REVIEW PAPER

Anthocyanins: Chemistry, Extraction, Stability, Significance and Application as a Biocolour

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Abstract

Colour is a vital constituent of food and its preferences are influenced by the geographical, sociological and cultural aspects of the populations. The food industry have a vast array of synthetic colours made available in the late 1800s and are used very extensively in various food products. In the last twenty years however, consumers have become increasingly aware of the ingredients in their foods and as such they require foods to be as 'natural' as possible. Natural colours, extracted from fruits, vegetables, seed, roots and microorganisms are often called "biocolours" due to their biological origin. These colors are chlorophyll, carotene, lycopene, anthocyanins, flavonoids and anthoxanthins. To identify the active health-promoting ingredients, focus is being made on the properties of the flavonoids, a large class of phenolic compounds that is abundant in fruits and vegetables. Anthocyanins (Greek *anthos*, flower and Greek *kyanose*, blue) are the largest group of water-soluble pigments in the plant kingdom known as flavonoids and a larger group of compounds known as polyphenols. Anthocyanins are responsible for the red, purple and blue hues in fruits, vegetables, flowers and grains. Different types of anthocyanin found in naturally are cyanidin, delphinidin, peonidin, malvidine and palargonidin. These are responsible for the attractive colour of Black currant nectar and they have been hypothesized as important antioxidant. Anthocyanin rich mixtures and extracts (not purified compounds) have been used historically to treat? An anthocyanin pigment is composed of an aglycone (an anthocyanidin) esterified to one or more sugars, and have four different structures, which are in equilibrium with one another but the relative amount of these structures,are varied and dependent on the pH and anthocyanin structure, Waste from industrial processes, such as wine or juice production is an excellent source of anthocyanin pigment with potential as a food colourant. An alcohol i.e. methanol or ethanol at different concentrations in combination with an acid i.e. hydrochloric acid or acetic acid or citric acid can be used for the extraction of anthocyanin. But the natural colours have lesser stability than synthetic or artificial colours. Due to highly reactive nature of anthocyanins, they degrade or react with other constituents in the medium, to form colourless or brown coloured compounds. The intensity and stability of the anthocyanin pigments is dependent on different factors. Various methods have been used to evaluate the degradation of anthocyanin i.e. pH differential method and substractive methods. Applications of anthocyanin extracts include coloration of acid fruit preparations, jams and preserves. However, relatively little is known about absorption of anthocyanin compounds after consumption in our body and of the mechanisms by which they exert their beneficial health effects. Increased knowledge of their bioavailability and therapeutic will result in better adoption of anthocyanin-based products as functional foods.

Keywords: Anthocyanins, bioavailability, therapeutic effects, Natural colours, biocolour, chemistry

Colour is the first characteristic perceived by our senses and is indispensable for rapid identification and ultimate acceptance of a product. Every type of food is associated with certain type of colour and addition of colour gives food an attractive and appetizing appearance, and enhances its acceptability

(Joshi *et al*. 2003). At the same time, Colour of a food is not a label flavour type, but provides information on quality and condition of the food also. In many food systems, colour acts as an indicator of condition of food such as fitness to eat, particularly microbiological quality. Colouring of foods has been an age-old practice, since invention of synthetic colourants and have found a widespread acceptability due to their good stability and colouring ability (Pattnaik *et al.* 1999). A number of foods are processed in one or the form before reaching consumer's table and thus, may lose their colour or develop some undesired colours during processing. The processors, thus are forced to replace the colour of the products, which could otherwise be colourless and unappealing during processing. With increasing public concern about the safety of synthetic colourants, natural pigments extracts are assuming greater importance, especially the, synthetic colourants or artificial dyes which have become the prime targets of this phenomenon because doubts have been raised in the public eyes regarding their safety.

As a result of constant research, new forms of natural colours for varied applications have been produced. Custom blending of natural colours offers new colours which have been developed through creation of compatible combined natural pigments. Further, extraction and processing of natural colours are eco-friendly and non-hazardous process (Chaudhari *et al*. 2004).

Natural colours have been able to perform better than synthetic colours, the classic example being the colouring of *chhedder* cheese with annatto. However, prior to the use of natural colours in a food formulation, consideration should be given as to the nature of the food system to be coloured and, in which phase the colour is desired. If the food system has both a water and an oil phase, the process to be used in production of the food product, the package to be used for the food product, the storage conditions to which the packaged product would be subjected and finally, nature of the colour to be used should also be considered.

Pigments which have since long been considered as potential colourants, are the natural substances in cells and tissues of plants, microorganisms and animals that impart colour to them (Masoodi *et al.* 2005). Natural colours, extracted from fruits, vegetables, seed roots and microorganisms are often called "biocolours" due to their biological origin. These have been proved to be safe and edible. Naturally occurring food colours are chlorophyll, carotene, lycopene, anthocyanins, flavonoids and anthoxanthins, (Downham and Collins, 2000; Joshi *et al*. 2012). In an endeavor to identify the active health-promoting ingredients, focus on the properties of the flavonoids has also been made. Most prominent among the flavonoids are the anthocyanins, which are of particular interest to the food industry due to their ability to impart vibrant colours and to enhance the health promoting qualities of the food product.

Anthocyanins (Greek *anthos*, flower and Greek *kyanose*, blue) are the largest group of water-soluble pigments in the plant kingdom and belongs to the family of compounds known as flavonoids which are part of an even larger group of compounds known as polyphenols (Mazza, 2007). Theseare responsible for the red, purple and blue hues in fruits, vegetables,flowers and grains, (Schemske and Bradshaw, 1999). Currently, anthocyanins in blue and purple corn are being used for the production of naturally coloured blue tortillas (Sugiyama Chemical Institute, 1977). Radish and potato extracts have colour characteristics very similar to those of Allura red (a red dye used in food applications) (Giusti and Wrolstad, 2003) and therefore have the potential for incorporation as food colourants. Incorporating anthocyanins as food colourants is not only valuable for improving overall appearance but also is very beneficial to our health. In this review, such issues connected with anthocyanins have been described.

SOURCES OF ANTHOCYANINS

Colourants naturally occurring in food are either produced by extraction from natural sources or reproduced by chemical synthesis (Bauernfeind, 1981). These are generally extracted from fruits,

vegetable, seeds, roots, insects and microorganisms (Joshi *et al.* 2003; Mahajan *et al.* 2000). Origin and some of the examples of biocolour are listed in Table 1.

of iarrhoea, delphinidin, peonidin, petunidin, pelargonidin and malvidin (Clifford, 2000). The types of anthocyanins from different sources are listed in Table 2.

Table 1: Origin and examples of biocolour

Origin	Example
• Natural colours obtained by direct use of approved pigmented vegetables and fruit products.	Dehydrated beet powder, Dried paprika powder, Dried turmeric powder, Dried saffron powder, Vegetable juice and fruit juice
• Natural colours obtained by extraction and concentration of colours derived from approved plant materials.	Anthocyanin: extract of berries, grapes, plum, vegetables; Betalaines: extract of vegetables; Carotenoids: annatto extract
• Natural colours obtained by extraction and concentration of colours derived from approved animal materials.	Laccania acid pigments from *Laccifera lacca*, Cocchineal extract and carmine from *Coccus cacti*
• Natural colours obtained by extraction and concentration of colours derived from approved microorganism	*Monascus* pigment from mold *Monascus purpurcus*, Phycocianin pigment from *Spirulina* spp., Pigments from *Rhodotorula, Yarrowia lipolytica, Cryptococcus sp.* and *Phaffia rhodozyma*
• Natural colours derived by heat processing of food grade carbohydrates	Caramel colour
• Natural pigment found in nature but chemically synthesized	β-Carotene, β-apo-8 carotenal and canthaxanthin
• Miscellaneous natural colourant	Lycopene pigment, Haemoglobin pigment, Kokam pigment, Phycocyanin, Riboflavin

Source: Mahajan *et al.* 2000 ; Downham and Collins, 2000; Romero-Cascales *et al.* 2005; Chaudhuri, 2004; Zaidi *et al.* 2008; Joshi *et al.* 2012, Joshi and Preema, 2014.

