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The effects of operational conditions on the total amount of anthocyanins extracted from Khorasan's native fig fruit "*Ficus carica*"

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ABSTRACT

Ficus carica is a large evergreen tree found in Iran. In this research, the extraction of anthocyanins from fig fruits was optimized using different SO_2 concentrations (100, 300, 600, 900, and 1200ppm), temperatures (10, 20, 40, 60, and 75 °C), and solvent to solid ratios (10, 20, 40, 60, and 75 L/kg). The extraction of anthocyanins changed with the concentration of SO_2, S/S, and temperature. Maximum yields of anthocyanins were obtained at an SO_2 concentration of 1000-1200ppm and 75L per kg of solvent of frozen fig fruits samples. It is recommended that temperatures of 35 - 40°C be used for the extraction of anthocyanins as higher temperatures will degrade these compounds.

Key Words: Anthocyanins, Fig fruits, Sulfur dioxide, Extraction, *Ficus carica*.

INTRODUCTION

Ficus carica grows in the tropical and subtropical regions of Iran. Different biologically-active compounds have been isolated from this plant. *Ficus carica* has been reported to include antioxidant, antiviral, antibacterial, hypoglycemic, cancer suppressive, hypotriglyceridaemic, and anthelmintic effects [1-3] Health benefits of consumption of fig, a natural antioxidant, products are discussed with reference to: reduction in risk of cardiovascular disease and prevention of cancer [4-5]. Polyphenol and bioactive compounds in fig fruit are excellent antioxidants that are enhanced by the presence of metals [6]. According to literature of medicine, Anthocyanins is a type of phenolic compounds that is of high pharmacia importance. Anthocyanins is well known to be useful in Cancers. Anthocyanins presented in fruits such as ficus carcia have been widely studied for their antioxidant properties [7]. The antioxidative phytochemicals, especially, the phenolic compounds found in vegetables, fruits and medicinal plants, have been in increasing focus for their potential role in the prevention of human diseases [8,9]. Several members of the genus ficus have been used traditionally in a wide variety of ethnomedical remedies all over the world [10]. Phytochemical investigations of some ficus species have revealed high contents of phenolic compounds [11-14]. Other studies reported the presence of antioxidant activity in some ficus species which was attributed to the antioxidant activity of their phenolic constitution [15].

Extraction of phytochemicals from permeable solid plant material, using liquid solvents, forms an important step in the manufacture of phyto-chemically rich products. Traditionally, acidified, aqueous solutions of organic solvents have been used to extract anthocyanins [16-19]. Unfortunately, organic solvents are potentially toxic, expensive and present environmental disposal problems for industrial utilization. Use of water as an extraction solvent could overcome these problems and provide a natural means of isolating anthocyanins from fruits if extraction efficiency is improved. Elevated extraction temperatures facilitate the release of phenolics from plant matrices, increase the solubilization rate of phenolics in solvents, and raise the diffusion coefficient, which in turn increases the extraction rate and reduces extraction time [20,21] . However, extraction temperatures higher than 70°C can cause rapid degradation and discoloration of anthocyanins [22,23].Addition of SO_2 to water stabilizes anthocyanins and increases their yield due to the formation of a stable anthocyanin-bisulfite complex. Moreover, SO_2 has been proposed to enhance the extraction of phenolics through two mechanisms: increased solubility and enhanced diffusion coefficient of the molecules through the solid [21]. Gao and Mazza reported that the extraction of anthocyanins from sunflower hulls was affected by the type of solvent, and sulfured water performed better than acetic acid and aqueous ethanol. They have endeavored in using SO_2 water as a solvent so as to reducte the use of organic solvent, as well as, reduce the cost of the extraction [24]. However, thermal degradation of anthocyanin pigment extracted using SO_2 water solutions has also been reported [25]. The optimum conditions of temperature and solvent to solid ratio (S/S) for the extraction of phenolics and particularly for the extraction of anthocyanins from black currants have thus far not been reported. Surface response methodology has been successfully used for optimization of anthocyanin yield in extraction from sunflower hulls [24]. This method has also been used to select the best solvent, temperature, and extraction [26].In this study, we searched the effectiveness of sulfured water (SSW) over different temperatures for isolating anthocyanins from *Ficus carica*.

