and citric acid. Higher recovery of anthocyanin pigment when acidified ethanol was used for extraction was reported by Xueming (2004) in mulberry and Nikkah *et al*., (2007) in three different berries (*Morus nigra*, *Morus alba* var nigar and *Fragaria* L.)

Clemente and Galli (2010) extracted anthocyanins from residue of processed grapes using 70 mL of ethanol 70% and 30 mL of HCl 0.1% at pH 2.0 and obtained high anthocyanin content (26.20 mg/100g). Guo-Ling Liu *et al*., (2012) reported that

Fig 1. Effect of extraction methods on anthocyanin content

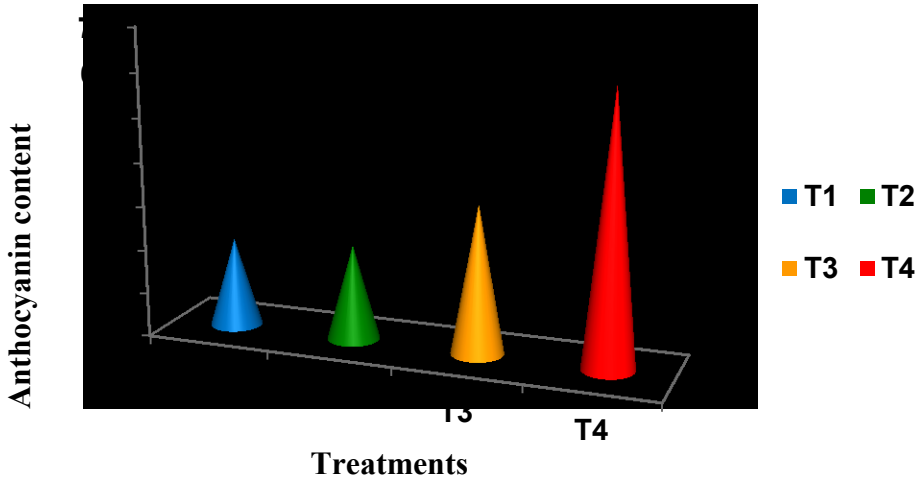
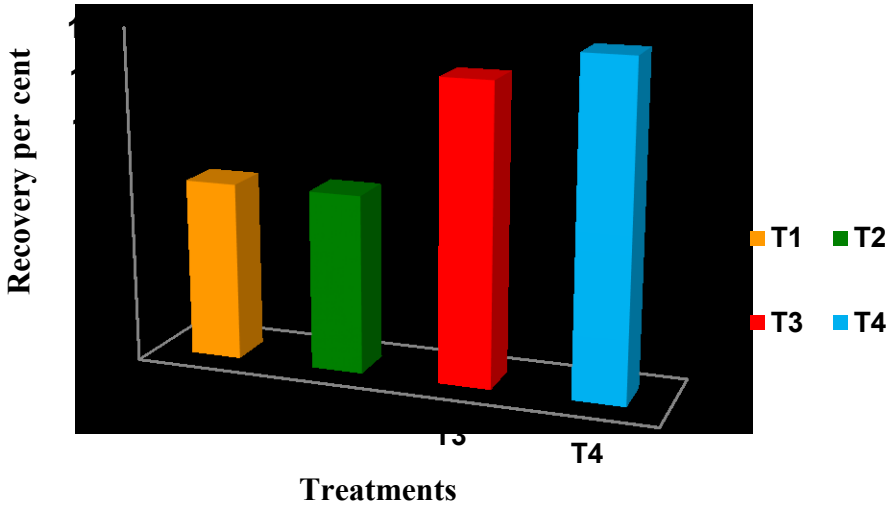


Fig 2. Effect of extraction methods on recovery of anthocyanin content



T_1 - Hot water extraction
T_2 - Acidified aqueous extraction (0.5% citric acid)
T_3 - Solvent extraction (20% ethanol)
T_4 - Acidified solvent extraction (20% ethanol+ 0.5% citric acid)

maximum yield of anthocyanin (4.358 ± 0.045 mg/g) from freeze-dried fruit skin of downy rose-myrtle (*Rhodomyrtus tomentosa* (Ait.) Hassk var. Gangren) was obtained using 60% ethanol containing 0.1% (v/v) hydrochloric acid as extraction solvent.

Chandrasekhar *et al*., (2014) obtained maximum anthocyanin content (390.6mg/L) from red cabbage when mixture of 50% (v/v) ethanol and acidified water was used.

The recovery of the crude extract of anthocyanin pigment depends upon the types/ accessions and the method of pigment extraction. In the present study, it was in range from 7.5% to 18 %.

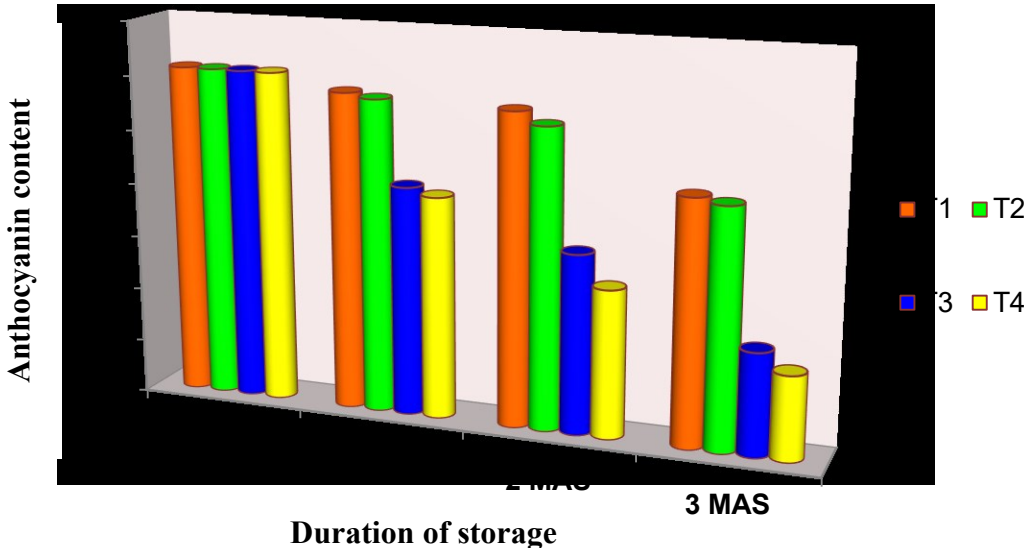
5.2.1. Storage stability of anthocyanin pigment

The effect of light and storage conditions on pigment stability was evaluated in two different containers viz., transparent glass bottles and amber coloured glass bottles at refrigerated and ambient conditions.

Anthocyanin pigment stored in amber coloured glass bottles at refrigerated conditions recorded highest anthocyanin content (58.53mg/100g) and better colour retention (Fig 3). Storage of anthocyanin pigment in amber coloured bottles may have prevented oxidation of pigment by protecting from light. The loss of anthocyanin content and colour in transparent glass bottles may be due to photo oxidation. In the aqueous extracts, light in comparison with dark also increased the destruction of pigments (Heidari *et al*., 2006).