The most common naturally occurring anthocyanins are the 3-O-glycosides or 3, 5-di-O-glycosides

Table 2: Type of anthocyanin from different source

Source	Major anthocyanin
Grapes	Malvidin glycosides
Blue morning glory flower (*Ipomoea tricolour.* Cv. Heavenly Blue)	Peonidin 3-(dicaffeylsophoroside)-5 glucoside
Sunflower hulls	Malonated glycosides of cyanidin
Black raspberry	Cyanidin 3-diglucoside
Plum	Cyanidin 3-glucoside
Peach	Cyanidin 3-glucoside
Strawberry	Pelargonidin 3-monoglucoside
Eggplant	Delphinidin-3-p-coumaroylrutinoside-5-glycoside
Red radish	Pelargonidin 3-sophoroside-5- glucoside

Source: Bridle and Timberlake, 1997; Mazza and Gao, 1994, 1996; Casal *et al.,* 2002; Guistand Wrolstad, 1996; Chiang and Wrolstad, 2005, Joshi and Preema, 2014.

ANTHOCYANIN AS BIOCOLOURS

People have developed increasing interest in food colourants of natural origin. Natural colourants are generally extracted from fruits, vegetables, seeds, roots and microorganisms. Out of these, anthocyanins are present in most of higher plants and provide a wide range of colours from orange to blue (Newsome, 1986; Francis, 1989). Concord grapes, grape skin extract (enocyanin), red cabbage, and fruit and vegetable juices (e.g. elderberries, blackcurrant, chokeberries etc.) are used as commercial sources of natural colourants (Francis, 1989; Murai and Wilkins, 1990; Skrede *et al.* 1992; La bell, 1993; Jackman & Smith, 1996). The total anthocyanins content of th different fruits are given in Table 3.

Table 3: Total anthocyanin content in different fruits

Fruits	Total anthocyanin (mg / 100 g fresh tissue)
Plum	44.1 – 231.29
Peach	7.64 – 50.1
Blackberries	70.3 – 201
Sour cherry	
Visciola Ninno	27.8 + 0.010
Amarena Mattarello	80.4 + 0.100
Visciola Sannicandro	74.6 + 0.050
Strawberry	
Hood strawberries	0.377 mg/ g fresh tissue
Tioga strawberries	0.276 mg/ g fresh tissue
Blueberry	99.9
Grapes	
Monastrell – B	776.5 + 58.9 mg/ Kg fresh tissue
Monastrell – J	1110 + 13.4 mg/ Kg fresh tissue
Cabernet iarrhoea	751.9 + 140.2 mg/ Kg fresh tissue
Syrah	914.9 + 28.8 mg/ Kg fresh tissue
Merlot	582.1 + 27.2 mg/ Kg fresh tissue

Source: Casals *et al.* 2002; Chiang *et al.* 2005; Federica *et al.* 2004; Abers and Wrolstad, 1979; Skrede *et al.* 2000; Tian *et al.* 2005, Joshi and Preema, 2014.

STRUCTURE OF ANTHOCYANIN

An anthocyanin pigment is composed of an aglycone (an anthocyanidin), esterified to one or more sugars (Fig. 1). Free aglycones rarely occur in food except possibly as trace components of degradation reactions. Only 5 sugars have been found as portions of anthocyanin molecules and in order of relative abundances as glucose, rhamnose, galactose, xylose and arabinose. Anthocyanins may also be 'acylated', which adds a third component to the molecule. One or more molecules of p-coumaric, ferulic, caffeic, malonic, vanillic or acetic acids can be esterified to the sugar molecule.

Anthocyanins have four different structures, which are in equilibrium with one another butthe relative amount of these structures in equilibrium are variable and dependent on the pH and anthocyanin structure, that is why some anthocyanins are more stable than others (Mazza and Minitiati, 1993; Bridle and Timberlake, 1997).
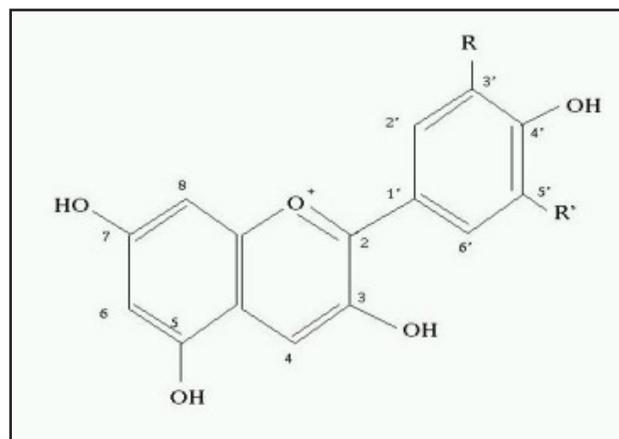

Fig. 1: A structure of an aglycone

The anthocyanins can be divided into a number of classes, depending upon the number of sugar molecules. The monosides have only one sugar residue, almost always in the 3rd position. The biosides contains 2 sugars, with either both on the 3rd position or one each on the 3rd and 5th positions, or rarely in the 3 and 7 positions. The triosides contain three sugars, usually at 2nd or the 3rd position with one on the 5th position very often. Three in a branched structure or a linear structure on the 3rd position or rarely with 2 on the 3rd position and one on the 7th position.

Table 4: Substitution on the flavylium cation structure to produce the major anthocyanidins

Anthocyanidins	Substituent on carbon number		
	$3^/$	$5^/$	$4^/$
Pelargonidin (II)	H	OH	H
Cyanidin (III)	OH	OH	H
Delphinidin (IV)	OH	OH	OH
Peonidin (V)	Ome	OH	H
Petunidin (VI)	Ome	OH	OH
Malvidin (VII)	Ome	OH	Ome

No anthocyanins with 4 sugar residue have been reported, so far but there is some evidence that

they exist. One anthocyanin with 5 sugar molecules and 4 acyl components has also been documented (Yoshimata, 1977). Major anthocyanidins are produced by the substitution of the flavylium cation structure (Table 4). Approximately, 140 anthocyanins have been reported in literature (Pattnaik *et al.* 1999).