MATERIALS AND METHODS

Plant specimens of figs (fruits) were collected in Quchan city, Iran. The air-dried fruits were homogenized. To achieve a standard size of particles, the ground material was sieved through a 1mm metal sieve. Large particles remaining on the sieve were further ground. The process was repeated until all the material passed through the sieve. The samples were stored at −18 ◦C before any further treatments. The frozen samples were dispersed in 2.5 L of solvent in an agitated 4L glass beaker. The solvent consisted of aqueous sulfur dioxide solutions prepared by dissolving sodium metabisulfite ($Na_2S_2O_3$) in water and acidifying it to pH 3.8 with acetic acid. The sulfur dioxide concentration, temperature, and weight of fruits varied for each extraction. The beaker was set in a thermostatic water bath at desired temperatures. Samples were calculated from S/S on a dry weight basis and added to the SO_2-water solvent when the desired temperature was reached. During extraction, 3mL samples of liquid were taken periodically. Extractions were finished when the extract and pomace reached equilibrium that was indicated by no further change in absorbance of the extracts read at 520nm. This procedure distinguishes this research from previous extraction studies, which have used a constant time [25,26] or time as an independent variable [27,28]. The technique used here allowed a more thorough extraction and thus evaluation of the maximum capability of each extraction condition. Besides, the equilibrium time rises as an additional dependent variable. Time to reach equilibrium was obtained from plots of phenolic concentration versus extraction time. Best-fit curves to reach a maximum asymptote and phenolics-predicted maximum values were obtained using Sigma Plot Software (SPSS Inc., Chicago, IL). Equilibrium times were considered as the times when curves reached the predicted maximum value. Partition coefficients *m* were calculated by eq. 1. Recoveries *F* (percent) of anthocyanins and total phenolics were calculated using eq. 2:

$$M = y_e/x_e \quad (1)$$

$$F\ (\%) = (C_{eq}/C_{fb}) \times 100 \quad (2)$$

Where C_{eq} and C_{fb} are the contents of a given marker compound in mg/g of frozen samples on a dwb in the final extract and in the frozen samples, respectively.

Periodic samples that were taken during extractions and aliquots of final extracts were filtered through 0.45 μm PVDF membrane disks using syringe filter holders. Samples were analyzed for anthocyanin by doing absorbance readings at 520nm, using a spectrophotometric method. Standard solutions used included cyanidin 3-glucoside. Samples of wet fruit (20 g) and frozen samples (10 g) were extracted in a blender, with 80% ethanol as described previously [29]. The composition of frozen samples was also determined by the equilibrium method [30] in which approximately 4g samples of frozen *Ficus carica* were soaked with 100mL of 1100ppm of SO_2-water solvent and set in an incubator at 20 °C for 60h. Supernatant was filtered through Whatman filter paper no. 541in a Büchner

funnel under vacuum, collected in a 100 mL volumetric flask, and analyzed as described above. The dry matter contents of the frozen samples were determined by drying 2-4g samples in a vacuum oven at 70°C for 30h.

RESULTS AND DISCUSSION

Optimization of extraction was carried out using the surface response methodology [31]. Selected experimental designs referred to as central composite designs had three factors and five levels. It consisted of 18 runs implemented in random order including four replicates of the center point. Independent variables were SO_2 concentration, temperature, and S/S. Sulfur dioxide concentrations were 100, 300, 600, 900, and 1200ppm. Lowest and highest values of S/S and temperature were 10 and 75 (Table 1). Phenolic yields, equilibrium times, partition coefficients of extracts were then measured.

