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Comparison of Microwave with Conventional Heating on Phytochemical Compounds of Cornelian Cherry (*Cornus mas* L.) Concentrate

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ABSTRACT

In this study the effects of concentration on tannin content, total anthocyanin content, total phenol content and antioxidant activity of cornelian cherry juice using conventional and microwave methods under various operational pressures (100, 38.5 and 12 kPa) were investigated. The final juice concentration of 42° Brix was achieved in 137, 125, and 93 minutes at 100, 38.5 and 12 kPa, respectively, by conventional heating. Applying microwave energy decreased required times to 115, 90, and 75 min. at 100, 38.5 and 12 kPa, respectively. Results showed that thermal treatment by microwave compared to conventional heating under low-pressure operation (12 kPa) caused less decrease in the phytochemical content (tannin content, total anthocyanin content, total phenol content and antioxidant activity) of cornelian cherry juice.

Keyword: Cornelian cherry concentrate, Conventional heating, Microwave heating, Phytochemical compounds**.**

INTRODUCTION

Fruits and vegetables are a good source of natural antioxidants, which contain many different significant scavengers that provide safety against radicals and decrease incidence and mortality rates of cancer and heart disorders along with several other health benefits (Shui and Leong, 2006). This good property is mainly attributed to the presence of different phytochemicals. Phytochemicals are a group of metabolites occurring in several plants and they contribute to many health benefits (McCann *et al*., 2005).

Red fruits contain natural antioxidants (Ozgen *et al*., 2009a). Literature indicates physical and chemical properties of cornelian cherry fruits, their antioxidant capacity, phenol and ascorbic acid, along with anthocyanin contents (Klimenko, 2004; Marinova *et al*., 2005; Vareed *et al*., 2006; Tural and Koca, 2008). Recent studies showed that cornelian cherry possesses significant phytochemical properties and antioxidant capacity (Rop *et al*., 2010; Pawlowska *et al*., 2010; Hassanpour *et al*., 2011; Popovic´ *et al*., 2012).

Cornelian cherry (*Cornus mas* L.) belongs to the *Cornaceae* family (Mamedov and Craker, 2004and it is an important source of phenolic compounds, anthocyanins, total flavonoids and ascorbic acids. Antioxidant activity is high in this fruit (Hassanpour *et al*., 2011)and it is rich in sugar, organic acids and tannins (Seeram *et al*., 2002). Therefore this fruit could be considered as a good source of natural antioxidants (Hassanpour *et al*., 2011).

Phenol and polyphenol are the most

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efficient antioxidants found in these fruits (Chipault, 1962; Bishov and Henick, 1977). Phenolic compounds are known to act as antioxidants due to their stable intermediates, which prevent oxidation of food components specially fatty acids and oils because of their ability to donate hydrogen or electrons (Cuvelier *et al*., 1992; Maillard *et al*., 1996).

Anthocyanins are among natural phenolic compounds, which are the largest group of pigments found in plants (Prodanov *et al*., 2005). These compounds play an important role in several fruits and vegetables and products derived from them (Yoshimoto *et al.,* 2001; Crisan *et al.,* 2013). These polyphenolic substances are glycosides of poly hydroxyl and poly methoxy derivatives of 2-phenylbenzopyrylium or flavilium salts. Anthocyanins are known as important antioxidants (Smith *et al*., 2000).

Tannins are water-soluble antioxidants (Gorunovic and Lukic, 2001), and high molecular weight compounds which constitute the third group of phenolics. They may be classified chemically into two main groups, hydrolysable and condensed tannins (Porter, 1989).

Concentrating fruit juices is a basic unit operation in fruit juice technology (Sulc, 1984), where the volume is reduced in the concentrated juice and leads to decreases in the cost of storage, transport and packaging. The increased concentration of solids also prevents microbial spoilage of the concentrate (Downes, 1990). During the concentration process, water is partly removed in the form of vapor from a boiling solution, without changes in solids composition such as vitamins, minerals and sugars. These components are left in the fruit juice concentrate (Toribo and Lozano, 1986). Anes *et al.,* (1999) reported that natural antioxidants in fruit juice readily undergo thermal degradation or participate in maillard reaction. It is evident that the amounts of natural occurring antioxidant compounds during concentration are significantly reduced (Rababah *et al*., 2011). However in terms of technology, finding an appropriate method that can improve the quantity and quality of fruit juice concentration is very important (Geedipalli *et al*., 2007). Combination of the low temperature and fast mass transfer by the vacuum system (Yongsawatidigul and Gunasekaran, 1996a) with the rapid energy transfer by microwave heating produce a rapid, low-temperature concentration (Bondaruk *et al*., 2007). In a similar way successful application of microwave vacuum technology in drying has been reported for cranberries (Yongsawatidigul and Gunasekaran, 1996a, b), carrots (Lin *et al*., 1998) and oregano (Yousif *et al*., 2000).

