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EXTRACTION OF ANTHOCYANINS FROM *CORNUS MAS* AND ITS APPLICATION IN THE NON-ALCOHOLIC DRINKS INDUSTRY

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ABSTRACT

In this study, anthocyanins of *Cornus mas* was used to produce carbonated drinks. For this propose, different levels of anthocyanins of *Cornus mas*, water, citric acid and sugar was mixed together. The produced *Cornus mas* based drinks were compared in the terms of taste through the classification test by a group of 20 assessors and their general acceptance level was assessed by the enjoyable test on a scale of 9 degrees. The obtained results were statistically analyzed (using the method purposed by Chiriboga and Francis, 1970) and the best formulation was determined. In the next step, drinks samples for secondary and minor creature were stored for two months to evaluate the shelf life and they were tested in terms of pH, acidity, turbidity, total counting of bacteria, molds and yeasts. It was finally found that pH, acidity and amount of microorganisms in any treatments were not significantly changed over the past two months storage and they were stable. However, environmental conditions and time had a significant effect on turbidity. So that the turbidity was significantly increased in the sample kept at room temperature and transparent bottles. Contamination of yeasts and molds was not observed in any treatments. The results obtained from sensory evaluation after the end of the storage period showed that none of the samples stored under different environmental conditions are not significantly different from each other and with new samples in terms of taste and appearance.

KEYWORDS: *Cornus mas*, Anthocyanins, Carbonated drinks

INTRODUCTION

Cornus mas or cornelian cherry is a tall shrubs or a small tree of the family of dicotyledonous flowering plants and *Cornus* genus whose tree reaches to a height of 8 meters and a diameter of 15 to 19 cm in Iran. This plant is native from southern Europe to West Asia (Iran, Azerbaijan, Armenia, Southeast Asia and Southern Europe). Its fruit is also called the Cornelian cherry or charcoal. *Cornus mas* fruit is tart. Ripe fruits of this plant have been used to produce compotes and jams or consumed as dried fruits such as dried cherries (Salbati, 258: Moein Persian Culture, 1971). Anthocyanin, which is a subgroup of flavonoids, is responsible for the red, purple and blue colors in many flowers, fruits and vegetables. Fruits, especially berries are natural rich sources of anthocyanin. Anthocyanins are significantly considered due to their beneficial effects on the health, especially because of their antioxidant, anti-cancer, anti-inflammatory and anti-angiogenic activity (Hassanzadeh, 2011; HajiFaraji, 1997; Hadjitarkhani, 1997). According to the results obtained in the study, it seems that the use of the *Cornus mas* fruit has hypoglycemic effects and may improve pancreas tissue changes in diabetes that these effects could be due to the presence of anthocyanin and other antioxidant compounds in this fruit (Shamsi et al., 1390: Tai et al., 2006).

Non-alcoholic drink is a general term used for all carbonated soft drinks. The main components of these drinks are mainly water, carbon dioxide, sweeteners and flavors. The sweetener can be selected from sugar, sugar alternatives or fruit juices. Coloring agents are also usually used for the attractiveness of the product. Soda and Coca-Cola are very common examples of non-alcoholic drinks. As many people consume drinks in our country and considering the harmful effects of these drinks that are including high amounts of glucose, searching new healthy formulations by the researchers and the food industry is required for this wide range of consumers. In this regard, we decided to produce a drink rich in natural oxidants according to the Iranian consumer tastes by using the *Cornus mas* extract, which contains different bioactive compounds (Mirzaei, 2009).

The hypothesis of this study are as follows:
Cornus mas extract based drink production can meet the Iranian consumer tastes.
Cornus mas extract based drink production can be a good alternative to cola drinks.
Industrial production of *Cornus mas* extract drink is an effective step towards localization of these drinks in the country. Anthocyanins extracted from *Cornus mas* can be used in various food formulations to replace edible synthetic colors.

MATERIALS AND METHODS

Anthocyanins extraction process: *Cornus mas* as a source for extracting the anthocyanin color is prepared from the local market. The method of Chiriboga and Francis (1970) was used to extract anthocyanin.
According to this method, 0.1% acidic ethanol was used to extract anthocyanin. were analyzed one kilogram of *Cornus mas* sample was weighted and poured in a blender and some previously prepared extraction solvent was added to it and it was stirred for 10 min. After homogenization, the resulted solution was filtered under vacuum using a Buchner funnel through Whatman paper No. 1. Finally, the used solvent was isolated using a vacuum evaporation device at 35 °C. After separation of the solvent, the concentrated substance remaining in the bottom of the balloon was almost pure anthocyanin. Some distilled water was added to the concentrated extract remaining in the bottom of the balloon and the solution was brought to 1,000 ml. The resulted solution was centrifuged for about half an hour at a speed of 8000 rpm and the upper transparent solution was separated for testing.