Janna *et al*., (2000) reported that ideal storage conditions for coloured anthocyanin pigments are low pH, dark and low temperature (4^0C). Turker *et al*., (2004) also reported that highest anthocyanin retention was at 4°C storage temperature with a half-life between 231 and 239 days. Roobha *et al*., (2011) reported that exposure to light spoilt the anthocyanin extract from *Musa acuminata* bract. According to Tomczak, (2012) highest degradation of anthocyanins was recorded in

Fig 3. Effect of light and storage conditions on anthocyanin pigment



T_1 - Amber coloured glass bottles (refrigerated condition)

T_2 - Transparent glass bottles (refrigerated condition)

T_3 - Amber coloured glass bottles (ambient condition)

T_4 - Transparent glass bottles (ambient condition)

MAS- Months after Storage.

samples with accessibility to light and oxygen. Devi and Joshi (2012) also obtained similar results.

5.2.2. Thermal stability of the anthocyanin pigment

The effect of high temperature on the anthocyanin content was studied at 70, 80 and 90^0 C and anthocyanin content was measured at intervals 30 minutes, 45 minutes and one hour of incubation at each temperature. Anthocyanin pigment was found to be sensitive to high temperature. Degradation of anthocyanin content was higher as the temperature of incubation was increased.

Initial anthocyanin content was 61.25mg/100g and it was 55.96, 54.74 and 53.57 mg/100g respectively at 70, 80 and 90^0 C after 30minutes of heating. The anthocyanin content recorded after one hour of heating was 53.07, 52.34 and 50.24 mg/100g at 70, 80 and 90^0 C respectively (Fig 4).

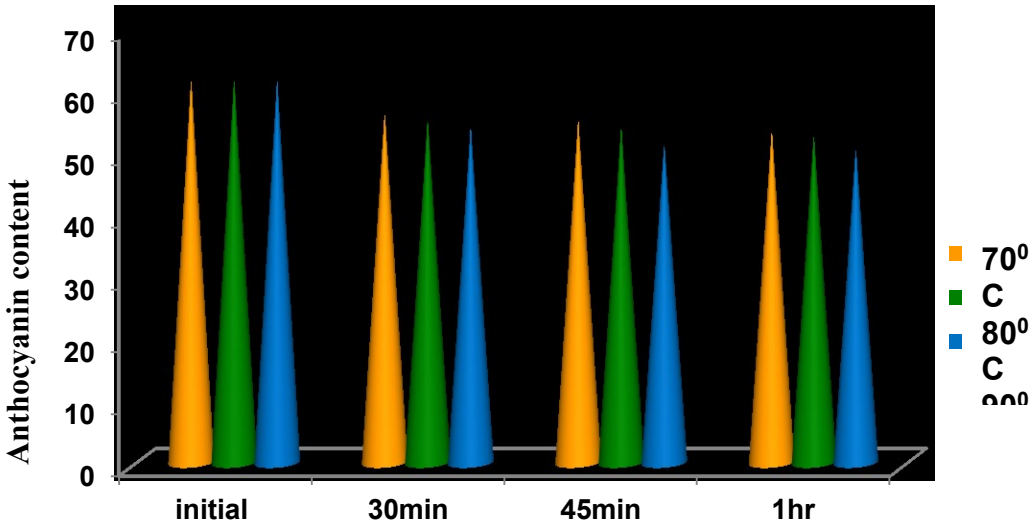
Garzon and Wrolstad (2002) and Kirca *et al*., (2007) also reported first-order reaction model for the degradation of monomeric anthocyanins from various sources. Laleh *et al*., (2006) reported that the speedy destruction of anthocyanin at higher temperatures could be due to hydrolyzation of 3-Glycoside structure, which has a protective effect in unstable anthocyanin.

5.2.3. Effect of pH on anthocyanin content

The effect of pH on pigment stability was studied at different pH ranging from 2.5 to 7 at room temperature at 1 hour, 1 day and then subsequently after 2, 3, 4, 5, 6, 13 and 20 days in the different buffered anthocyanin extracts.

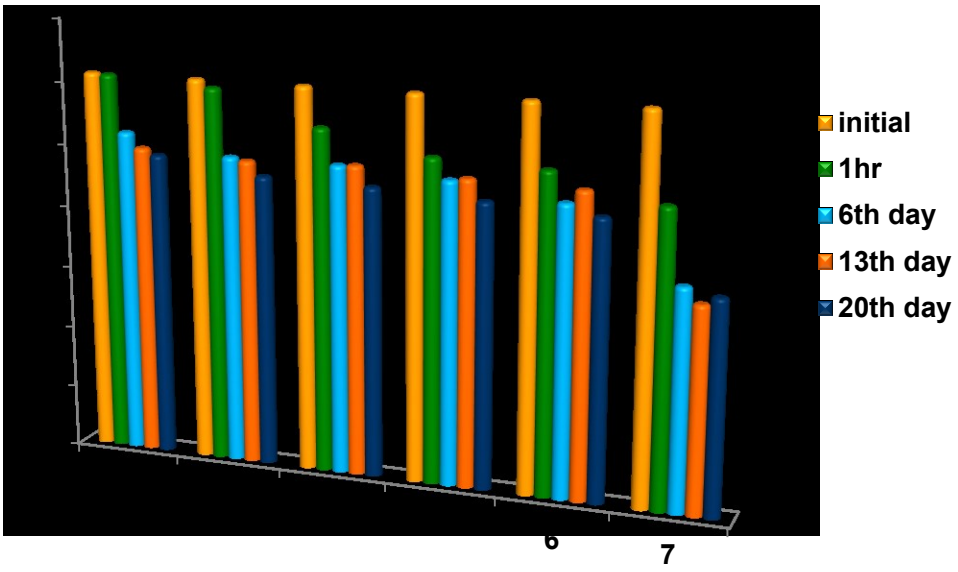
At pH of 2.5 and 3 the colour was reddish tinge and it changed to a yellowish tinge as the pH was increased from 3 to 7. A decreasing trend was followed in anthocyanin content throughout the study period as the pH increased (Fig 5).

Fig 4. Effect of temperature on anthocyanin content



Temperature (^{0}C) and Period of

Fig 5. Effect of pH on anthocyanin content



Treatments (pH)

The anthocyanin content was highest at pH 2.5 (61.1mg/100g) followed by pH 3 (60.03mg/100g) and was least at pH 7 (47.16mg/100g) one hour after incubation. It decreased to 48.81, 46.75, 46.41, 45.63, 44.76, and 34.25mg/100g at pH 2.5, 3, 4, 5, 6, and 7 respectively 20 days after incubation at room temperature. The decrease was highest at neutral pH.