EXTRACTION OF ANTHOCYANIN FROM WASTE OF DIFFERENT FRUITS, VEGETABLES AND FLOWERS

Waste from fruits and vegetable industrial processes, such as wine or juice production is an excellent source of anthocyanin pigment which could possibly be utilized as a food colourant. An overview of the techniques employed in extracting the anthocyanin has been presented in Table 5. Absolute ethanol was used to facilitate subsequent concentration steps. Citric acid chelate techniques employed in extracting the anthocyanin have also been presented in table 5 flow sheet of anthocyanin extraction, using various treatments are shown in Fig 2.
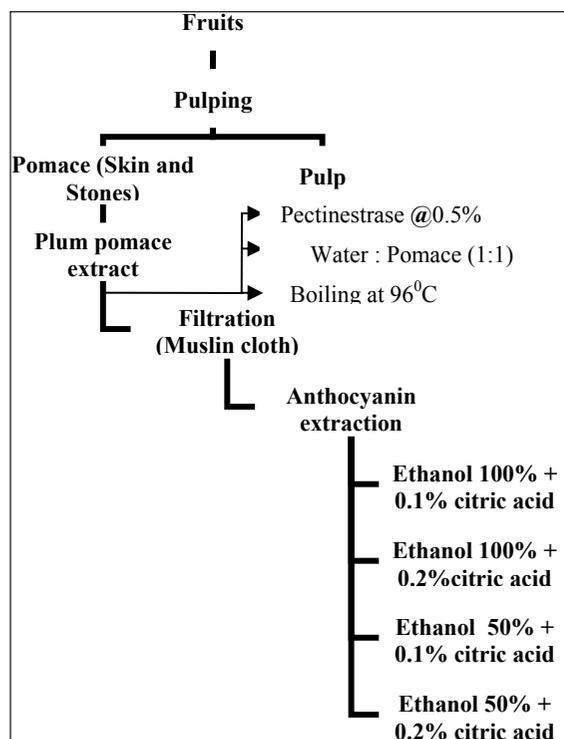

Fig. 2: Flowsheet of anthocyanin extraction from plum
Source: Preema, 2012.

Metals may have an added protective effect throughout the processing of the spray dried powder. It is less corrosive than HCl and would still act to stabilize the anthocyanin structure in the cationic form by maintaining a low acid pH. The citric acid can be added to the single strength extract such as that a 10 to 1 concentrate would have a pH of 3-3.2. This pH is chosen because the dried powders are used in colouring low acid food products.

While using methanol as a solvent, the methanol in the pigment solution can be removed by distillation and the resulting aqueous solution absorbed on an Amberlite C G-50 resin. The resin absorbs the anthocyanin and many of the impurities can be rinsed off the column with water. Preema (2008) reported that while using ethanol (50%) as solvent along with citric acid (0.2%), the anthocyanin content of plum var Santa Rosa was found to be higher (325 mg/100 ml) than those reported by Cevallos-Cavals *et al.* (2006).

However, Xavier *et al.* (2008) extracted anthocyanins from red cabbage. Batch studies under several extraction conditions have indicated that acetic acid in aqueous solution (10% V/V) was the best solvent, used in the proportion of 0.25 g of red cabbage mL^{-1}. At this condition, column assays were carried out and showed that the pH, recirculation and mass of red cabbage had statistically significant effects, where the optimum operation conditions found for the process was pH 2.3, recirculation volume of the solvent was 0.83 L and mass of red cabbage was 50g. The schematic view of the column extraction system is shown in Fig. 3.

Solvent extraction time, size of ground hulls, pH of extracting solvent, hull/ solvent ratio and concentration of SO_2 in water were found to be significant factors affecting yield of extracted anthocyanins. Ethanol-acetic acid-water was found more effective extractant than acetic acid. Although these extraction methods are commonly used but these methods are lacking in one or the other way, so new methods should be developed for better results.

Table 5: Comparison of anthocyanin extraction techniques from different waste

Type of waste	Extraction Techniques	Source
Fruits		
Grape	Absolute ethanol (100%) with 0.1% HCl	Heidari *et al.* 2006
Grape	Methanol with citric acid (0.01%)	Clydesdale *et al.* 1978
Concord grape	Labsolute ethanol (100%) with 0.01% citric acid	Main *et al.* 1978
Vitis vinifera var Grenache noir	Methanol 1-M with HCl (99:1 v/v).	Sarni-Manchado *et al.* 1996
Blue grapes	Acidified methanol (75ml 3M HCl + 425 ml methanol)	Thakur and Arya, 1989
Concord grape	Methanol acidified with 0.01 % citric acid	Calvi and Francis, 1978
Blueberries	MeOH/ Formic acid/ Water (70/2/28)	Gao and Mazza, 1994
Bilberry, rabbiteye, blueberry and blackcurrant	90% ethanol with 0.1% H_2SO_4	Jun – ichiro Nakajima *et al.* 2004
	Filtrates collected after centrifugation were applied to non-ionic polymeric absorbent. Then, elution with acidified ethanol (0.05% citric acid)	
Blackberries	Methanol acidified with 0.1 % HCl	Julin *et al.* 1992
Blackberries	Liquid nitrogen powder + acetone: water (70/30 v/v) + acetone: chloroform at 1:2 v/v	Chiang and Wrolstad, 2005; Rodriguez-Soana and Wrolstad, 2001
Black raspberry	Dichloromethane-methanol (1:1 v/v)	Tian *et al.* 2005; Huang *et al.* 2002
Tart cherry (*Prunus cerasus)*	Homogenized with water at 10,000g for 10 min at 4^{0}C	Amitabh *et al.*1992
Strawberry	Polyvinyl-polypyrrolidone (PVPP) resins with water were used for isolating anthocyanin. Then, anthocyanin were extracted from the resin by methanol with 0.1% HCl	Skrede *et al.* 1992; Wrolstad and Putnam, 1969
Plum (*Prunus salicinia* Lind)	50% ethanol with 0.2% citric acid in 10:1 concentration	Joshi and Preema, 2014
Cranberry	Methanol with 0.03% HCl at a 5:1 solvent:pomace ratio	Jackman *et al.* 1996
Lychee	Acidified ethanol (1.5 N HCl: 95% ethanol; 15:85 v/v)	Lee and Wicker, 1991
Fig (*Ficus carica* L.)	Acetone and 0.1N NH_4OH (9:1 v/v). Re-extraction with1:1 (v/v) acetone +diethyl ether	Antoine *et al.* 1976
Vegetables		
Sweet potato	1% HCl in water	Bassa and Francis, 1987
Red radish cv. Fuego and Red fleshed potato tuber	1.Chemical purification : Acetone/ Chloroform 2. Juice processing	Guisti and Wrolstad, 1996a; Rodriguez-Soana *et al.* 1999
Red radish	Samples added with liquid nitrogen powder then added to 2L acetone/ Kg of skins. Futher re-extraction with acetone 30:70 (v/v). Filtrates combined with chloroform (1:2 acetone: chloroform v/v)	Hong and Wrolstad, 1990; Monica and Ronald, 1996
Pomogranate	Reverse-phase high-performance liquid chromatography and Sephadex LH-20 chromatography.	Zhang *et al*. 2011

