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Effect of SO_2 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 SO_2 (25, 50, 100 ppm). 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.

Key words: Anthocyanin, SO_2, degradation, berries, stability

INTRODUCTION

There is worldwide interest in using food colorants from natural sources to replace artificial colorants. Anthocyanins are a group of naturally occurring phenolic compounds, which are responsible for the attractive colors of many flowers, fruits (particularly in berries), vegetables and related products derived from them. These polyphenolic substances are glycosides of polyhydroxy and polymethoxy-derivatives of 2-phenylbenzopyrylium or flavilium salts (Wang, and Xu, 2007).

The 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. Likewise other flavonoids, the significance of anthocyanins has been discussed in relation to a wide range of physiological functions such as improvement of vision, anticancer activity and also in their implication in neural dysfunction and cognitive decline (Cantuti-Castelvetri *et al*., 2000; Hou *et al*., 2004; Aruoma *et al*., 2003; Kowalczyk *et al*., 2003).

Anthocyanins have four different structures, which are in equilibrium and include flavylium cation, quinoidal base, carbinol pseudobase and chalcon (Fig. 1). The relative amounts of these structures in equilibrium are varied and depend on the pH and anthocyanin structure (Mazza and Minitiati, 1993). Some anthocyanins are more stable than other depends on their molecular structure.

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).

So far, anthocyanins have not been broadly used in foods and beverages. Since they are not as stable as synthetic dyes.

Sulphur dioxide, often abbreviated to sulphite or SO_2, is used extensively in modern winemaking. It's use is predominantly for its suppression of yeast and bacterial action and its anti-oxidant properties. It is possible to make good wine without using sulphites, but these results in less control, consistency or biological stability. Sulphit is a natural by product of yeast and as much as 41 ppm has been recorded in fermentations where no SO_2 has been added (Abrahamson, Lyle, 1991).

The initial anthocyanin content and condition of processing and storage have been shown to influence the color quality of muscadin wine and similar products. (Ballinger *et al*., 1973; Ballinger *et al*., 1974; Bates, 1980; Flora, 1976; Flora *et al*., 1977; Flora *et al*., 1978; Nesbitt *et al*., 1974).

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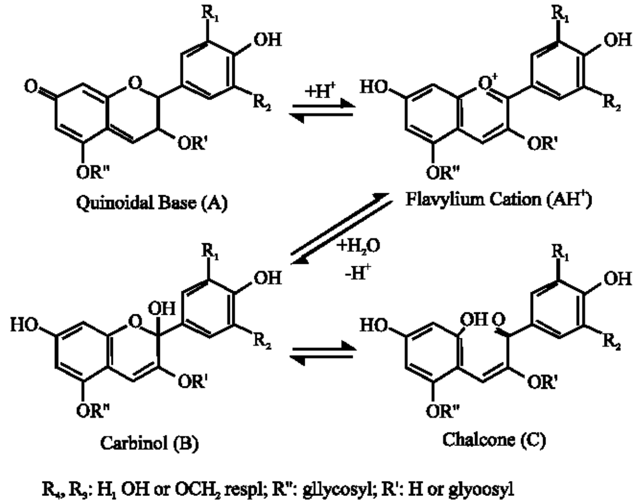

Fig. 1: Structural transformation of anthocyanins in aqueous solution

Sulfur dioxide is used widely in the wine industry as an antioxidant and inhibitor of undesirable microbial growth, but is known to bleach or lighten the color of wine due to formation of anthocyanin-4-bisulfit, a colorless compound (Burroughs *et al.*, 1975; Jurd, 1964; Robinson *et al.*, 1966; Timberlake and Bridle, 1967) Sulfur dioxide addition has a negative effect on red wine colour (Robert, 2002).

As, the usage of sulfur dioxide in wine and fruit juice industry is necessary, therefore, monitoring of suitable amount of it, is important. As, it could play its protective role and don't change the stability of color. The purpose of this study was to examine the effect of three concentration of sulfur dioxide on stability of anthocyanin pigments in berries and determine of suitable concentration.

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. The experiments were done in December to February in 2006-2007 at biochemistry lab in Urmia university.

