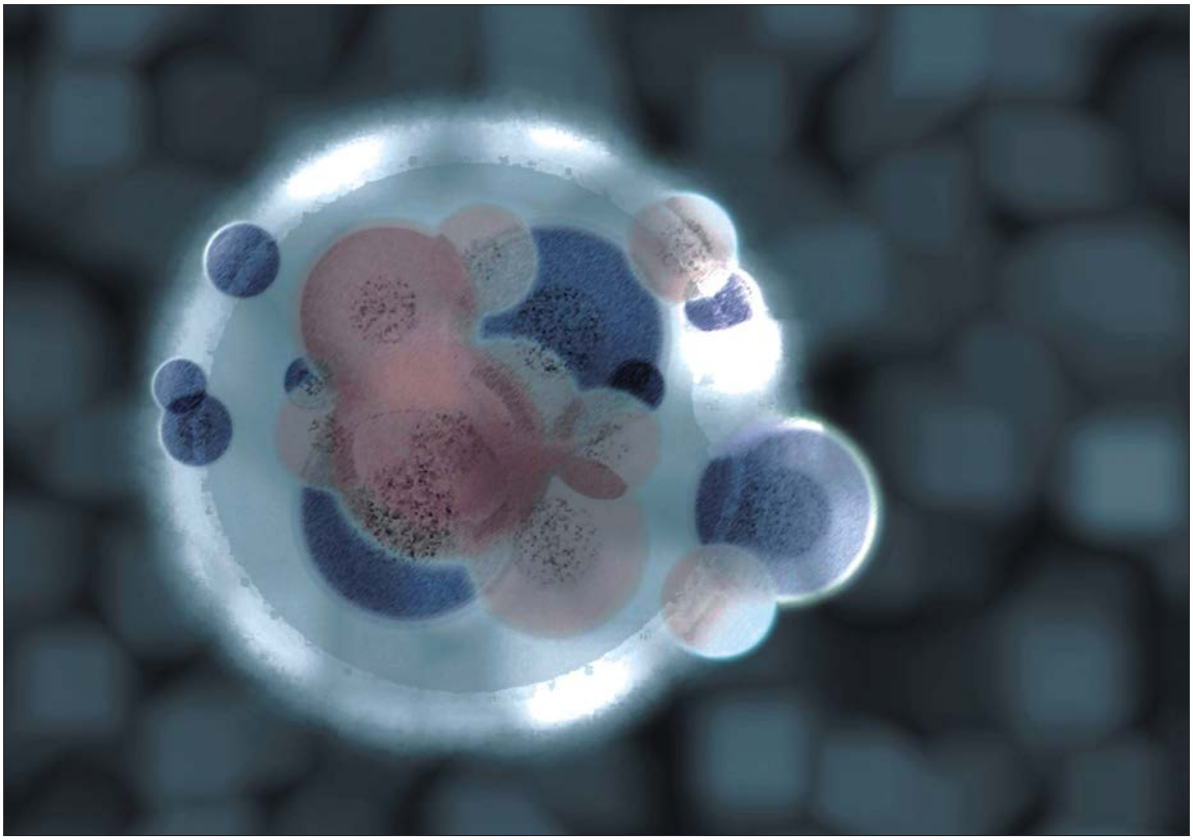

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Effect of Sugar Treatment on Stability of Anthocyanin Pigments in Berries

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Abstract: Anthocyanins are natural pigments widely distributed in nature. Anthocyanin color molecules are a subclass of flavonoids. They are responsible for the reds, purples and blues in many flowers, fruits and vegetables. Fruits and berries are the most sample sources of anthocyanins in nature. Berries and fruits are an important part of the Finnish diet. In many researches the positive effect of fruit and berry intake on human health has been reported. Anthocyanins are considered to contribute to the healthiness of fruits and berries for their antioxidant, anti-carcinogenic, anti-inflammatory and anti-angiogenic properties for example. Anthocyanins can also improve the nutritional value of processed foods by preventing oxidation of lipids and proteins in the food products. However, the stability of anthocyanins becomes most significant also in this case, as in the case of color quality. In this study the anthocyanin pigment was extracted from the three different berries (*Morus nigra* L., *Morus alba* var. *nigra* and *Fragaria* L.). Using the soaking and wetting in ethanol (1% acidified). The extracted anthocyanin pigments then were exposed to three different concentration of sugar (sucrose) (20, 40 and 60%). Three groups of anthocyanin solutions keeping in darkness and refrigerator for 63 days and per 3 week, the quantity of anthocyanin absorbance recorded in 520 nm. In this study, according to statistical analysis, primary concentration (20%) of sugar (sucrose) has protective effect on Anthocyanins, but in higher concentration this effect is decreased.