Flower

|		
• *Tradescantia pallida*	0.1% HCl with water. Acid extract was purified using a cation exchange resin. Then, pigment were eluted from column with 0.1% HCl in methanol	Zulin *et al.* 1992
• Purple sunflower seeds	3 solvents system used i.e. (50:1:49) ethanol-acetic acid-water(EAW), 0.01M acetic acid (AAc) or water containing SO$_2$	Gao and Mazza,1996
• Zebrina	0.1% HCl with water	Teh and Francis, 1988
• Roselle (*Hisbiscus sabdariffa)*	Water extraction	Esselen and Sammy, 1973
• Rose	Ethanol 96% and HCl 0.1 M as a solvent with ratio 4:1 in 24 hours	Wahyuningsih *et al.* 2017
• *Delonix regia*	Acidified-water-based media and assisted-ultrasound or stirring extraction procedures.	*Adjé et al.* 2010
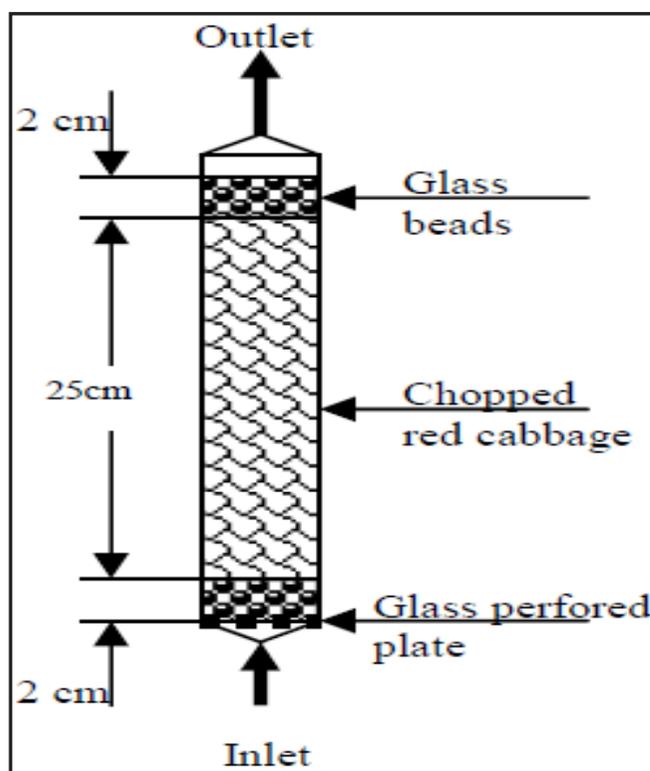
Fig. 3: Schematic view of the column extraction system

STABILITY OF ANTHOCYANIN

It is well established that the natural colours have lesser stability than synthetic or artificial colour and because of the highly reactive nature of anthocyanins, they degrade or react with other constituents in the medium, to form colourless or brown coloured compounds. The presence of an ox-onium ion adjacent to carbon 2 makes the anthocyanin particularly susceptible to nucleophilic attack by such compounds as SO$_2$, ascorbic acid, hydrogen peroxide and even water. As written earlier also anthocyanins have been reported to be very unstable (Shrikhande, 1976). Although anthocyanins are less stable in various environmental conditions, they include varieties of colours which make them an attractive colouring agents in food industries (Markakis, 1982; Francis, 1989). Anthocyanins have four different structures, which are in equilibrium and the relative amount of these structures in equilibrium are varied and dependent on the pH and anthocyanin structure, and that make some anthocyanins are more stable than others depending on their molecular structure (Mazza and Minitiati, 1993). The example of this is the Malvidin glycosides, the major anthocyanin in grapes, which due to dimethyloxylation of the molecules are more stable than other anthocyanin (Bridle and Timberlake, 1997). Another factor in increasing the stability of the anthocyanin has been found to be the co-pigmentation (Francis, 1989; Malien-Aubert *et al.* 2001). Investigation on comparison of drying of the anthocyanin concentrates from plum var Santa Rosa by spray drying and freeze drying also reported acceptable stability while using 30°B maltodextrin as its carrier. (Preema and Joshi, 2012a). Anthocyanins were intensively investigated for beverage uses in model system which contained about 13-15%

sucrose and or glucose (Chiriboga and Francis, 1973; Palamidis and Markakis, 1975; Calvi and Francis, 1978; Clydesdale *et al.*, 1978; Bassa and Francis, 1987; Teh and Francis, 1988). Evaluation of the stability of plum anthocyanin powder in squash based and RTS based model solution gave encouraging result where the stability were maintained till 3 months (Preema and Joshi, 2012b and Preema and Joshi, 2012c).

Similar to this loss of colour in strawberries has been observed after thawing of frozen fruits and during processing and storage of strawberry products i.e. juice, concentrates, wine, preserves and purees (Wrolstad *et al.* 1970; Abess and Wrolstad, 1979; Sistrunk and Cash, 1970). It has been found that the anthocyanin disappeared as monomeric compounds and was transformed into polymeric form (Markaris, 1982; Baranac *et al.* 1996; Francia – Aricha *et al.* 1997; Johnston and Morris, 1997). This transformation resulted in a colour change to a more brownish shade. Knowledge of the changes that the anthocyanin pigments undergo with processing is important with respect to their role in colour quality. An Acylated anthocyanin pigment shows greater stability during processing and storage (Bassa and Francis, 1987; Hong and Wrolstad, 1990; Murai and Wilkins, 1990; Rommel *et al.* 1992; Shi *et. al.* 1992). These acylated pigment also respond differently to pH change than do non-acylated ones (Price and Wrolstad, 1995). The presence of acylating groups has been believed to protect the oxonium ion from hydration, thereby preventing the formation of hemiketal (pseudobase) or chalcone forms (Brouillard, 1981; Francis, 1989).

Factors effecting stability of anthocyanin

The intensity and stability of the anthocyanin pigments is dependent on different factors (Mazza and Minitiati, 1993; Francis, 1989) as discussed here:

pH: The molecule of anthocyanin exists in a state equilibrium between four primary structures. At low pH (2 and below), the red form of the anthocyanin is favoured but as the pH is increased, the red form converts itself into blue form and a non-coloured form. Therefore, the hue of an anthocyanin solution becomes bluer and less intense as pH increases.

While the blue form revert back to the red form, the non-coloured form of the anthocyanin can either revert back to the red form or can undergo an irreversible hydrolysis of the flavilium portion of the anthocyanin that prevents reversion to the red form. This mechanism can be examplified by citing the case of that flower that changes its colour from bud formation till flowering. The major anthocyanin in blue morning glory flower is stable in a neutral aqueous solution.

Wahyuningsih *et al.* (2017), have also reported similar result. So, anthocyanin can be used as food colorant depending upon the pH of the product Lukton *et. al.* (1956) studied the rate of anthocyanin breakdown in pure solution and in strawberry juice in nitrogen and oxygen. In both the cases, it was much faster and pH dependent in oxygen whereas in nitrogen the pH had little effect. The rate of oxidation seems to be directly dependent upon the percentage of pigment existing in the form of the pseudobase and inversely proportional to the amount of flavylium cation. Meschter (1953) had found previously that the rate of anthocyanin breakdown was dependent on pH and that lowering the pH resulted in a greater degree of stabilization. Sistrunk and Cash (1970) attempted to improve the stability of anthocyanins in strawberry puree and reported that lowering the pH contributed far more to colour stability than did any other method. Wrolstad *et al.* (1970) stated that pH was a far better measure of the acid component than was the total acidity.

Bathochromic shifts were observed at low pH. At pH values below about 1 for anthocyanin in aqueous solution, addition of co pigment could reduce a bathochromic shift, but no increase in absorbance took place Infact, a small hypochromic shifts did occur (Mohammad *et al.* 2006). Hypochromic shifts were however, observed at higher pH. A higher chroma at pH 7 for red sweet potato compared to the purple corn was due to the presence of a purple-blue quinonoidal base with higher absorbance. Generally, this form is less stable and the absorbance decreases dramatically after a few minutes (Brouillard, 1982) (Fig. 4).
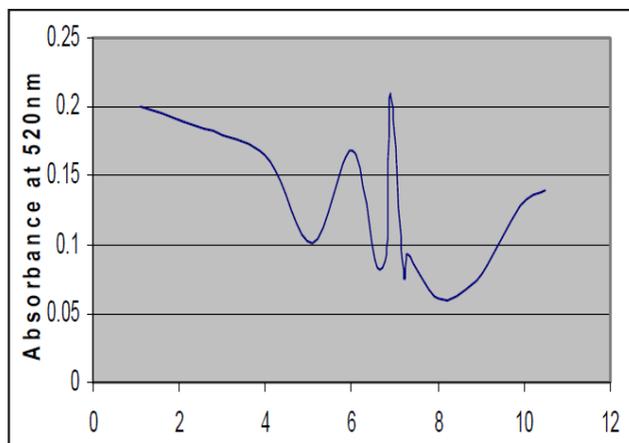
Fig. 4: Stability of Anthocyanins samples taken after 20 days storage at various pH

Source: Brouillard, 1982.