According to Pang Xue Qun *et al*., (2001) the colour of anthocyanins faded, turned brown and became unstable as pH increased; however, it became stable at low temperatures. Increasing the pH of samples from 2 to 6 accelerated the pigment destruction in the aqueous extract (Heidari *et al.,* 2006). The effect of pH on thermal stability of anthocyanins was studied by Kirca *et al*., (2007) at six different pH levels (2.5-7.0) in citrate phosphate buffer solutions and significant decrease in anthocyanin stability was observed at pH above 5.0. According to Hunjaroen and Chantanawarangoon (2008), anthocyanins were most stable at pH 2.5 followed by 4.0, 6.0 and 8.0, respectively. Roobha *et al*., (2011) also observed that increase in pH spoiled anthocyanin from *Musa acuminata* bract.

5.3. Evaluation of pigment stability in processed food products

Guava RTS beverage

The effect of ascorbic acid, pH, colour and storage conditions on the anthocyanin pigment was evaluated in guava RTS beverage. The RTS beverage was prepared according to FSSAI standards and stored for three months under ambient and refrigerated conditions and observations recorded fortnightly. Results obtained are discussed below.

5.3.1. Effect of storage conditions on pH of RTS beverage

The pH of the RTS beverage registered a decreasing trend during three months of storage. At 15 DAS there was no significant variation in pH of RTS beverage.

Minimum changes in pH were observed in RTS beverage prepared with 0.3% citric acid and 0.01% ascorbic acid and stored at refrigerated condition (T_{10}) throughout the period of study (Fig 6). The per cent change in pH observed in T_{10} was 1.69, 2.08, 5.37, 7.96, 7.96 and 8.78 respectively at 15, 30, 45, 60, 75 and 90 DAS.

Maximum change in pH was recorded in RTS beverage prepared without additives citric acid and ascorbic acid and stored under ambient conditions (T_8).

The pH of RTS beverage was found to be influenced by storage temperature. RTS beverage stored at refrigerated conditions had higher pH values compared to that stored under ambient conditions during the study period. Variations in pH during storage may be due to change in chemical properties which are affected by storage conditions (Yadav *et al*., 2010).

Decrease in pH during storage was attributed to simultaneous increase in titrable acidity as reported by Sogi and Singh (2001) in kinnow RTS beverage and Choudhary and Dikshit (2006) in guava RTS beverage. A decline in pH towards acidic region was noticed as the storage duration of beverage increased. Similar trend of decreasing pH was also reported by Nath *et al*., (2005). Hamaran and Amutha (2007) also reported similar results in case of banana and sapota beverage stored at different temperatures for 180 days.

According to Yadav *et al*., (2013) pH significantly decreased with increase in storage periods irrespective of sugar level and storage temperatures.

5.3.2. Effect of storage conditions on acidity of RTS beverage

Acidity of the RTS beverage was found to increase during storage (Fig 7).

Maximum change in acidity was observed in product coloured with synthetic colour (T_8) during three months of storage. The per cent change in acidity observed in T_8 was 15.81, 27.77, 74.07, 92.59, 98.14 and 105.55 respectively at 15, 30, 45, 60, 75

Fig 6. Effect of storage conditions on pH of RTS beverage

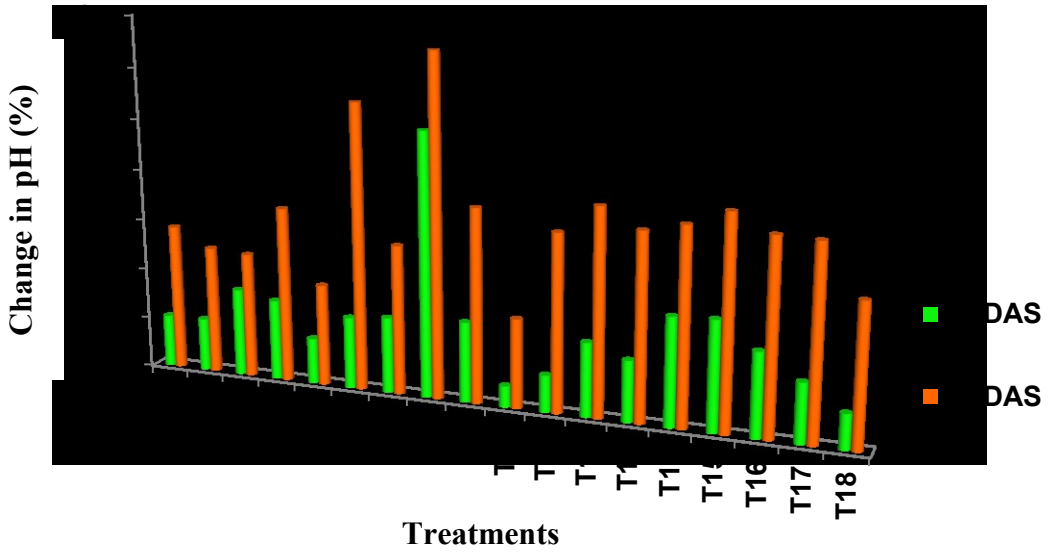
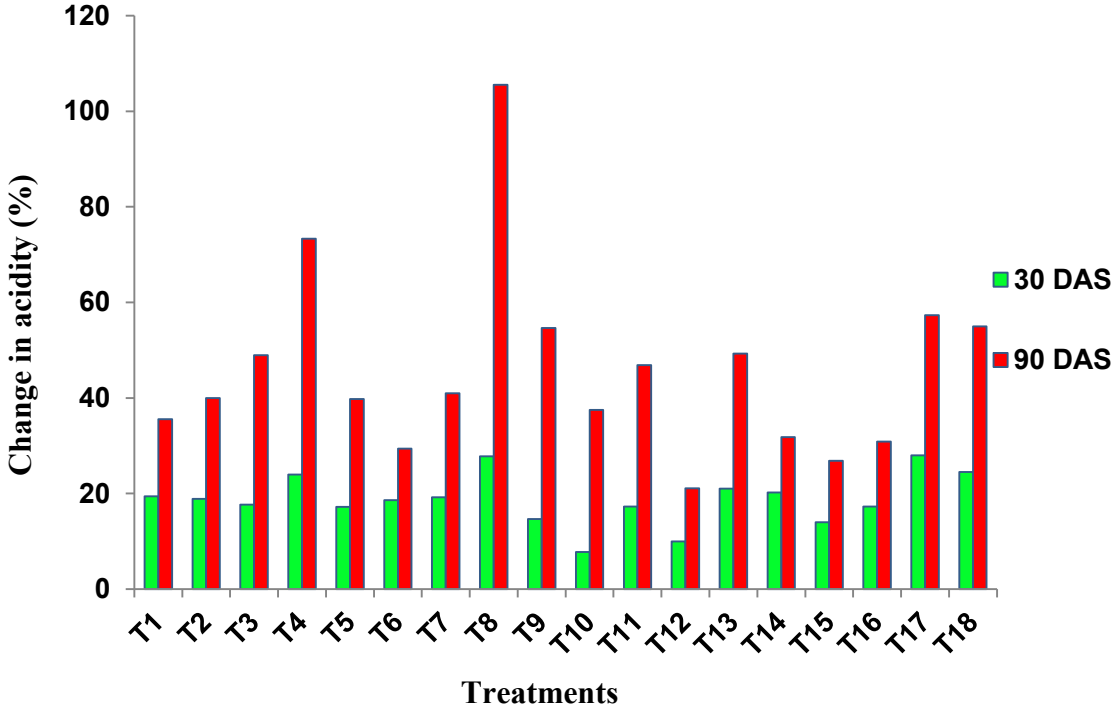


Fig 7. Effect of storage conditions on acidity of RTS beverage



and 90 DAS. Minimum change was recorded in product coloured with anthocyanin pigment and 0.5% citric acid and 0.01% ascorbic acid (T_{12}). The per cent change in acidity observed in T_{12} was 6.66, 10, 14.44, 17.77, 16.66, and 21.11 respectively at 15, 30, 45, 60, 75 and 90 DAS.