Key words: Anthocyanin, sugar, degradation, berries, stability

INTRODUCTION

Anthocyanins, a large group of water soluble pigments, are responsible for red to purple and red to blue colors of fruits and vegetables, commonly used in acidic solutions as a red pigment in soft drinks, jams, confectionary and bakery products, have been identified to possess strong chemo preventive activities such as anti-mutagencity, anti-hypertension, anti-oxidative potential and reduction of liver injury (Yoshimoto *et al*., 2001). It is well recognized that diets rich in fruits and vegetables afford protective effect against the development of chronic disease such as cardiovascular disease and some cancers (Block, 1992). Grapes and berries are the chief dietary sources of anthocyanins. (Rechkemmer and Pool-Zobel, 1996).

The recent interest in the field of anthocyanin chemistry has been generated by restriction and limitation of the use of synthetic dyes as food ingredients. Because of low toxicity of anthocyanins (Timberlake and Henry 1986), they have a high potential as a food colorant as the substitute of synthetic red dyes. Studies concerned with anthocyanin production using plant tissue cultures have therefore become very important. Pigment recovery from the fresh materials involves such limitations as variability and seasonal availability of raw materials, fresh material losses and pigment degradation caused by storage and the extraction process (Masayuki Nakamura *et al*., 1999).

Anthocyanins have four different structures, which are in equilibrium and include flavylium cation, quinoidal base, carbinol pseudo base and chalcon. The relative amounts of these structures in equilibrium are varied and depend on the pH and anthocyanin structure (Mazz and Minitiati, 1993). Some anthocyanins are more stable than other depends on their molecular structure. The example of this is the Malvidin glycosides, the major anthocyanin in grape, which due to dimethyloxylation of the molecules are more stable than other anthocyanins. Moreover, hydroxylation of organic acids results in more stable molecules in most cases (Bassa and Francis, 1987; Francis, 1989).

Fruits and berries are the most sample sources of anthocyanins in nature. In fruits and berries, anthocyanins are mainly located in the peel, like in apples and grapes, but they are also found in the pulp, as in the case of cherries or blue berries. Berries and fruits are an important part of the Finnish diet. (Anonymous, 2003). In many researches the positive effect of fruit and berry intake on human health has been reported (Hollman *et al*., 1996a, b; Youdim *et al*., 2002; Knekt *et al*., 2002).

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Anthocyanins are considered to contribute to the healthiness of fruits and berries for their antioxidant, anti-carcinogenic, anti-inflammatory and anti-angiogenic properties for example (Clifford, 2000; Kong *et al*., 2003; Rossi *et al*., 2003). Anthocyanins can also improve the nutritional value of processed foods by preventing oxidation of lipids and proteins in the food products (Kähkönen *et al*., 2001, 2003; Viljanen *et al*., 2004).

Blackberries are of particular interest in this regard, due to the high anthocyanin and phenolic contents that contribute to its noted antioxidant capacit (Wang and Lin, 2000).

In particular, cyaniding-3-glucoside, a common anthocyanin in many soft fruits, including blackberry, has also been reported to have the highest antioxidant capacity of 14 different anthocyanin tested (Wang *et al*., 1997; Mazza and Miniati, 1993).

In strawberries, pelargonidin-3-glucoside is common anthocyanin that has the antioxidant capacity too. (Sondheimer and Kertesz, 1948a; Wrolstad and Putnam, 1969; Goiffon *et al*., 1991; Lopes-Da-Silva *et al*., 2002).