Extraction efficiency is determined using the weighting method and the total amount of pigment is determined based on absorption spectrum (spectrophotometry). A sample of the extract is added to the soft drink and coloring quality and utility of the extracts are sensory evaluated from the perspective of consumers. Sample preparation and formulation of drink: The prepared *Cornus mas* extract was used to produce the *Cornus mas* extract based carbonated drink. Different ratios of carbonated water and *Cornus mas* extract (4 levels) were mixed with sugar and citric acid. The four samples (each with 4 replicates) were prepared. After preparation of the samples, they were poured into glass bottles using the past-mix method and after capping, they were pasteurized in hot water at a temperature of 70 ° for 15 minutes and then rapidly cooled to a temperature of 30 ° C (Khurdiya. 1990: Khurdiya. 1989).

A desktop drink maker machine model SIPP-525, Italy was used to produce carbonated water. The injection pressure of carbonic gas into the water was set 5 bar. In order to purify and reduce the hardness of the consumption water for preparing the drink, a resin purification device manufactured by Alborz Company was installed in the inlet water to the drink maker machine (Kohnen, 2005). Sensory evaluation to select the best formulation: In this plan, an assessor team containing 20 persons, who had previously undergone the necessary training, was used to compare the characteristics corresponding to taste in the produced formulations. Accordingly, the samples were evaluated by assessors in 4 steps and the top samples of each step were compared using a separate test. The taste-rating test and the enjoyable test with the method of 9 degrees were used to compare the characteristics corresponding to taste (Hassani, 2004).

Evaluating the shelf life of the drink: After selecting the best formulation, the assessment of physicochemical changes during the storage period was used to evaluate the effect of temperature, light and time on the shelf life of the product. Samples were stored in an environment with natural fluctuations in temperature and light conditions, refrigerator temperature and the presence of light, refrigerator temperature and colored bottles for 2 months. Trials of pH, Brix, turbidity, acidity and mold count was performed in triplicate at the maintenance interval of 20-day. At the beginning of the maintenance period, the mentioned tests were also performed on samples and the obtained results were considered to study the changes over time and different environmental conditions (Hassanzadeh 2003).

Measurement of pH:
The measurement of pH was performed before freezing the samples using a pH meter (Methrom Model 827) (Iran National Standard, 1994 and 1971). Brix measurement: Brix of samples was measured using a desktop Abbe refractometer device at 20 ° C (Iran National Standard, 1994 and 1971). Turbidity measurement: The turbidity of samples was determined using the HACH Turbidimeter and the obtained results were reported in NTU (Iran National Standard, 1994 and 1971).

Acidity measurement: In order to measure the acidity, 1 normal percent sodium hydroxide in the presence of phenolphthalein reagent was used (Iran National Standard, 1994 and 1971). Measurement of the growth of mold: growth medium of Sabouraud dextrose agar was used for culturing mold using the surface culture method (Kohnen, 2005; Williams, 1984). Sensory evaluation: In order to compare the effect of treatments on taste and appearance of drinks, the sensory evaluation was used. A this stage, the samples, which had been stored at different environmental temperatures for 2 months, were compared with each other and with fresh samples in terms of taste and appearance properties by a 20-person evaluation group (Hassanzadeh 2003). Statistical analysis of data: The data resulted from experiments were evaluated using randomized complete block design with four replications at 5%.

RESULTS AND DISCUSSION

(According to the general characterizations):

The results of the measurement of the acidity of the samples showed that the addition of the anthocyanin at different levels had no significant effect on the acidity. The highest acidity was related with the samples containing 1% anthocyanin and the lowest acidity was related with the sample containing 2 and 3% anthocyanin, but there was no significant difference between samples in terms of acidity. Acidity of all samples was stable during storage period (2 months) and there was no significant difference between treatments. Therefore, the time had no effect on acidity. These dinks acidity is created by adding citric acid to samples and carbonic acid resulting from the dissolution of carbon gas in the water. As shown in the table, the initial acidity of this drink is 0.08.