Acidity of the RTS beverage stored under ambient conditions was higher as compared to that under refrigerated conditions. The increase in acidity may be attributed to lower pH values and chemical reactions that take place at ambient conditions.

However irrespective of storage conditions maximum change in acidity and pH was observed for RTS beverage prepared with synthetic colour (without citric acid and ascorbic acid)

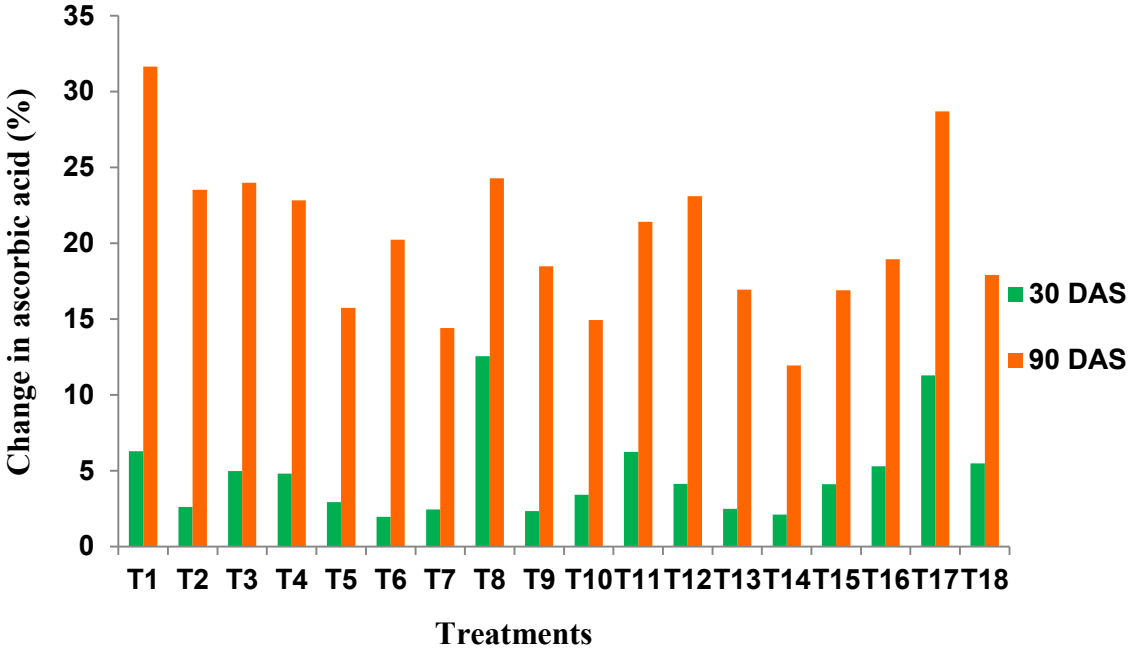
Bhardwaj and Mukherjee, (2011) attributed the increase in acidity during storage due to degradation of sugars. BhavyaSree and Vanajalata (2015) reported that the acidity increased during storage due to release of acids from pulp or juice particles due to autolysis of cells and simultaneous decrease of pH. Similar results have been reported by Islam *et al*. (1996) in mango based beverages, Attri *et al*. (1998) in pear-apricot and plum beverage, Choudhary and Dikshit (2006) in guava RTS beverage and Ilamaran and Amutha (2007) in banana and sapota carbonated beverages.

5.3.3. Effect of storage conditions on ascorbic acid content of RTS beverage

A decline in ascorbic acid content of the RTS beverage was noticed as the storage duration increased.

A minimum change in ascorbic acid was observed in RTS beverage prepared with 0.4% citric acid and 0.02% ascorbic acid and stored at refrigerated condition (T_{14}) throughout the period of study (Fig 8). The per cent change in ascorbic acid

Fig 8. Effect of storage conditions on ascorbic acid of RTS beverage



Ambient conditions

T_1 - AC + 0.3% citric acid (CA) + 0.01% ascorbic acid (AA)

T_2 - AC + 0.4% CA + 0.01% AA

T_3 - AC + 0.5% CA + 0.01% AA

T_4 - AC + 0.3% CA + 0.02% AA

T_5 - AC + 0.4% CA + 0.02% AA

T_6 - AC + 0.5% CA + 0.02% AA

T_7 - AC + 0.5% CA

T_8 - Synthetic colour alone

T_9 - Anthocyanin pigment (AC) alone (control)

Refrigerated conditions

T_{10} - AC + 0.3% citric acid (CA) + 0.01% ascorbic acid (AA)

T_{11} - AC + 0.4% CA + 0.01% AA

T_{12} - AC + 0.5% CA + 0.01% AA

T_{13} - AC + 0.3% CA + 0.02% AA

T_{14} - AC + 0.4% CA + 0.02% AA

T_{15} - AC + 0.5% CA + 0.02% AA

T_{16} - AC + 0.5% CA

T_{17} - Synthetic colour alone

T_{18} - Anthocyanin pigment (AC) alone (control)

observed in T_{14} was 2.05, 6.58, 8.64, 10.69, 11.11 and 11.93 respectively at 15, 30, 45, 60, 75 and 90 DAS.

Maximum change in ascorbic acid was recorded in RTS beverage prepared with 0.3% citric acid and 0.01% ascorbic acid and stored at ambient condition (T_1).

The per cent change in ascorbic acid observed in T_1 was 2.17, 6.28, 26.93, 35.68, 37.19 and 31.63 respectively at 15, 30, 45, 60, 75 and 90 DAS.

Irrespective of storage conditions, significant variation was observed in ascorbic acid content between treatments. RTS beverage stored under ambient conditions had more ascorbic acid content compared to that stored under refrigerated conditions.

Addition of ascorbic acid resulted in degradation of anthocyanin pigment in the RTS beverage stored under ambient condition, whereas there was no change seen in the RTS beverage stored under refrigerated conditions.

Ascorbic acid content decreased significantly at all storage intervals. These losses of ascorbic acid could be attributed to the effect of processing, storage time and exposure to light. **(**Balaji and Prasad, 2014). Similar decreasing trends for ascorbic acid contents in different fruit beverages were also reported by Gomez and Khurdiya (2005) in Kinnow juice blend. Byanna and Gowda (2012) also observed a decreasing trend of ascorbic acid in RTS beverage from sweet orange.