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 Wattman 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 SO_2: To examine the effect of SO_2, three various levels of SO_2 (25, 50, 100 ppm) selected. Ninety milliliter of anthocyanin solution spilled in three groups of test tube with three repeats in each group. Certainly at first, the pH of anthocyanin solution regulated with pH meter (pH2) subsequent, different concentration of SO_2 added to anthocyanin solution. Three groups of anthocyanin solutions were kept in darkness within refrigerator for 63 days and every three weeks, the quantity of anthocyanin absorbance in 520 nm recorded.

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

RESULTS AND DISCUSSION

Effect of SO_2: In blackberry (*morus nigra*), effect of time and different concentrations of SO_2 alone weren't significant, also mutual effects of time and concentration don't have significantly differences. But, totally in the case of concentration, gradually with increase in SO_2 concentration, quantities of absorbance were increased. In the case of time, lowest absorbance concerned with third time and highest was primary time.

In *morus alba var nigra*. Effect of time and concentration were significant only, but their mutual effect, don't significant. In the case of time, the quantity of absorbance means from primary to third times have significant differences with zero time mean, but don't have significant differences within themselves. In the case of concentration, different concentration means have significant differences with zero concentration means. The highest absorbance related to third concentration, i. e. highest concentration of SO_2 (100 ppm) and lowest were related to zero concentration, i.e., with out SO_2.

In Fragaria (strawberry), effect of time was significant, but effect of concentration weren't. In the case of time, along time we observed decreased absorbance but in the case of concentration, although they haven't significant differences, gradually with increase in SO_2 concentration, observed increased absorbance of anthocyanin.

DISCUSSION

Sulfur dioxide is used widely in the wine and fruit juices as an antioxidant and inhibitor of undesirable microbial growth, but is known to "bleach" or lighten the color of wine due to the formation of anthocyanin-4-bisulfite, a colorless compound (Burroughs, 1975; Jurd, 1964; Robinson *et al.*, 1966; Timberlake, 1967).

SO2 conjugated with anthocyanin and produce colorless product; as follow:

$$AH + HSO_3 \rightarrow AH\text{-}SO_3H$$

An equilibrium constant, for this reaction is high and expressive that little amount of SO_2 may decolorize most amounts of anthocyanins (Timberlake and Bridle, 1967). Bisulfit rapidly react with flavylium ions and if eliminated from system, reaction rapidly acts in revers direction. Within acidified solution with pH1, the decolorization reaction is reversible. ks is obviously dependent to pH. But this is from this truth that SO_2 exists in wines balancing and when decreased equilibrium from state that react whit anthocyanin converted to state that can't react with anthocyanin demonstrated that effect of free SO_2 is much more from pH (Sapers and Simmons, 1998). Each dye that cause disrupted binary bonds in system, result in colorless. Existence of positive oxonyum ions on C-2 gets anthocyanins susceptible to invasion nucleofilic compound such as SO_2 and H_2O_2. after addition SO_2, whit formation of flavon-4-sufonic acid, anthocyanins getting colorless. With deletion this material from environment in low pH condition, the anthocyanin's color is reversible. Although this decreasing in color isn't demanded in many foods, but SO_2 used along preparation process of foods (Sapers and Simmons, 1998).

The potential use of anthocyanins as food color additives has been restrained by their sensitivity to nucleophilic additions leading to their decolourization. This could occur in presence of sulfur dioxide as well, this one being widely used in the food industry as preservative. Even though this phenomenon has been observed for many years, the involved reaction still is not thoroughly understood. A former mechanism was considering the chalcone 3, resulting from the opening of carbinol2 (in equilibrium with anthocyanin 1) to be involved, leading to a chalcone-bisulfite adduct 4. More recent works ruled out this proposal and showed that the colorless compound, formed upon bisulfite addition either to C_2 or to C_4 of the anthocyanin, should be a sulfonate 5 or 6, similar to the carbinol 2 (Fig. 2).

Sims and Morris (1984) reported that Sulfur dioxide levels higher than 25 ppm free SO_2 severely bleached the color of red muscadine wine and lessened browning in high pH wine only.