Therefore, preservation of anthocyanins is important. The intensity and stability of anthocyanin pigments is dependent on various factors including structure and concentration of pigments, pH, temperature, light, intensity, quality and presence of other pigments together, metal ions, enzymes, oxygen, ascorbic acid, sugar and sugar metabolites, sulfur oxide, etc. (Mazza and Minitiati, 1993; Francis, 1989).

Sucrose protected anthocyanins from degrading during frozen storage and also prevented browning and the formation of polymeric pigments, which is probably due to the inhibition of enzymatic reactions or the hindering of different condensation reactions by sucrose (Wrolstad *et al*., 1990). Also the lowering of water activity by sugars can be protective against anthocyanin degradation (De Ancos *et al*., 1999b).

The effect of added sugar on the anthocyanin stability depends on its structure, concentration and type of sugar. Wrolstad *et al*. (1990) reported that when the sucrose concentration increased by 20%, the stability of the anthocyanins from strawberry also increased. On the other hand, at low concentration of sucrose (86 g L^{-1}) the degradation of anthocyanins from red cabbage, blackcurrant and elderberry extracts was higher in soft drinks compared to buffer systems both at pH 3, whilst the opposite was observed for grape extract (Dyrby *et al*., 2001). In another study, the anthocyanins stability of grape marc, elderberry and blackcurrant extracts was lower in all sucrose (100 g L^{-1}) added systems as compared to the control at pH values of 3, 4 and 5, whereas the browning index did not change with addition of sugar (Malien-Aubert *et al*., 2001). Addition of 20 g L^{-1} of sucrose to a drink model system (pH 3) containing red cabbage and grape extracts did not influence the thermal and photo stability of the anthocyanins (Duhard *et al*., 1997). Although most anthocyanin extracts showed lower stability in sugar added systems, no statistical analysis was carried out to verify the significance of this difference.

Rizzolo *et al*. (2003) reported that total anthocyanins slightly decreased during storage, even if not statistically significant, at -10 and -20°C, while they did not change at -30°C. No influence of sugar addition was observed.

Veridiana and Mercadante (2007) Considered that addition of sugars and salts had a negative effect on the anthocyanin stability from both sources and that the açai added systems were significantly more stable than those of acerola, these results show that the viability of anthocyanin natural extract addition to soft drinks strongly depends on the composition of the anthocyanin source.

In this research, we study the effect of three concentration of sugar (sucrose) on stability of anthocyanin pigments in berries.

MATERIALS AND METHODS

Sample preparation: Samples of *Berries* were obtained locally. *Berries* fruits were washed with distilled water and kept frozen at -18°C until use.

Methods: Extraction was carried out by the methods of Chiriboga and Francis (1970). Briefly, after taking the samples out of the freezer, they left at room temperature for 30 min to defrost. Then 1000 g from each samples was put into a mixer and after adding ethanol solvent was mixed for 10 min. Then the products were filtered in Bochner funnel vacuum and Whatman filter (grade 1), the remains of the each mixture left on the filter paper was washed again with the above mentioned solvent and filtered again to get a clear liquid. The filtered product then placed in a balloon container within a vacuum evaporator at 35°C to separate the ethanol-acid solvent. The balloon container was separated from the vacuum evaporator and distilled water was added to dissolve the powder, which was formed at the bottom of the balloon container. The product then transferred to a 1000 mL container and brought the volume to 1000 mL using distilled water and centrifuged at 8000 rpm, the supernatant was separated and kept for further analysis.

Treatment with sugar: To examine the effect of sugar, three various levels of sucrose (20, 40 and 60%) was selected. Ninety milliliter of anthocyanin solution spilled in three groups of test tube with three repeat in each group. Certainly at first, the pH of anthocyanin solution regulated with pH meter (pH 2) subsequent, different concentration of sugar added to anthocyanin solution. Three groups of anthocyanin solutions were kept in darkness within a refrigerator for 63 days and every three weeks, the quantity of anthocyanin absorbance recorded in 520 nm.

Statistical analysis: Statistical analysis of the data was performed by ANOVA using Microsoft SAS. All experiments were repeated three times.