Ascorbic acid: Beattie *et al.* (1943) in studies on processed fruit juices reported a marked decreased in the level of ascorbic acid in the presence of air, which was accompanied by changes in colour of the product. This is due to the interaction between the ascorbic acid and the anthocyanin pigments present in the fruit juice. Further studie Sonheimer and Kertesz (1953) indicated that the maximal loss of anthocyanins occurred under the conditions favourable to ascorbic acid oxidation. The formation of hydrogen peroxide from ascorbic acid oxidation has been demonstrated earlier Sonheimer and Kertesz (1952) studied its effect on strawberry anthocyanins. Pratt *et al.* (1954) also confirmed the participation of ascorbic acid in anthocyanin degradation and implicated riboflavin in this reaction as well. The effect of ascorbic acid on the stability of the strawberry anthocyanin Pelargonidin 3-monoglucoside was later investigated by Markakis *et al.* (1957) in the presence and absence of oxygen. Contradictory results were however reported by Sistrunk and Cash (1968) as they demonstrated that at lower temperatures ascorbic acid may in fact exert a beneficial effect but for a short period of time.

Sugars: Tinsley and Bockian (1960) using model systems found that by increasing the sugar concentration, the amount of extractable strawberry anthocyanins decreased due partly by the breakdown products i.e. furfural, 5-hydroxymethylfurfural,

levulinic acid and formic acid. Arabinose, levulose and sorbose appeared to have a far greater degradative effect on the pigments than either maltose or sorbitol. Furfural, 5-hydroxymethylfurfural being the sugar degradative product appear too condensed with anthocyanins to form a brown coloured-complex. The minimal loss of pigment was however, found in the presence of glucose, whereas the maximal loss occurred in the presence of furfural. Hydroxymethylfurfural did not however, produce as large a reduction in pigment as furfural.

Metal complexing effects: Anthocyanins containing *o*-dihydroxyl groupings form metal complexs, which result in a shift from red to stable blue and violet pigmentations, i.e. cyanidin 3-glucoside (Jurd and Asen, 1966; Asen *et al.* 1969; Wrolstad *et al.* 1970). Pink discolouration during the commercial canning of processed pears was shown to be due to the decomposition of the leucoanthoccyanins present in the fresh fruits to cyanidin (Luh *et al.* 1960; Nortji, 1964). Chandler and Clegg (1970) however, suggested that the pink discolouration was due to the formation of an insoluble tin-cyanidin complex while Lueck (1970) reported that the iron derived from the must undergo oxidation to the ferric state before the discolouration occurs. The formation of the water-insoluble iron rutin complex appeared to be dependent on the relative amounts of tin and iron derived from can since both are of direct competition with the rutin molecule. The tin reacted with the rutin molecule and form a bright yellow water soluble complex which was still fairly acceptable in appearance. It is far more stable than the iron rutin complex. So, it was found that the addition of EDTA to the brine used in canning of asparagus also prevented discolouration by chelating with any metal present.

Temperature: Prolonged heat exposure causes browning in some of colours, particularly anthocyanins, grape skin extract, fruits and vegetables juices and destroy others. Preema (2008) observed that higher the storage temperature greater was the degradation of anthocyanin. This has been reported earlier in case of stability of grapes anthocyanin that increasing the storage temperature accelerated

greatly the pigment degradation in the beverage (Palamidis and Markakis, 1975).

In darkness, it has been found that at 38°C after 135 days of storage only 23% of original amount of hot water extracted pigment was left in the beverage, while at 3.5°C under the same condition of storage, 92% of the pigment was retained. Maccarone *et al.* (1985) have studied the stability of anthocyanin in red orange juice at 15°, 25° and 30° C during a 15 day period and found that the increase in temperature accelerates the destruction of anthocyanins.

Jenshi *et al.* (2011) have demonstrated that increasing time and temperature resulted in changes in anthocyanin and the copigmentation complex which resulted an increase in the visible spectrum (hyperchromic effect) and an increase in max (bathochromic shift) in the main peaks (Mohammad *et al.* 2006). These changes are shown in Fig. 5.
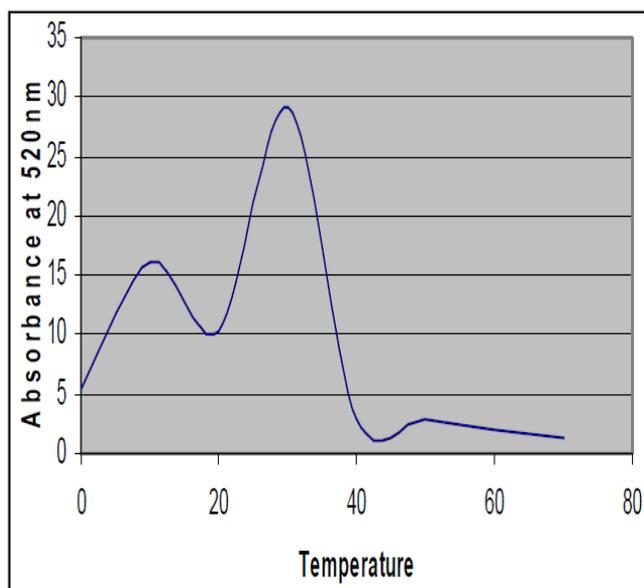

Fig. 5: Stability of anthocyanins in various temperature (light)

Source: Jenshi *et al.,* 2011.

Light: Palamidis and Markakis (1975) had also studied the role of light on the stability of anthocyanin in grapes juice and showed that exposure of the pigments to light accelerate their destruction. It was shown that after placing the juice sample containing anthocyanins in dark for 135 days at 20 °C, almost 30% of the pigments were destroyed but placing the same sample at the same temperature and for same period of time in the presence of light destroyed more than 50% of total pigments.

Jenshi *et al.* (2011) also recorded the effect of eight different temperatures 0, 10, 20, 30, 40,50, 60 and 70°C on level of anthocyanin extracted from *Musa acuminata* bract during 5 days. Anthocyanins were measured in separate instances. Out of these, the anthocyanin at 40°C, 50°C, 60°C, and 70°C. There was an increase in absorbance of *Musa acuminata* bract anthocyanin which was stable at temperature 10°C, 20°C and 30°C (Fig. 6). Timberlake (1989) suggested that light increases the flavylium cation construction,but in the absence of light the amount of chalcone in the extract containing anthocyanin was higher than its flavyliumcation.
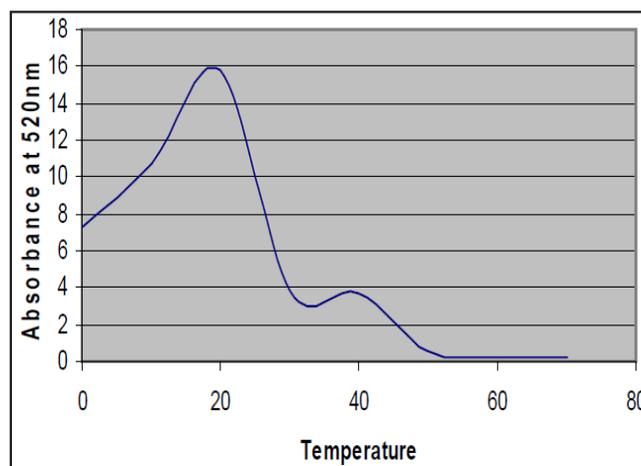

Fig. 6: Stability of anthocyanins in various temperature (dark)

Source: Jenshi *et al.,* 2011.