TABLE 1: CENTRAL COMPOSITE EXPERIMENTAL DESIGN FOR THREE VARIABLES

Run	Temp (^{0}C)	S/S[a](ml/g)	SO_2 conc[b](ppm)
1	20(-1)[c]	20(-1)	300(-1)
2	20(-1)	20(-1)	900(+1)
3	20(-1)	60(+1)	300(-1)
4	20(-1)	60(+1)	900(+1)
5	60(+1)	20(-1)	300(-1)
6	60(+1)	20(-1)	900(+1)
7	60(+1)	60(+1)	300(-1)
8	60(+1)	60(+1)	900(+1)
9	10(-1.68)	40(0)	600(0)
10	75(+1.68)	40(0)	600(0)
11	40(0)	10(-1.68)	600(0)
12	40(0)	75(+1.68)	600(0)
13	40(0)	40(0)	100(-1.68)
14	40(0)	40(0)	1200(+1.68)
15	40(0)	40(0)	600(0)
16	40(0)	40(0)	600(0)
17	40(0)	40(0)	600(0)
18	40(0)	40(0)	600(0)

[a]Solvent to solid ratio expressed in ml/g of frozen samples on a dry weight basis (dwb).
[b]Calculated as ppm equivalents of sulfur dioxide.
[c]Numbers in parentheses are the coded values of variables in the experimental design.

TABLE 2: COMPOSITION OF FROZEN *FICUS CARICA*[a]

Sample	Total Phenolics[b]	Anthocyanins[c]
1[d]	86.9	14.5
2	90.8	13.6
3	87.2	13.2
average	88.2	13.77
1[e]	21.8	14.2
2	19.8	13.8
3	18.9	12.9
average	20.2	13.6

[a] Phenolic concentrations in mg/g of frozen ficus carcia on a dry weight basis (dwb) expressed as equivalents of the following:
[b] chlorogenic acid; [c] cyanidin 3-glucoside. d,e Phenolics determined by equilibrium with a 1100 ppm SO_2 water at 100 mL/g solvent to solid ratio at 20°C for 60h (d calculated from spectrophotometric determination; e calculated from HPLC determination).

Anthocyanin content of ficus carcia (Table 2) was higher than values reported in the literature.

The model developed by surface response analysis for total phenolic yield (Table 3) was significant at low levels of probabilities, and variability could be very well explained by the model, surface response models for yields of anthocyanins (Table 3). Total phenolics increased with SO_2 concentration in the whole range of S/S, but the increase was greater at a higher S/S. Anthocyanin extraction also increased with a rise in SO_2 concentration. Yields of extraction anthocyanin from ficus carcia increased with higher SO_2 concentration.

In a similar study, J. E. Cacace and G. Mazza reported extraction of anthocyanins and other phenolics from black currants with sulfured water in 2002. [32]

TABLE 3: SURFACE RESPONSE FOR EXTRACTION YIELD, PARTITION COEFFICIENT, AND EQUILIBRIUM TIME OF TOTAL PHENOLICS AND ANTHCYANINS

	Total phenolic			Anthocyanin		
Run	yield[a]	Partition coeff	Equil time[b]	yield[c]	Partition coeff	Equil time[b]
1	18.2	0.12	115	11.8	0.24	119
2	25.0	0.16	50	12.4	0.31	49
3	26.3	0.05	58	12.1	0.09	65
4	45.2	0.10	41	13.1	0.10	48
5	16.8	0.11	42	11.2	0.19	30
6	28.5	0.25	13	12.9	0.35	31
7	19.3	0.04	15	12.8	0.06	19
8	31.8	0.28	9	13.1	0.08	18
9	29.2	0.07	129	12.9	0.11	131
10	16.7	0.18	17	9.2	0.09	15
11	16.5	0.28	58	10.5	0.11	62
12	34.2	0.10	26	12.6	0.09	26
13	16.8	0.03	76	11.2	0.08	86
14	34.5	0.23	26	11.5	0.10	32
15	29.1	0.08	32	12.6	0.09	37
16	24.3	0.11	28	11.7	0.10	31
17	25.9	0.05	18	12.2	0.11	39
18	24.2	0.09	34	12.8	0.12	35

[a] *Total phenolic yields in mg/g of frozen ficus carcia on a dwb expressed as equivalents of chlorogenic acid.*
[b] *In minutes.*
[c] *Anthocyanin yield in mg/g of frozen ficus carcia on a dwb expressed as equivalents of cyanidin 3-glucoside.*