Tiwari and Deen (2015) reported that vitamin C content continuously decreased from the first day (2.38 mg/100g) to the end of storage (1.93 mg/100g) in RTS beverage from bael and aonla. The decrease in vitamin C content might be due to the oxidation of ascorbic acid into dehydro ascorbic acid. The losses of vitamin C in RTS beverage of different fruit based beverages during storage at ambient temperature were also reported in other studies (Tiwari, 2000; Mandal, 2003).

5.3.4. Effect of storage conditions on colour of RTS beverage

Colour of the RTS beverage was Light yellowish pink (19B) initially in all the treatments except T_8 and T_{17} which was vivid red (45A).

RTS beverage stored at refrigerated conditions retained colour throughout the storage period compared to ambient condition where there was a greater loss of the colour. The colour instability can be attributed to the effect of pH and photo oxidation. Under ambient conditions the RTS beverage treated with ascorbic acid and citric acid had greater loss of colour compared to beverages with citric acid alone, but in refrigerated conditions there was no much change in colour. Durge *et al*., (2013) reported retention of anthocyanin up to 18.2% in coloured rice flour by addition of 1% citric acid.

De Rosso and Mercadante, (2007) also reported that there was greater colour fading, indicating the increase of L* and decrease of a* and C* values in acai anthocyanin solutions due to higher level of ascorbic acid.

Anu, (2009) investigated the colour stability of wine and squash coloured with anthocyanin pigment from Malay apple and observed colour fading in these products during storage. Palamidis and Markakis (1975) reported the effect of temperature on the stability of anthocyanin in soft drinks and have observed that increase in the storage temperature greatly accelerated the destruction of pigments.

Oxidative reactions which take place in presence of light also might have accelerated the fading of colour. Khudriya and Anand (1982) opined that low pH exerted a positive influence on the pigment stability of anthocyanin in phalsa juice. They also reported that sugar and light had little effect on the decomposition of anthocyanin. For better colour stability of anthocyanin in food products, modified pH, storage atmosphere and suitable colour stabilizers are to be tried.

5.3.5. Effect of storage on sensory attributes of guava RTS beverage

Guava RTS beverage was organoleptically evaluated by selected panel of judges. Sensory scores followed a declining trend during storage period.

Initially highest mean scores for appearance (7.90), colour (8.00), taste (8.40) and overall acceptability (8.00) were recorded for RTS beverage prepared with 0.3% citric acid and 0.01% ascorbic acid and stored at refrigerated condition (T_{10}) and flavor (7.80) and texture (7.60) in RTS beverage prepared with 0.4% citric acid + 0.01% ascorbic acid and stored at ambient condition (T_2).

Three months after storage highest mean scores for appearance (7.50), colour (7.75), flavor (7.50) and texture (7.37) taste (7.25) and overall acceptability (7.50) were recorded for RTS beverage prepared with 0.3% citric acid and 0.01% ascorbic acid and stored at refrigerated condition (T_{10}). The products stored under refrigerated conditions recorded highest scores in organoleptic evaluation as compared to those kept at ambient conditions.

Satkar *et al*., (2013) reported that the score for overall acceptability of bitter gourd RTS beverage decreased during storage. The RTS drink which was stored at refrigerated temperature was found to be more acceptable after 3 months of storage. Kumar and Manimegalai, (2005) reported that the sensory quality attributes were highly acceptable even after 3 months of storage at refrigeration in whey based papaya fruit juice blended RTS beverage. According to Choudhary and Dikshit, (2006) the organoleptic quality of guava RTS and nectar gradually decreased during the storage.

5.3.6. Effect of storage on pH of mixed fruit jam:

pH of the mixed fruit jam followed decreasing trend during the entire period of storage. It ranged from 4.06 to 4.30 initially and it was 3.46 to 3.92 at the end of storage period. pH of the mixed fruit jam stored under refrigerated condition was

higher compared to ambient conditions during storage. This may be due to lower rate of chemical reactions at refrigerated conditions compared to ambient conditions.

Jam quality parameters such as total phenolics, antioxidant activity, anthocyanins, colour, acidity, soluble solids and texture can be affected during storage (Amakura *et al*., 2000). Kopjar *et al*., (2010) reported that during storage there was slight oscillation of pH values in comparison to samples after preparation in bitter orange and sweet orange jams during storage. However Gogus et *al*., (2000) reported that in orange jam, pH values ranged between 3.5 and 3.7 and there was no considerable change during the storage of the samples.

5.3.7. Effect of storage on colour of mixed fruit jam:

There was no significant change in colour of mixed fruit jam during storage. The colour of the mixed fruit jam initially was light yellow (14D) in T_1 and T_4, vivid red (52A) in T_2 and T_5 and moderate reddish orange (41C) in T_3 and T_6.

At ambient conditions, there was a slight darkening of the mixed fruit jam compared to that stored under refrigerated conditions. This might be due to light reactions under ambient conditions, which have affected the anthocyanin pigment.

Colour of the jam is one of the fundamental attributes influencing the acceptability of the product among consumers (Connor *et al*, 2002). According to Kovacevic *et al*., (2015) there was a loss of anthocyanins, and colour during processing in strawberry purees and jams. The stability is affected by temperature, pH, water activity, presence of carbohydrates and enzymatic activity (Lee and Wrolstad, 2006).

Howard *et al*., (2010) evaluated the colour of jams stored for 6 months and found that the product stored at 4°C maintained higher levels of anthocyanins, and have less colour changes than those stored at 25°C.

5.3.8. Effect of storage on organoleptic quality of mixed fruit jam:

The scores obtained for colour, appearance, flavour, taste and overall acceptability of mixed fruit jam decreased slightly with the increase in storage period.

One month after storage highest mean scores for appearance (7.96) and colour (7.96) were recorded in product with synthetic colour under refrigerated condition (T_2). Highest scores for flavour (7.76) and texture (7.23), odour (7.46) and overall acceptability (7.56) were highest in control under refrigerated condition (T_1) and for taste (7.93) and after taste (7.53) it was highest in T_4 (control under ambient condition)

Three months after storage highest mean score for flavour (7.53), texture (7.6), taste (7.86) and overall acceptability (7.33) was for product prepared with synthetic colour and stored under refrigerated condition. However highest score for colour was for the product prepared with synthetic colour and stored under ambient condition. Comparatively lower scores obtained for mixed fruit jam stored under ambient conditions may be attributed to darkening of the pigment when exposed to light.

The products kept under refrigerated conditions recorded highest scores for flavour, texture, taste and overall acceptability compared to those kept at ambient conditions three months after storage.

According to Shivani *et al*., (2010) colour, appearance, flavour, taste and overall acceptability of jamun jam and chutney decreased significantly with the increase in storage period. Loss of organoleptic quality and appearance of a jam after certain period is common. Temperature plays an important role in inducing certain biochemical changes in the jam which leads to development of off flavour and reduction an organoleptic quality and appearance.