RESULTS AND DISCUSSION

The results of statistical analysis in this study represented that a long 63 days, different groups of Anthocyanins with three levels of sugar (20, 40 and 60%) have different effects. Chiefly, in three samples, there is significant difference between various times means and various concentration means, separately.

But in *Murus alba* var. *nigra* and *Fragaria* L. (strawberry), mutual effect of time and concentration is significant too (Fig. 1b and c)

In all samples at the end of 63 day, getting most destruction in Anthocyanins. Higher absorbance is represented in concentration of 20% (Fig. 1a-c). Therefore at this point, there is highest value of anthocyanin and this concentration is moderate concentration for preserve of Anthocyanins in this fruits.

Chiefly, sugars are presented in fruits and during food preparation process, usually added to variant productions such as fruit extracts and jams. Demonstrated that, sugars decreased stability of anthocyanins (Meschter, 1953; Thakur and Arya, 1989). In studies that were performed by Daravingas and Cain (1968) all tested sugars (sucrose, fructose, glucose and xylose) in same method, increased anthocyanins degradation. Evident example of destructed productions of sugar effects is phorphorals (Meschter, 1953).

Rosso and Mercadante (2007) demonstrated that the addition of sugars and salts had a negative effect on the anthocyanin stability.

Anthocyanins reactions with destructed production of sugar, cause formation of brown-color polymerized pigments (Krifi *et al*., 2000).

In some cases, represented that sugars protects Anthocyanins. Sucrose preserves anthocyanins that freezing during storage and also inhibits from browning and formation of polymeric pigments that probably resulting inhibited from enzymatic reaction or with prevention from different condensation reactions with sucrose (Huang, 1956). Reduction of water activity with sugar can also prevent from anthocyanin destruction (De Ancos *et al*., 1999a).

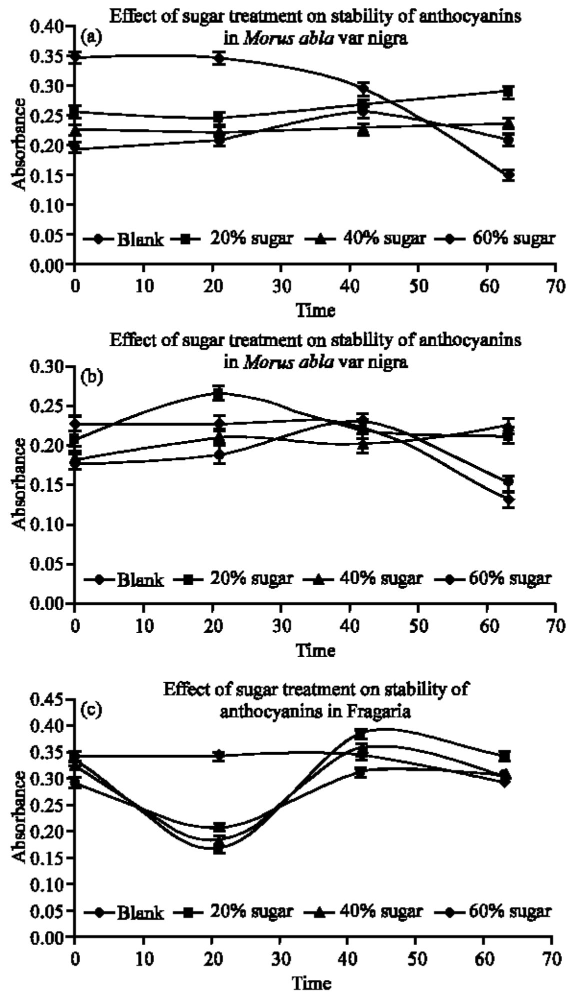

Fig. 1: Changes in anthocyanin content of enhanced juices with sugar during storage, at 0, 21, 42 and 63 days. In every three graph observed that quantity of anthocyanin absorbance later adding sugar, relative to blank were decreased

Wrolstad *et al*. (1990) represented that the protective effect of sugar on anthocyanin pigments could be occurring during freezing or thawing, rather than storage. Also they demonstrated that browning and development of polymerized color were also significantly reduced by sugar addition (Wrolstad *et al*., 1990).