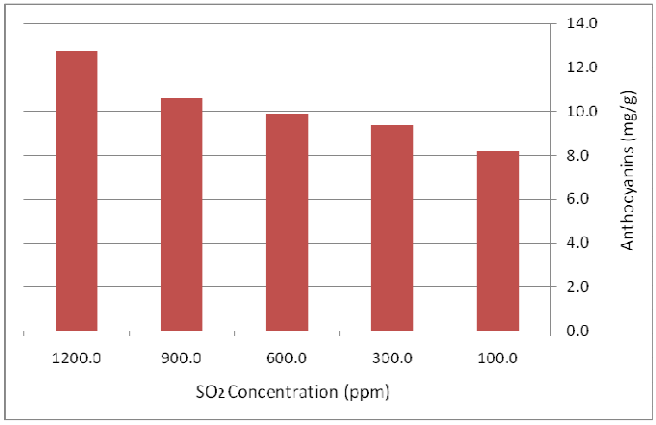

FIG. 1: ANTHOCYANINS EXTRACTED FROM FROZEN *FICUS CARICA* WITH DIFFERENT SO2 CONCENTRATION

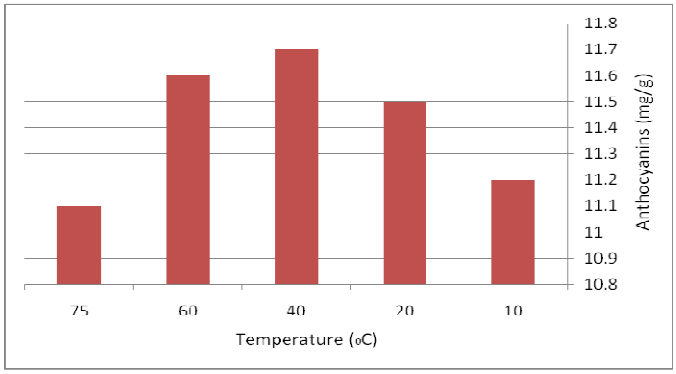

FIG. 2: THE EFFECTS OF TEMPERATURE AT A CONSTANT SO2 CONCENTRATION OF 1200PPM ON EXTRACTION OF ANTHOCYANINS.

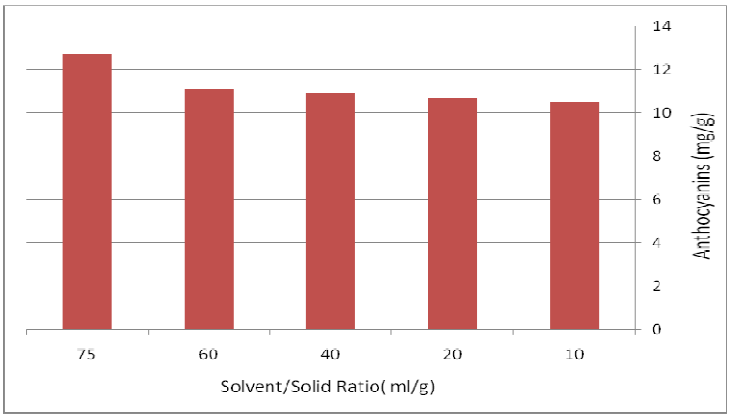

FIG. 3: THE EFFECTS OF SOLVENT/SOLID RATIO (ML/G) AT A CONSTANT TEMPERATURE (40°C) AND SO2 CONCENTRATION OF 1200PPM ON EXTRACTION OF ANTHOCYANINS.

CONCLUSION

Our results indicate that a maximum yield of phenolics is possible at an SO_2 concentration of 1000-1200ppm. Equilibrium reaching times for the extraction of total phenolics and anthocyanins were reduced with an increase in extraction temperatures. However, it is recommended that temperatures of 35 - 40°C be used for the extraction of anthocyanins, as higher temperatures will degrade these compounds. Increasing the solvent ratio increased anthocyanins extraction, and a maximum yield was obtained with 20L of solvent/kg of samples of frozen *Ficus carica*.

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