Pathak, (1988) reported that colour degradation during storage may be due to non-enzymatic reaction such as organic acid with sugar or oxidation of phenols which leads to the formation of brown pigments.

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Summary

6. SUMMARY

The project entitled “Extraction and utilization of anthocyanin pigments from jamun (*Syzygium cumini* Skeels.)” was carried out in the Department of Processing Technology, College of Horticulture, Vellanikkara.

The objectives of the study were standardization of method of extraction of anthocyanin pigment and evaluation of pigment stability to pH, light, temperature and storage conditions. The stability of the pigment in two processed products viz., guava RTS beverage and mixed fruit jam was studied under the project.

Jamun fruits were collected from KAU main campus and pigment was extracted by four different extraction methods. Highest content of anthocyanin (61.07mg/100g), recovery per cent (13.75), colour hue (1.30) and colour intensity (1.13) were obtained for acidified solvent extraction method (20% ethanol + 0.5% citric acid). Hence this extraction method was selected for further studies.

The effect of light and storage conditions on pigment stability was evaluated in two types of containers viz., transparent glass bottles and amber coloured glass bottles under ambient and refrigerated (4°-7°C) conditions. Degradation of anthocyanin pigment was higher (74.61%) in transparent bottles stored at ambient conditions (T_4) as compared to that stored under refrigerated conditions. Storage in amber coloured bottles under refrigerated conditions was found to be best for anthocyanin pigment.

The influence of high temperature on the anthocyanin content was studied at 70, 80 and 90^0 C and anthocyanin content was measured at intervals 30 minutes, 45 minutes and one hour of incubation at each temperature. Anthocyanin pigment was found sensitive to high temperature. Degradation of anthocyanin content was higher as the temperature of incubation was increased. Anthocyanin content decreased from an initial value of 61.25mg/100g to 50.24mg/100g one hour of heating at 90^0 C.

The impact of pH on pigment stability was studied at different pH ranging from 2.5 to 7 at room temperature, at 1 hour, 1 day and then subsequently after 2, 3, 4, 5, 6, 13 and 20 days in the different buffered anthocyanin extracts. At pH of 2.5 and 3 the colour was reddish tinge and it changed to a yellowish tinge as the pH was increased from 3 to 7. A decreasing trend was followed in anthocyanin content throughout the study period as the pH increased. Least decrease in anthocyanin content was observed at pH 2.5. At pH above 6 there was drastic reduction in anthocyanin content. The anthocyanin content was found to decrease from an initial value of 61.25mg/100g to 34.28mg/100g, 20 days after incubation at a pH of 7.

Guava RTS beverage was prepared from clarified fruit juice. The effect of antioxidant (ascorbic acid), pH and storage temperature on stability of anthocyanin pigment in RTS beverage was evaluated under this experiment.

The pH of the RTS beverage decreased significantly with advancement in storage period. Minimum changes in pH were observed in RTS beverage prepared with 0.3% citric acid and 0.01% ascorbic acid and stored at refrigerated condition (T_{10}) throughout the period of study. The per cent change in pH observed in T_{10} was 1.69, 2.08, 5.37, 7.96, 7.96 and 8.78 respectively at 15, 30, 45, 60, 75 and 90 DAS. Maximum change in pH was recorded in RTS beverage prepared without additives citric acid and ascorbic acid and stored under ambient conditions (T_8).

Acidity of the RTS beverage was found to increase progressively during storage period. Maximum change in acidity was observed in product coloured with synthetic colour (T_8) during three months of storage. The per cent change in acidity observed in T_8 was 15.81, 27.77, 74.07, 92.59, 98.14 and 105.55 respectively at 15, 30, 45, 60, 75 and 90 DAS. Minimum change was recorded in product coloured with anthocyanin pigment and 0.5% citric acid and 0.01% ascorbic acid (T_{12}). The per cent change in acidity observed in T_{12} was 6.66, 10, 14.44, 17.77, 16.66, and 21.11 respectively at 15, 30, 45, 60, 75 and 90 DAS.

A decreasing trend in the ascorbic acid content of the RTS beverage was observed with increase in duration of storage. Least change in ascorbic acid was observed in RTS beverage prepared with 0.4% citric acid and 0.02% ascorbic acid and stored at refrigerated condition (T_{14}) throughout the period of study. The per cent change in ascorbic acid observed in T_{14} was 2.05, 6.58, 8.64, 10.69, 11.11 and 11.93 respectively at 15, 30, 45, 60, 75 and 90 DAS.

Maximum change in ascorbic acid was recorded in RTS beverage prepared with 0.3% citric acid and 0.01% ascorbic acid and stored at ambient condition (T_1). The per cent change in ascorbic acid observed in T_1 was 2.17, 6.28, 26.93, 35.68, 37.19 and 31.63 respectively at 15, 30, 45, 60, 75 and 90 DAS.

RTS beverage stored under refrigerated conditions retained colour throughout the storage period as compared to ambient condition where there was a greater loss of the colour. Colour of the RTS beverage was light yellowish pink (19B) initially in all the treatments except T_8 and T_{17} which was vivid red (45A). The colour instability can be attributed to the effect of pH and photo oxidation. Under ambient conditions, the RTS beverage treated with both ascorbic acid and citric acid had greater loss of colour compared to beverages with citric acid alone, but in refrigerated conditions there was not much change in colour.

Guava RTS beverage was organoleptically evaluated by selected panel of judges. The products kept under refrigerated conditions recorded highest scores for flavour, texture, taste and overall acceptability compared to those kept at ambient conditions three months after storage.

Pigment stability in mixed fruit jam was also studied by storing the prepared product under ambient and refrigerated conditions for a period of three months.

pH of the mixed fruit jam followed a declining trend during storage, least change in pH was observed in control under refrigerated condition (T_1) during

storage. At the end of storage period greatest change in pH (14.95%) was observed in product coloured with anthocyanin pigment (T_6) and stored under ambient condition.

There was no significant change in the colour of the mixed fruit jam. A slight darkening of the colour was seen in the bottles stored at ambient condition.

Mixed fruit jam was organoleptically evaluated by selected panel of judges. The products kept under refrigerated conditions recorded highest scores for flavor, texture, taste and overall acceptability compared to those kept at ambient conditions three months after storage. Highest scores for colour, appearance, odour, flavor, texture, taste, after taste and overall acceptability were obtained for product coloured with synthetic colour.