Ready-to-serve base model solutions were used for evaluation of the stability of plum anthocyanin powder for 3 months interval. Results obtained revealed that in dark conditions the rate of colour change was lesser than Day and UV light. The decrease in red colour was also observed during the storage interval of 3 months. Slight decrease in the 'a' value during the different storage conditions and storage interval (Preema, 2008).

Enzyme: The decolourization of anthocyanins by glucosidases (anthocyanase) was reported by Huang (1955, 1956). An enzyme isolated from *Aspergillus niger* exert a decolourizing effect on pigment extracts obtained from berry juice. Wagenknecht *et al.* (1960) later suggested that anthocyanase was in part responsible for the loss of red colour associated with scald in sour cherries. Peroxidases have also been implemented with the decolourization of anthocyanins. Grommeck and Markakis (1964) investigated the effect of peroxidase on the stability of anthocyanins isolated from red tart cherries and strawberry. Since the enzymes capable of decolourizing anthocyanins are present in many fruits and vegetables, the possibility of inactivating them and thereby, stabilizing these pigments was investigated.

MEASUREMENT OF ANTHOCYANINS

Oxidation of phenolic compounds is the maincause of browning in fruits (Macheix, *et al.* 1990). Enzymatic browning is mainly associated with PPO, which is able to act on phenolic compounds in the presence of oxygen (Nicolas *et al.* 1994). Polyphenol oxidase and its substrates varie and change markedly in fruits and vegetables (Mayer and Harel, 1991). Underhill and Critchley (1994) have suggested that changes in anthocyanin is one of the causes of browning. However, degradation of anthocyanins is the result of coupled oxidation in the presence of other phenolics (Kader *et al.* 1998). Cyanidin-3-glucoside declined with storage or browning, which implies that anthocyanins contribute to the browning possibly through the coupled oxidation.

EVALUATION OF ANTHOCYANINS

Degradation index

The analysis of the total anthocyanin in juice or other products stored for an appreciable length of time introduces problems which prevents the use of the method developed for cranberries (Fuleki, 1967). During storage, a considerable amount of brownish degradation products accumulate which have an absorption maximum around 415nm (Ponting *et. al.* 1960), though they also absorb to some extent at 510 mm. This could introduce an error if a simple direct total anthocyanin determination method, similar to that developed for cranberry was used. Frequently a simple direct total anthocyanin determination method cannot be applied because of interference by chlorophyll or its degradation products formed either from sugar-amino acid reaction (Dickinson and Gawler, 1956), or from the degradation of anthocyanin. In such cases, indirect methods are used to determine the total anthocyanin content.

Methods

pH differential method: This method was developed by Sondheimer and Kertesz (1948) and was based on the fact that the absorptivity of anthocyanin is markedly dependent on pH. Differential pH measurements was used between solutions of pH 2 and 3.4 to determine the concentration of anthocyanin in strawberry products. A solution of congo red was used as the standard of colour intensity. Swain and Hills (1959) suggested the use of different measurements between solutions of pH 1.0 and 0.5.

Substractive methods: This method is based on optical density (OD) measurements taken before and after destroying the colour due to anthocyanin in the plants extracts or removal of the degradation products before measuring the concentration of anthocyanin.

Dickinson and Gawler (1956) bleached the colour due to anthocyanin with sodium sulfite while Swain and Hills (1959) destroyed the anthocyanin by oxidation with hydrogen peroxide. In both the cases, pure anthocyanin was used to established the standard curve. It was shown that the reagent used had no effect on the interfering background material. In another attempt, Lempka *et al.* (1966) developed a total anthocyanin determination method for stored juices based on removal of the degradation products on an ion exchange column prior to the absorption measurement. Ribereau-Gayon (1964) compared the pH differential method (difference between pH 0.6

and 3.5) with the substractive methods using sodium bisulfite on redwines of different age and both the methods gave comparable results, but the sulfite bleaching method always gave higher values (10-25 mg/L). Differential absoption of anthocyanins has been utilized for the calculation of an index which is indicative of the proportion of degraded anthocyanin in the sample and is quite useful when the original anthocyanin content is not known and a measure of the state of anthocyanin degradation is needed.

Clydesdale et al. (1978) found that although chemical analysis showed 50% degradation of pigments, visual evaluation was still quite high at these degradation levels. It was concluded that grapes anthocyanin pigments in a spray dried form (Main et al. 1978) have potential albeit limited, use in dry packed food products.

MEDICINAL/THERAPEUTIC VALUE OF ANTHOCYANIN

Anthocyanins used to be incorporated in the human diet many centuries ago. They were components of the traditional herbal medicines used also by North American Indians, the Europeans, and the Chinese. The average intake of dietary flavonoids is estimated at about 23 mg/day for Holland (Hertog et al. 1993) and 650 mg/day for USA (Kühnau, 1976, Middleton et al. 2000). The daily intake of anthocyanins in humans has been estimated at 180-215 mg/d in USA (Kühnau, 1976). This value is considerably higher than the intake of other flavonoids such as flavones and flavonols in the Dutch diet (23 mg/d, measured as aglycones) (Hertog et al. 1993). Major sources of anthocyanins are blueberries, cherries, raspberries, strawberries, black currants, purple grapes and red wine. Servings of 100 g of berries can provide up to 500 mg of anthocyanins (Mazza and Miniati, 1993). Anthocyanin rich mixtures and extracts (though not purified compounds) have been used historically to treat many conditions as diverse as hypertension, pyrexia, liver disorder, dysentery and diarrhoea, urinary problems including kidney stones and urinary tract infections, and the common cold. They have been purported to yield improvements to vision

and blood circulation. Recent studies using purified anthocyanins or anthocyanin-rich extracts on in vitro experimental systems have confirmed the potential potency of these pigments. Demonstrable benefits includes protection against liver injuries; significant reduction of blood pressure, improvement of eyesight, strong anti-inflamatory, anti-cancer and antimicrobial activities, inhibition of mutation caused by mutagens from cooked food and suppression of proliferation of human cancer cells (Oliveira et al. 2016).

Antioxidant properties of anthocyanin

Oxidative damage in the human body plays an important causative role in disease initiation and progression (Jacob and Burri, 1996; Kelly, 1998). Damage from free radicals and reactive oxygen species has been linked to some neurode generative disorders (Floyd, 1999; Youdim and Joseph, 2001) and cancers (Goodwin and Brodwick, 1995), and oxidation of low-density lipoproteinis; a major factor in the promotion of coronary heart disease (CHD) and atherosclerosis (Frankel et al. 1993; Steinberg, 1997). Diets high in fruits and vegetables and low in cholesterol and fats are inversely correlated with the incidence of CHD and cancer (Hertog et al. 1993; Hertog et al. 1995; Knekt et al. 1996).

Natural antioxidants from fruits and vegetables provide a measure of protection that slows the process of oxidativedamage (Jacob and Burri, 1996). Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many fruits and vegetables (Luo et al. 2002; Vinson et al. 1999).