PH also did not show a significant change by adding anthocyanin, athough the highest pH of the sample was observed in the sample containing 4% anthocyanin and the lowest pH was observed in the sample containing 1% anthocyanin that this sample was not significantly different from the other samples. Studying the pH of the samples over time indicates that the time had no significant effect on samples, so storage in the refrigerator and out of the refrigerator has no significant effect on the pH. In this study, no change in acidity and pH of the drink indicates that factors such as temperature and pH as well as packaging and product composition are so that they inhibit performing an effective reaction on pH and acidity.

Research on pomegranate juice over a period of three months showed that the condition does not have a significant effect on pH and total soluble solids (Hosseinnejad 1995). Impurities in the consumption water and sugar involve in transmission of turbidity factors. As the storage at ambient temperature compared to the temperature of the refrigerator accelerates increasing the turbidity of drinks, the microbial activity can be also known as a factor of turbidity. Over time, the turbidity of the samples has been increased and the effect of time has been significant on the turbidity. Drink samples of clear stored in transparent bottles have more turbidity because light catalysis reactions, which cause turbidity (Sawada, 1999). In general, it can be concluded that increasing the temperature and time accelerates the turbidity of the samples and intensifies the turbidity.

In order to assess the sensory characteristics of samples, 20 assessors were used and the samples were evaluated in terms of taste and overall acceptability. There was no significant differences between sensory properties of fresh samples and samples storing in bottles for 2 months. Therefore, storage in different conditions has no effect on the sensory and appearance characteristics of the samples within two months of storage. Thus, it seems that the stability of the samples has been appropriate based on the used method for thermal safety and type of formulation and packaging and so, the produced drink has an appropriate sensory stability.

Findings

The results of sensory tests showed that the *Cornus mas* carbonated drink has a relatively favorable position in terms of the level of public acceptance so that nearly 70% of assessors have expressed interest in consuming it. Study of the trend of microbial and physiochemical changes showed that this type of drink has an appropriate stability. So that the impact of parameters affecting the quality of the product containing compounds and compositions, packaging and pH is in such a way that during the 2-month storage, product quality parameters including pH, acidity, total soluble solids and microbial level are not changed. However, the increase in turbidity of the samples is significantly more severe under ambient temperature and the presence of light. Thus, considering that the increase in turbidity of the samples is severe under ambient temperature and the presence of intense light, the production of carbonated drinks with fruit extract can

be considered as an appropriate alternative to industry drinks, as these products have no suitable physicochemical stability.

Table 1: The physicochemical properties *Cornus mas* drinks immediately after production

	pH	Acidity	Brix, NTU	Turbidity percent	Gas volume g/100ml
Cornus mas drink	4.5	0.08	13	4.5	0.5

Table 2: The general characterizations of the sample

		Control	Anthocyanin 1%	Anthocyanin 2%	Anthocyanin 2%	Anthocyanin 2%
PH	First month	4.15 ±0.01	4.24 ±0.05	4.18 ±0.04	4.09 ±0.05	4.25 ±0.02
	Second month	4.76 ±0.02	4.62 ±0.06	4.27 ±0.03	4.15 ±0.07	4.05 ±0.05
Acidity	First month	0.08 ±0.78	0.09 ±0.80	0.07 ±0.49	0.07 ±0.61	0.08 ±0.78
	Second month	0.09 ±0.79	0.07 ±0.90	0.07 ±0.55	0.08 ±0.75	0.08 ±0.69
Turbidity	First month	4.9 ±0.2	4.6 ±0.4	4.7 ±0.3	4.5 ±0.3	4.7 ±0.3
	Second month	5.7 ±0.4	5.2 ±0.4	4.9 ±0.2	5.5 ±0.2	5.4 ±0.2
	Refrigerator temperature	4.9 ±0.2	4.6 ±0.4	4.7 ±0.3	4.5 ±0.3	4.7 ±0.2
	Environment temperature	6.8 ±0.5	6.6 ±0.4	5.9 ±0.5	5.8 ±0.3	6.7 ±0.5
Mos	First month	45.38 ±6.92	41.94 ±2.90	43.33 ±1.69	46.67 ±2.67	41.45 ±5.98
	Second month	48.73 ±7.71	46.74 ±8.86	45.52 ±5.42	47.44 ±5.64	45.75 ±4.22
Taste, appearance and sensory properties		3.14 ±0.68	3.21 ±0. 55	3.00 ±0.36	3.13 ±0.59	3.10 ±0.60
	Total acceptance	3.45 ±0.67	3.7 ±0.49	3.56 ±0.81	3.39 ±0.77	3.25 ±0.87

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