The effect of added sugar on the anthocyanin stability depends on its structure, concentration and type of sugar. Wrolstad *et al.* (1990) reported that when the sucrose concentration increased by 20%, the stability of the anthocyanins from strawberry also increased, that is according to present results.

Tsai *et al.* (2004) reported that data on the anthocyanin Degradation Index (DI) half-life of anthocyanin and activation energy of anthocyanin degradation showed that sucrose was a good anthocyanin protector. They demonstrated that activation energy was 14.78, 17.32 and 18.21 kcal mol^{-1} for the 20, 40 and 60% sucrose systems, respectively.

Anthocyanins exhibit greater stability under acidic condition, but under normal processing and storage conditions readily convert to colorless derivatives and subsequently to insoluble brown pigments.

The stability of the anthocyanins is influenced by several factors. Some of them have been discussed by several authors and it would appear that PPO plays an important role in the degradation of anthocyanins from fruits and vegetables. Wagenknecht *et al.* (1960) reported an anthocyanin-decolorizing system in cherries that acts as an oxidizing enzyme.

Kader *et al.* (1997) demonstrated that mechanism of browning that occurs after fresh High bush blueberry fruits are crushed and demonstrated that PPO, CG and anthocyanin play an important role in the browning reactions. Then in 1998, they investigated the mechanisms of anthocyanin degradation in the presence of PPO and CG using model solutions with pure substrates.

In aqueous solution an equilibrium of four anthocyanin chromophores exists, which is determined by pH (Fig. 2). At very low pH values the equilibrium is dominated by the red flavylium cation (AH+). At higher pH values AH+ is transformed to the colorless carbinol (B) by hydration at C_2 and further to the likewise colorless chalcone (C) through opening of the ring structure. Simultaneously, the blue quinonoidal base (A) is formed from the flavylium cation in small amounts by increasing the pH value (Brouillard, 1982). Since the flavylium cation dominates the colour and its susceptibility towards degradation is comparatively low, anthocyanin colour is most stable at low pH values (<3) (Jackman and Smith, 1996). Colour changes that occur during anthocyanin degradation can be quantified by colour measurement. The loss of anthocyanins is most significantly characterized by a reduction of redness (Hubbermann, 2005). Besides pH several agents may promote the degradation of anthocyanins such as oxygen, ascorbic acid, sugars and sugar degradation products and different enzymes (Jackman and Smith, 1996). Additionally, the water activity of systems as well as anthocyanin concentration on its own influence colour stability (Katsaboxakis *et al.*, 1998; Garzon and Wrolstad, 2001; Skrede, 1992).

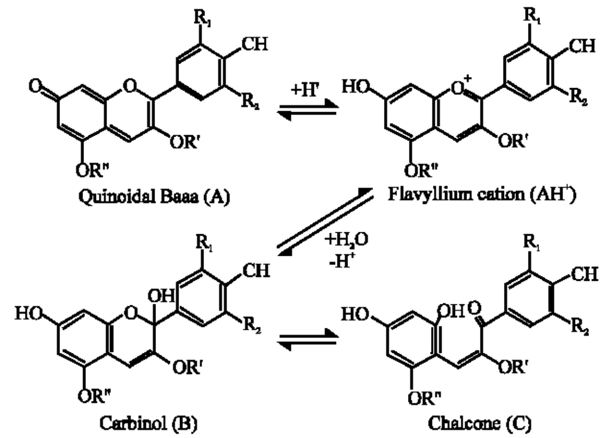

Fig. 2: Structural transformation of anthocyanins in aqueous solution

CONCLUSIONS

Attractive color is one of the most important sensory characteristics of fruit and berry products. However, the color of red berry products is unstable and susceptible to degradation. Maintaining a strong and stable color in berry wines and juices is problematic during processing and storage. In this study, according to statistical analysis, sugar (sucrose) concentration of 20% of has protective effect on Anthocyanins, but in higher concentration this effect is decreased. Therefore, using of sugar only, isn't effective and should be used freezing for decrease enzymatic activity and prevent of browning, for using of sugar to preservation of anthocyanins.

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