Anthocyanins contribute greatly to the antioxidant properties of certain colourful foods, such as grapes and cranberries (Wang et al. 1997). Glycosylation and hydroxylation of the anthocyanidin backbone affects antioxidant activity (Wang et al. 1997). The antioxidant activity of aqueous plant extracts has not been extensively studied, due to the presence of water-soluble antioxidant vitamins and sugars that may mask the activity of polyphenols. The polarity and complexity of water extracts can make it difficult

to isolate pure components (Degenhardt *et al*. 2000). Yet the aqueous fruit extracts often contain potent polyphenolic antioxidants, such as anthocyanins and tannins (Wang *et al*. 1997).

Anthocyanins are potent antioxidant superior to classical antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoulene (BHT), and α-tocopherol (Wang *et al.* 1997, Fukumoto and Mazza, 2000). Glycosylation of an anthocyanin decreases radical scavenger activity compared with theaglycone, as it reduces the ability of the anthocyanin radical to delocalize electrons.

Accordingly, Fukumoto and Mazza (2000) reported an increased antioxidant activity with increase in the hydroxyl groups and decreased antioxidant activity with glycosylation of anthocyanidins. The antioxidant activity (scavenging free radicals,metal chelation, protein binding) of anthocyanins including the protection of LDL against oxidation,has been demonstrated in a number of different *in vitro* systems (Teissedre *et al*. 1996, Aviram and Fuhrman, 2002). Burdulis *et al*. (2009) revealed that the strongest antioxidant activity is possesed by blueberry cultivar 'Berkeleyi'.

Antimicrobial properties of anthocyanin

Antimicrobial berry compounds, especially dietary flavonoids, may have important applications in the future as natural antimicrobial agents for the food industry as well as for the applications (Puupponen *et al*. 2005). Among different berries and berry phenolics, cranberry, cloudberry, raspberry, strawberry and bilberry especially possess considerable antimicrobial effects against, e.g. *Salmonella* and *Staphylococcus* (Puupponen *et al*. 2005). Several mechanisms of action in the growth inhibition of bacteria are involved, such as destabilization of cytoplasmic membrane, permeabilization of plasma membrane, inhibition of extracellular microbial enzymes, direct actions on microbial metabolism and deprivation of the substrates required for microbial growth. Antimicrobial activity of berries may also be related to anti adherence of bacteria to epithelial cells, which is a prerequisite for colonization and infection of many

pathogens. It was found that myricetin inhibited the growth of all lactic acid bacteria derived from the human gastrointestinal tract flora but did not affect the *Salmonella* strain (Puupponen *et al.* 2001). Extracts from common Finnish berries inhibited the growth of Gram-negative, but not Gram-positive bacteria. Other authors reported that there was no correlation between Gram-positive or Gram-negative bacterial status and susceptibility to the berries (Cavanagh *et al*. 2003).

Life Style of Diseases

The diverse nature of physiological activities, the consumption of anthocyanin of bacteria playing a significant role in preventing lifestyle-related disease such as cancer, diabetes and cardiovascular and neurological diseases (Izabela and Wei, 2004). They also exhibit antioxidant activities and therefore, may contribute to the prevention of heart disease (Harborne and William, 2000; Ames *et al.* 1993; Ames, 1983; Hou, 2003; Bagchi *et al.* 2004). Anthocyanin, have been used globally as a medicine or a source of health food/dietary supplement. The amounts and distribution of anthocyanin in the berries however, differ depending on their plant species, cultivation conditions and producing districts. Consequently, the antioxidant activity may be different among various berry extracts, in particular, the berry anthocyanin extracts in the commercial market (Jun *et al.* 2004)

NUTRITIVE VALUE OF ANTHOCYANIN

Vision

The beneficial health effect that anthocyanins have on vision was one of the first reported health properties (Ghosh and Konishi, 2007). Theidea that bilberry can be used to enhance night vision arose from reports of the British Royal Air Force aviators eating bilberry jam to improve their night vision during World War II (Muth *et al*. 2000). The role of anthocyanins on vision has also been demonstrated in a few animal studies. In a recent study by Kalt *et al.* (2008) using a pig model, the distribution of anthocyaninsin tissues such as the liver, eye and brain tissue was

investigated. The results suggested that anthocyanins can be accumulated in tissues, even beyond the blood-brain barrier. Anthocyanins from blackcurrant have also been examined for their effects on night vision and asthenopia (eyestrain). In contrast to bilberry that contains 15 anthocyanins, black currants only contain 4 anthocyanins: delphinidin-3-glucoside; delphinidin-3-rutinoside; cyanidin-3-glucosideand cyanidin-3-rutinoside. Nakaishi *et al.* (2000) report that an oral intake of 12.5, 20 or 50 mg of blackcurrant anthocyanins was significant to decrease the dark adaptation threshold in a dose-dependant manner. This finding strongly suggests that blackcurrant anthocyanins are absorbed and display physiological activities. However, the reports on the effects of anthocyanins on vision are conflicting. The results discussed *in vitro*, animal and human studies demonstrate that oral intake of anthocyanins or anthocyanin-rich extracts may have potentialas a drug for treating opthalmological diseases such asmyopia and glaucoma. However, more extensive research using animal and human intervention trials is necessary to further support and validate the data to support the described ocular health benefits.

Diabetes

It is suggested that, a diet low in fat and high in fruits and vegetables, especially rich in polyphenols may reduce the risk of obesity and type-2 diabetes, a condition associated with insulin resistance. Insulin resistance is a disorder in which insulin inadequately stimulates glucose transport in skeletal muscle and fat and inadequately suppresses hepatic glucose production (Ghosh and Konishi, 2007). Cancer compounds, including drugs and naturally occurring components have been identified as potential chemopreventive agents and out of these, only the compounds capable of inducing differentiation or apoptosis of cancer cells are the primary cancer chemopreventitive and/or chemotherapeutic agents (Fimognari *et al*. 2004). Liu *et al.* (2002) demonstrated that the proliferation of human hepatocellular liver carcinoma is inhibited in a dose dependant manner by raspberries. However, the individual compounds responsible for inhibition of the tumour

cells were not identified. In an earlier elderly cohort study, those who consumed strawberries less than once/week had an odds ratio of 0.3 for developing cancer at any site compared to subjects who did not consume high berry consumption (Colditz *et al*. 1985). Other epidemiological studies have linked coloured fruits and vegetables to a lower incidence of human breast cancer (Almendingen *et al*. 2004) and colourectal polyp recurrence(Adlercreutz, 1998). Despite the data suggesting that anthocyanin containing fruits and vegetables can help reduce and delay the onset of different types of cancers (liver, leukemia, colourectal, skin and breast cancer), further studies are necessary to demonstrate the effectiveness of their clinical development in orderto be used in chemopreventive interventions. Studies should focus on both the specific anthocyanidin(s) and/or anthocyanin(s) worthy of development as well as on the tissue types that might be particularly susceptible to cancer prevention by anthocyanins. In a latter study by Liu *et al.* (2005), they investigated the role of black raspberry extracts as an angiogenesis-inhibiting agent. The epidemiological data is not abundant.

APPLICATION OF ANTHOCYANINS AS FOOD COLOURANTS

Generally for maintenance of good manufacturing practices, level of anthocyanin is used as an indicator to evaluate the quality of coloured food (Boyles and Wrolstad, 1993). Anthocyanin profiles have been used to determine the quality of fruit jams. From anthocyanin profile, it can be easily detected that labeled blackcherry jam which is prepared from common red cherries is real or not (Chattopadhyay *et al*. 2008). Besides, adulteration of blackberry jam with strawberries can also be detected efficiently by the analysis of pelargonid in andcyanidin-3-glucoside content (Viguera *et al*. 1997). Additional Good availability, a low price and high yield crops whose pigments possess an acceptable tinctorial strength are also valued (Stintzing and Carle, 2004).