The objective of this work was to investigate the effect of thermal methods of concentration, including conventional heating and microwave heating at different operational pressures, on the total phenolic content, tannin content, total anthocyanin content and antioxidant activity of cornelian cherry juice during concentration.

MATERIALS AND METHODS

Cornelian cherry juice preparation

Cornelian cherries (*Cornus mas* L.) were obtained from local market in Karaj, Iran and were selected in terms of appearance. After appropriate washing and cleaning, the fruits were soaked in water overnight, the juice was extracted manually by pressing and then a fabric filter was used to separate the skin and pyrene. The single-strength clarified juice (7% of total soluble solid) was frozen at -18˚C and used for further experiments.

The cornelian cherry juice was obtained following the procedures shown in Figure 1.

Content of total anthocyanin, tannin content and total phenolic of juice were 20.0460±0.1942 (mg cyanidin-3-glucoside 100 mL^{-1}), 44.94±0.0916 (mg 100 mg^{-1}) and 174.53±0.0173 (mg galic acid 100 mL^{-1}), respectively and antioxidant activity (IC_{50}) was 0.500±0.0055 .

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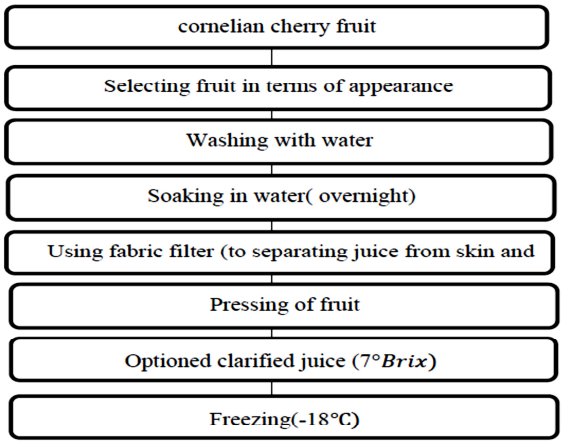

Figure 1. Flow chart of cornelian cherry juice production.

Concentration methods

Microwave Heating

A programmable microwave (Butane MR-1, Iran, using a maximum output of 900W at 2,450 MHz) was used to produce cornelian cherry juice concentrate using microwave energy. Vacuum pump (Robin-air - Owatonna, MN, USA) was used to concentrate the juice at different operational pressures (12, 38.5 and 100 kPa) (Figure 2). Cornelian cherry juice (400 ml) was concentrated from an initial concentration of 7° Brix to a final concentration of 42° Brix. After concentration, the time of process was recorded and concentrates were collected for further experiments (Maskan, 2006). The schematic diagram is shown in Figure 2.

Conventional Heating

Four hundred ml of cornelian cherry juice with an initial total soluble solid content of 7° Brix was put in a round bottom flask and was concentrated by rotary vacuum evaporator (Heidolph, Heizbad HB Contr, Germany) under 12, 38.5 and 100 kPa pressure until reaching the desired final concentration of 42° Brix. During the evaporation process, the sample was heated by immersing it in a soybean oil bath and temperature of the sample and the time of process were recorded (Maskan, 2006).

Evaporating at Atmospheric Pressure

To obtain sample control, an

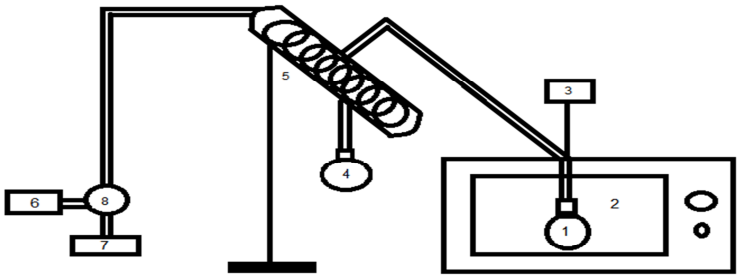

Figure 2. The schematic diagram of microwave vacuum, (1) Airtight jar; (2) Microwave heating chamber; (3) Thermometer ; (4) Collectors jar ; (5) Condenser ; (6) Vacuum pump; (7) Pressure controller; (8) Vacuum control valve.

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electromagnetic heater was used to concentrate juice at atmospheric pressure. Four hundred of juices with initial concentration of 7° Brix were concentrated and the final Brix was reached to 42. During concentration, stirring was performed by magnet (Maskan, 2006).