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Appendices

APPENDIX – I

Effect of storage conditions on pH of RTS beverage

Treatments		pH					
	Initial	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS
T_1	3.36	3.22	3.18	3.16	3.12	2.96	2.88
T_2	3.2	3.08	3.03	2.88	2.82	2.81	2.80
T_3	3.1	2.93	2.83	2.81	2.80	2.71	2.72
T_4	3.5	3.38	3.22	3.05	2.92	2.96	2.90
T_5	3.11	2.93	2.97	2.90	2.86	2.83	2.83
T_6	3.3	3.15	3.06	3.03	2.98	2.86	2.20
T_7	3.65	3.58	3.37	3.26	3.18	3.17	3.11
T_8	4.3	4.09	3.19	3.09	3.17	3.10	3.10
T_9	3.55	3.49	3.26	3.08	3.05	2.99	2.87
T_{10}	3.35	3.29	3.28	3.17	3.08	3.08	3.01
T_{11}	3.4	3.28	3.27	3.20	3.12	2.95	2.81
T_{12}	3.11	2.92	2.88	2.87	2.75	2.60	2.48
T_{13}	3.32	3.19	3.12	3.02	2.88	2.76	2.71
T_{14}	3.6	3.49	3.21	3.10	3.01	2.95	2.91
T_{15}	3.3	3.17	2.94	2.94	2.78	2.70	2.61
T_{16}	2.9	2.73	2.66	2.50	2.46	2.36	2.35
T_{17}	4.25	4.06	4.00	3.97	3.80	3.61	3.45
T_{18}	3.66	3.54	3.53	3.50	3.22	3.19	3.15
CV%		2.175	2.756	1.907	1.744	2.002	1.911
CD (0.05%)		0.119	0.145	0.097	0.087	0.097	0.090

Two way factor analysis of pH of guava RTS beverage

Treatments	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	Mean
T_1	1.69	2.09	5.37	7.96	7.96	9.95	10.39
T_2	3.64	5.20	9.89	11.77	11.97	12.50	3.32
T_3	5.37	8.60	9.35	9.67	12.36	12.25	3.99
T_4	3.42	7.90	12.85	16.38	15.33	17.14	6.40
T_5	5.68	4.50	6.64	7.82	8.78	8.78	9.03
T_6	4.72	25.73	28.06	26.14	27.90	27.90	11.77
T_7	1.91	7.48	10.68	12.69	12.96	14.61	8.76
T_8	4.54	7.07	8.08	9.59	13.13	33.13	7.51
T_9	2.06	7.98	13.14	14.08	15.77	18.96	9.64
T_{10}	3.96	5.15	5.75	7.14	11.70	14.28	4.44
T_{11}	3.43	3.62	5.88	8.13	13.23	17.35	13.43
T_{12}	6.10	7.28	7.50	11.57	16.39	20.15	13.78
T_{13}	3.81	6.02	8.83	13.25	16.66	18.27	12.21
T_{14}	2.96	10.64	13.79	16.20	18.05	19.16	15.70
T_{15}	3.83	10.80	10.80	15.65	18.18	20.70	16.54
T_{16}	5.86	8.27	13.79	15.17	18.62	18.96	16.35
T_{17}	4.31	5.88	6.43	10.58	14.90	18.82	18.15
T_{18}	3.18	3.46	4.18	11.84	12.84	13.93	18.31
Mean	9.73	15.35	11.64	10.48	8.60	10.78	
CD for comparing treatments (Factor A) - 1.22							
CD for comparing storage (Factor B) - 0.70							
CD for comparing interactions (AXB) - 3.00							

APPENDIX – II

Effect of storage conditions on acidity of RTS beverage

Treatment		Acidity (%)					
	Initial	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS
T_1	0.3	0.30	0.32	0.35	0.37	0.40	0.40
T_2	0.3	0.30	0.35	0.36	0.38	0.41	0.42
T_3	0.32	0.36	0.37	0.43	0.44	0.47	0.47
T_4	0.25	0.27	0.31	0.35	0.36	0.40	0.43
T_5	0.31	0.33	0.36	0.39	0.41	0.42	0.43
T_6	0.34	0.37	0.40	0.41	0.43	0.44	0.44
T_7	0.33	0.37	0.39	0.42	0.44	0.46	0.46
T_8	0.25	0.27	0.32	0.33	0.35	0.38	0.39
T_9	0.25	0.27	0.28	0.32	0.33	0.36	0.38
T_{10}	0.24	0.26	0.28	0.27	0.30	0.31	0.33
T_{11}	0.27	0.29	0.31	0.32	0.35	0.36	0.39
T_{12}	0.3	0.32	0.33	0.34	0.35	0.35	0.36
T_{13}	0.23	0.25	0.27	0.29	0.30	0.30	0.34
T_{14}	0.28	0.30	0.33	0.34	0.35	0.35	0.36
T_{15}	0.31	0.34	0.35	0.37	0.37	0.38	0.39
T_{16}	0.27	0.29	0.31	0.31	0.32	0.35	0.35
T_{17}	0.18	0.20	0.23	0.31	0.34	0.35	0.37
T_{18}	0.2	0.22	0.24	0.26	0.27	0.29	0.31
CV%		4.181	4.091	3.317	3.489	2.379	3.458
CD (0.05%)		0.021	0.022	0.019	0.021	0.015	0.023

Two way factor analysis of acidity of guava RTS beverage

Treatments	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	Mean
T_1	1.11	7.77	16.66	25.55	34.44	35.55	7.28
T_2	1.11	18.88	20.00	27.77	36.66	40.00	9.28
T_3	14.58	17.70	35.41	37.50	47.91	48.95	10.70
T_4	10.66	24.00	41.33	44.00	61.33	73.33	17.36
T_5	6.45	17.20	26.88	33.33	36.55	39.78	18.09
T_6	9.80	18.62	20.58	27.45	31.37	29.41	20.79
T_7	12.12	19.19	27.27	33.33	41.41	41.01	26.81
T_8	14.81	27.77	74.07	92.59	98.14	105.55	43.08
T_9	9.33	14.66	28.00	33.33	45.33	54.66	32.04
T_{10}	9.72	19.44	15.27	25.00	29.16	37.50	32.60
T_{11}	9.87	17.28	20.98	29.63	33.33	46.91	29.84
T_{12}	6.66	10.00	14.44	17.77	16.66	21.11	38.16
T_{13}	8.69	21.01	28.98	30.43	31.88	49.27	41.38
T_{14}	9.52	20.23	23.81	26.19	25.00	31.78	36.76
T_{15}	9.67	13.97	19.35	20.43	23.65	26.88	42.22
T_{16}	9.87	17.28	16.04	20.98	29.63	30.86	44.50
T_{17}	8.00	28.00	34.66	40.00	54.66	57.33	23.44
T_{18}	11.66	24.50	30.00	38.33	45.00	55.00	49.89
Mean	25.87	27.98	27.85	28.63	40.62	23.80	
CD for comparing treatments (Factor A) – 2.95							
CD for comparing storage (Factor B) – 1.70							
CD for comparing interactions (AXB) – 7.23							