A major drawback of anthocyanins as food colourants is their decrease in tinctorial strength in low acid

media. However, research in the development of anthocyanin containing food colourants has identified glucosylation and acylation to greatly improve the stability of the anthocyanin to pH changes, heat treatment and light exposure (Stintzing and Carle, 2004). Vegetable sources such as radish, purple sweet potato, black carrot, red-fleshed potato or red cabbage have been shown to provide a higher percentage of acylated anthocyanins than fruits which reflect a higher tinctorial strength of the respective extracts at food pH (Stintzing and Carle, 2004). Of these, radishes and red potatoes in particular have the potential to be used as an alternative for Federal Food Drug and Cosmetic Red No. 40 (Allura red). For example, colour measurements showed that acylated pelargonid in derivatives extracted from red radishes imparted a red colour to maraschino cherries close to that of Allura red at pH 3.5. The colour dilution assay developed by Hofmann (1998) is an efficient way of ranking the tinctorial power of isolated pigments in wine, fruit and vegetable extracts, as good anthocaynin source. It has been found as high ratio of monoacylated structures increasing colour retention at food pH as well as small amounts of non-anthocyanic phenolics which are known to oxidize easily (Stintzing *et al*. 2002). Red wine has been shown to be an exceptional source for food colouring. Its co-pigments are reduced to form stable complexes during fermentation and generated intermediate products further react to yield colour stable anthocyanin derivatives (Boulton, 2001).

Red rice is commonly used as a food colourant in China (Yoshinaga, 1986). Red rice is approved by the Chinese Ministry of Health as a modern food additive to increase the colour and delicacy of meat, fish and soybean products a part of the Chinese diet (Ma *et al*. 2000). Successful food applications have also been reported for red cabbage and radish extracts, made only possible after the development of suitable procedures to remove or drastically reduce the concentration of aroma and flavour compounds (Giusti and Wrolstad, 2003). Other applications of anthocyanin extracts include colouration of acid fruit preparations, jams and preserves. It is highly dependent on the nature and quality of the fruit (fresh, frozen or sulphited) and whether proteins are present. For example, extracts containing other phenolics or oliogomeric pigments above a certain level that cannot be used to colour jellies as they form precipitates with gelatine (Bridle and Timberlake, 1996).

In confectionary sugar, grape extract (0.4% w/w) produces a clear ruby red colour in boiled sweets, which can be adjusted with colourants from other fruit sources. Giusti and Wrolstad (2003) investigated the viability of acylated anthocyanins from red radish, red cabbage, black carrot and grape skin extract to colour dairy products such as yogurt and sour cream having pH levels around 4.2-4.5. The radish and carrot alone or in combination could provide a desirable red hue for dairy applications at concentrations as low as 5 mg monomeric anthocyanin/100 g sample. Since the shelf-life of these tested dairy products are only a few weeks under refrigeration, the stability of anthocyanin extracts would not be affected making them viable alternatives. With increasing pH value, discolouration occurs, but if the product being coloured contains components capable of acting as co-pigments, colour could be retained and also light-stabilised to a certain extent (Bridle and Timberlake, 1996). Therefore, the use of acylated anthocyanins with improved colour and stability to heat, light and pH could hold a promise for incorporating anthocyanins as natural food colours.

It is predicated that in the future, the production and addition of anthocyanins as natural food colourants will steadily increase, following the current trend of getting away from synthetic colours (Horbowicz *et al*. 2008). Regulatory policies concerning the use of anthocyanin based colourants differ considerably from country to country. Many countries have started the approval of colourant usage to defined food products only, and do not permit their use in such commodities as milk and fruit juice. The European Union, Australia, Canada, Cyprus, Finland, Japan, New Zealand, Norway, South Africa, Sweden, Switzerland, and the USA restrict the anthocyanin usage to defined food products (Wrolstad, 2004).

Countries that do have general approval for food use of anthocyanin colourants are Chile, Columbia, Iran, Israel, South Korea, Malta, Peru, Saudi Arabia and the United Arab Emirates. In the European Union, all anthocyanin-derived colourants are recognized as natural colourants under classification E 13. In the U.S., 4 of the 26 colourants that are exempt from certification and approved for food use are anthocyanin derived: grape skin extract, grape colour extract, fruit juice, and vegetable juice (Wrolstad, 2004). In addition to grapes, the more common fruit and vegetable sources include red cabbage, blackcurrants, radishes, elderberries, chokeberries, blackberries, black raspberries, and black currants. Blue wheat could also be a promising source for the production of anthocyanin-based colourants (Abdel-Aal *et al.*, 2008).

MARKETING POTENTIAL

A large global market exists for food colourants of about US $ 5 billion annually of which synthetic colours enjoy a share of $ 4 billion, while only about $ 1 billion is expended for natural food colourants. However, there is an annual growth rate of 4% for natural food colourants compared to 2 to 3% for synthetic colourants. The potential market for astaxanthin pigments is approximately $ 200 million per year. Based on current production statistics, it can be stated that more efficient means to produce natural material will cost 20 to 30% less than the synthetic material (Kishore *et al.*, 2004).

In Japan and European countries, the demand for synthetic colours has reduced. However, in the United States and much of the rest of the world, demand for synthetic colours continues to grow annually. The current popularity of natural colourants is basically due to; the public distrust of food additives in general and the perceived solution of safety with food colourants (Kishor *et al.*, 2004).

FUTURE STRATEGIES AND CONCLUSION

The earth is bestowed with plants containing a wide range of healthful and attractive pigments. But, worldwide, 70% of all the plants have not been

investigated at all, and the chemical composition of only 0.5% has been exhaustively studied (Wissgott and Bortlik, 1996). Despite centuries of interest in natural pigments, our knowledge of their sources from plants, distribution, availability and properties is limited. The increasing market demand for dyes and the dwindling number of dye-yielding plants forced the emergence of several synthetic dyes like aniline and coal-tar. However, low colour value and longer time make the cost of dyeing with natural dyes considerably higher than, synthetic dyes with the and so novel plant pigments must be searched for in unprospected land or in the sea but their proper collection, documentation, assessment and characterization, cultivation confronted with the various drawbacks of commercial natural food colourants (instability to light, heat or adverse pH).

Thus, to make the biocolours popular, new sources of plant pigments need to be made available in sufficient quantities for industrial extraction, large-scale cultivation, harvesting and storage facilities and new ways of formulating existing pigments, improvement of existing sources through breeding and selection of high yielding strains and application of biotechnological tools including plant cell and tissue cultures, genetic engineering and other modern techniques are required to improve the quality and quantity of dye production and to enhance their stability during processing and storing. Rapid advancements in food technology and analysis have allowed for efficient extraction, processing and identification of anthocyanin compounds from various fruits, vegetables and grains to be increasingly incorporated into the food and beverage industry as food colourants.

To sum up further improved understanding of these processes could enable the development of new food products, both fresh and manufactured, with greater therapeutic efficacy. Currently, relatively little is known about absorption of anthocyanin compounds after consumption and of the mechanisms by which they exert their beneficial health effects. Increased knowledge of their bioavailability and therapeutic effects will result in better adoption of anthocyanin-

based products as functional foods. Currently, only a small percentage of anthocyanin-containing fruits, vegetables and grains are being integrated into the food and beverage industry. Thus, greater publicity of the health benefits of anthocyanins should increase consumption of these products and thus, their development and use as food colourant.

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