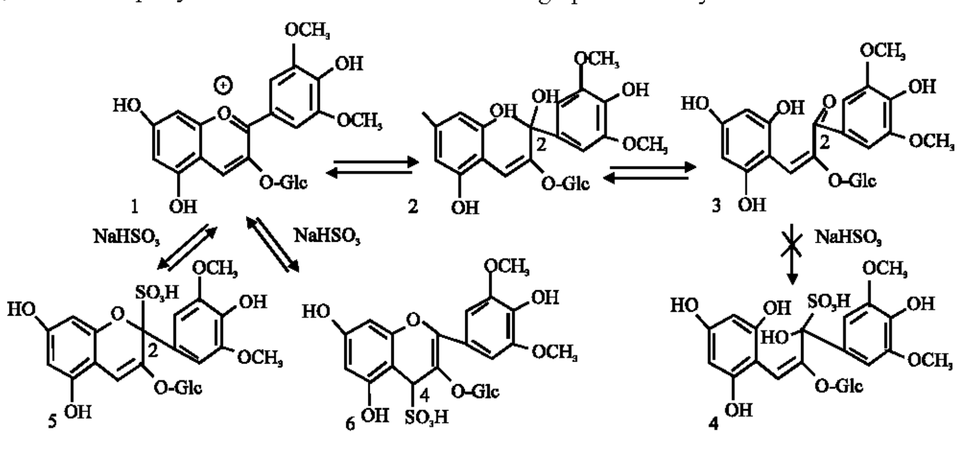

Fig. 2: Formation of colorless compound, upon bisulfite addition either to C_2 or to C_4 of the anthocyanin

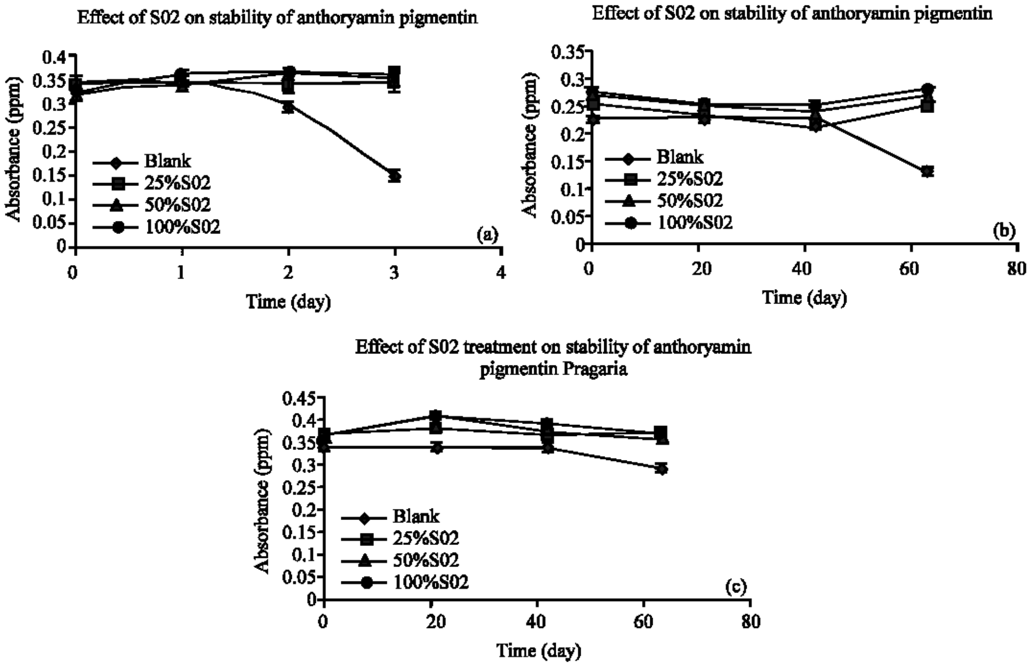

Fig. 3: Changes in anthocyanin content of enhanced juices with SO_2 during storage, at 0, 21, 42 and 63 days. Mean of 3 measurements±SE Blank, is anthocyanin solution without SO_2

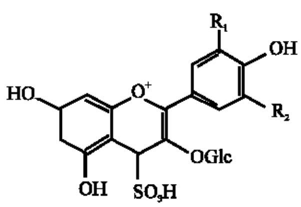

Fig. 4: Colourless bisulfite addition compound showing blocked site at C_4

The main effects of SO_2 indicate that high SO_2 levels did not inhibit browning or anthocyanin loss during storage (Fig. 3).

Robert Paul has been reported that Sulfur dioxide addition has a negative effect on red wine colour. The aromatic nature of the anthocyanin's C ring is destroyed by the reaction of the anthocyanin with sulfur dioxide to form a bisulfite addition compound. This results in a colourless molecule (Fig. 4).

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