APPENDIX – III

Effect of storage conditions on ascorbic acid of RTS beverage

Treatment		(Ascorbic acid mg/100g)					
	Initial	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS
T_1	49	48.00	47.33	46.33	45.83	45.33	33.50
T_2	48.6	47.66	47.33	43.66	40.66	39.83	37.16
T_3	45.6	44.66	43.33	37.00	35.33	34.83	34.66
T_4	46.22	45.00	44.00	43.00	38.66	36.16	35.66
T_5	44.3	43.33	43.00	39.50	39.66	38.16	37.33
T_6	40.12	39.66	39.33	39.16	32.16	32.00	32.00
T_7	36.22	35.66	35.33	31.66	31.83	31.16	31.00
T_8	46.88	45.00	41.00	39.00	36.00	36.00	35.50
T_9	42.2	41.00	41.83	39.83	37.83	37.33	37.16
T_{10}	55.2	54.00	51.73	40.33	35.50	34.66	45.00
T_{11}	48.35	47.33	45.33	40.33	39.33	38.00	38.00
T_{12}	48.33	47.33	46.33	38.66	39.83	37.50	37.16
T_{13}	40.33	39.66	39.33	37.83	36.66	33.83	33.50
T_{14}	40.5	39.66	37.83	37.00	36.16	36.00	35.66
T_{15}	36.5	35.33	35.00	32.00	31.83	31.000	30.33
T_{16}	36.6	35.33	34.66	31.16	30.66	29.83	29.66
T_{17}	40.2	39.00	35.66	32.83	31.83	29.83	28.66
T_{18}	40.2	39.00	38.00	37.33	35.50	34.33	33.00
CV%		2.190	2.372	2.763	2.528	2.002	2.331
CD (0.05%)		1.545	1.629	1.745	1.524	1.171	1.345

Two way factor analysis of ascorbic acid of guava RTS beverage

Treatments	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	Mean
T_1	2.04	3.40	5.44	6.463	7.48	31.63	1.992
T_2	1.92	2.60	10.15	16.32	18.03	23.52	2.453
T_3	2.04	4.97	18.86	22.51	23.61	23.97	2.726
T_4	2.64	4.80	6.96	16.34	21.75	22.83	3.441
T_5	2.18	2.93	10.83	10.45	13.84	15.72	5.413
T_6	1.13	1.96	2.37	19.82	20.23	20.23	5.861
T_7	1.52	2.44	12.57	12.11	13.95	14.41	9.101
T_8	4.01	12.54	16.80	23.20	23.20	24.27	16.419
T_9	2.84	0.86	5.60	10.34	11.53	14.92	11.247
T_{10}	2.17	6.28	26.93	35.68	37.19	18.47	15.329
T_{11}	2.10	6.23	16.58	18.64	21.40	21.40	19.594
T_{12}	2.06	4.13	19.99	17.58	22.40	23.09	13.543
T_{13}	1.64	2.47	6.19	9.08	16.10	16.93	17.496
T_{14}	2.05	6.58	8.64	10.70	11.11	11.93	21.615
T_{15}	3.19	4.11	12.32	12.78	15.06	16.89	16.856
T_{16}	3.46	5.28	14.84	16.21	18.48	18.94	22.988
T_{17}	2.98	11.27	18.32	20.81	25.78	28.68	18.938
T_{18}	2.98	5.47	7.13	11.69	14.59	17.91	18.553
Mean	9.21	12.64	11.30	15.41	13.90	11.93	
CD for comparing treatments (Factor A) – 1.39							
CD for comparing storage (Factor B) – 0.80							
CD for comparing interactions (AXB) – 3.41							

APPENDIX – IV

Score card for organoleptic evaluation of mixed fruit jam

Name of the judge:

Date:

Score							
Characteristics	**Refrigerated condition**				**Ambient condition**		
	T_1	**T_2**	**T_3**		**T_4**	**T_5**	**T_6**
Appearance							
Colour							
Flavour							
Texture/consistency							
Odour							
Taste							
After taste							
Overall acceptability							

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Signature:

EXTRACTION AND UTILIZATION OF ANTHOCYANIN PIGMENTS FROM JAMUN (*Syzygium cumini* Skeels.)

By

Naresh N.
(2013-12-121)

ABSTRACT OF THE THESIS

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Master of Science in Horticulture

Faculty of Agriculture
Kerala Agricultural University

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ABSTRACT

The project entitled “Extraction and utilization of anthocyanin pigments from jamun (*Syzygium cumini* Skeels.)” was undertaken at the Department of Processing Technology, College of Horticulture, Vellanikkara during 2013-15. The objectives of the study were standardization of method of extraction of anthocyanin pigment and evaluation of pigment stability to pH, light, temperature, storage conditions and in processed products.

Method for extraction of anthocyanin pigments from jamun was standardized. Among the four extraction methods compared, highest content of anthocyanin (61.07mg/100g), recovery per cent (13.75), colour hue (1.30) and colour intensity (1.13) were obtained for acidified solvent extraction method (20% ethanol + 0.5% citric acid).

The effect of light and storage conditions on pigment stability was evaluated. Storage in amber coloured bottles under refrigerated conditions was found to be best for anthocyanin pigment due to lesser degradation of the pigment compared to that stored under ambient conditions.

The effect of temperature on the anthocyanin content was studied at 70, 80 and 90^0 C and anthocyanin content was measured at intervals 30 minutes, 45 minutes and one hour of incubation at each temperature. Anthocyanin content decreased from an initial value of 61.25mg/100g to 50.24mg/100g one hour of heating at 90^0 C.

The effect of pH on stability of anthocyanin pigment was studied at different pH ranging from 2.5 to 7 and incubating it for different intervals from one hour to 20 days at room temperature. Anthocyanin content was found to decrease with increase in pH and the least decrease was found at pH 2.5. Anthocyanin content was found to decrease from an initial value of 61.25mg/100g to 34.28mg/100g 20 days after incubation at a pH of 7.

Least changes in pH were observed in RTS beverage prepared with 0.3% citric acid and 0.01% ascorbic acid and stored at refrigerated condition (T_{10}). Acidity of the RTS beverage followed an increasing trend during storage, greatest change in acidity was observed in T_8 (product coloured with synthetic colour). Ascorbic acid was found to decrease with increase in duration of storage, minimum changes in ascorbic acid was observed in RTS beverage prepared with 0.4% citric acid and 0.02% ascorbic acid and stored at refrigerated condition (T_{14}).

RTS beverage stored under refrigerated conditions retained colour throughout the storage period as compared to ambient condition where there was a greater loss of the colour. Addition of ascorbic acid was found to hasten the colour degradation of the beverage under ambient conditions, but under refrigerated conditions there was not much change in colour.

Pigment stability in mixed fruit jam was also studied by storing the prepared product under ambient and refrigerated conditions for a period of three months. pH of the mixed fruit jam followed a declining trend during storage, least change in pH was observed in T_1 (control under refrigerated condition) during storage. There was no significant change in the colour of the mixed fruit jam stored under refrigerated conditions. A slight darkening of the colour was seen in the bottles stored at ambient condition.

Guava RTS beverage and mixed fruit jam was organoleptically evaluated by selected panel of judges. The products kept under refrigerated conditions recorded highest scores for flavour, texture, taste and overall acceptability compared to those kept at ambient conditions three months after storage.