Tannin Content

Tannins amount in concentrates were measured by titration of potassium permanganate and Tannin was expressed in gram per gram of the concentrate (Isiri, 2007). Twenty five grams of cornelian cherry concentrate was mixed with 300 ml of distilled water and boiled for 1 hour. With boiling, part of the water evaporates, so the amount of evaporated water was re-added into the solution. After cooling the solution, the volume was set to 500 ml and was filtered with Whatman filter paper No. 5. Two hundred ml of the filtrate was mixed with 800 ml of distilled water. Twenty ml indigo reagent (5, 5'-indigodisulfonic acid sodium) was added slowly and carefully. Then they were titrated with standard solution of potassium permanganate (N. 10). A Color change from green to blue and golden yellow was observed. 1 g of active carbon was added to the remaining filtrate, stirred for 10 minutes and filtered with Whatman filter paper No. 5. The resulting solution was titrated with potassium permanganate similar to the previous step until the advent of the golden yellow color. Tannin content (g 100 g^{-1}) was calculated using the equation:

$$T=\frac{(a-b)\times 0/0035\times 100}{m} \quad (1)$$

Where, *m* is sample weight (g); *a* and *b* are the volume of potassium permanganate in the first and second titration respectively (ml); *T* is tannin content (g 100 g^{-1}).

Total Anthocyanin Content (TAC)

Concentration of anthocyanins was determined by the pH differential method (Lako *et al*., 2007) using two buffer systems–potassium chloride buffer, pH= 1 (0.025M) and sodium acetate buffer, pH= 4.5 (0.4M). 0.4 ml of the concentrate was mixed with 3.6 ml of corresponding buffers and read against a blank (distilled water) at 510 and 700 nm in UV–vis spectrophotometer (PerkinElmer Lambda 25 Spectrometer). Absorbance (A) was calculated as:

$$A=(A_{510}-A_{700})_{pH_1}-(A_{510}-A_{700})_{PH_{4.5}} \quad (2)$$

The *TAC* of the samples (mg cyanidin-3-glucoside 100 mL^{-1} of mulberry juice) was calculated using the equation:

$$TAC=\frac{A\times MW\times DF\times 100}{MA} \quad (3)$$

Where, *A* is absorbance; microwave is molecular weight (449.2); *DF* is the dilution factor (10); and *MA* is the molar absorptivity of cyanidin-3-glucoside (26900).

Total Phenolic Content (TPC)

The Total amount of Phenolic Compounds (TPC) was measured by spectrophotometry and the Folin and Ciocalteu reagent. Concentration of phenolic compounds was expressed in mg gallic acid per 100 g of the concentrate (Slinkard and Singleton, 1977).

Folin and Ciocalteu method is the most common method for measuring the phenolic compounds. This method is based on reducing Folin by phenolic compounds in alkaline solution and the maximum absorbance at 760 nm wavelengths. Water - Ethanol mixture was used for the extraction of phenolic compounds. One ml of cornelian cherry concentrate sample was mixed with 1 ml hydrochloric acid 6M, then 5 ml solution of ethanol and water were added and mixed, the mixture vortexed and was placed in a water bath at 90°C for 2 hours and after cooling the tube at room temperature, the solution was diluted with 10 ml of distilled water. One ml of this solution with 5 ml of diluted Folin and Ciocalteu and 15 ml of the sodium carbonate (7 g 100 mL^{-1}) were mixed and finally 100 ml of distilled water

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was added. Its absorption was read by a spectrometer (Perkin Elmer Lambda 25 Spectrometer) at a wavelength of 760 nm.

To evaluate total phenol content (milligrams per 100 grams of gallic acid), gallic acid's standard curve was used. Absorption at different concentrations of gallic acid was measured at wavelength of 760 nm.

Determination of Antioxidant Activity

The antioxidant capacity was carried out by *DPPH* (2, 2-DiPhenyl-PicrylHydrazyl) system (Brand-Williams *et al*., 1995).

First set the spectrophotometer (Perkin Elmer Lambda 25 Spectrometer) with pure methanol. Different concentrations of *DPPH* were made in order to draw the standard curve, and absorbance was measured by a spectrophotometer at 515 nm. 2.5 mg of DPPH was dissolved in 100 mL methanol (this solution was prepared daily and was kept in the darkness) and 3.9 mL of the above-mentioned *DPPH* solution was mixed with 0.1 mL of methanol. Absorption was measured at 515 nm and initial concentration of *DPPH* was calculated using a standard curve. Different concentrations of juice was prepared and 0.1 ml of juice at different concentrations was mixed with 3.9 mL of *DPPH* solution and after being kept in the darkness for half an hour, absorption at 515 nm was measured. The amount of remaining *DPPH* was calculated using the standard curve. The amount of remained DPPH (%) at the steady-state condition was calculated as follows:

$$\%\,\mathrm{DPPH}_{rem} = \frac{\mathrm{DPPH}_{remained}}{\mathrm{DPPH}_{initial}} \times 100 \quad (4)$$

For evaluation of antioxidant capacity, IC_{50} factor was used. The amount of remaining *DPPH* at steady state was plotted against the sample concentration to obtain the IC_{50} value. Value is defined as concentration of the juice that is able to decrease the initial *DPPH* concentration by 50%.

Statistical Analysis

The results were reported as an average of three replicates. The data were analyzed by SPSS_{16} and one way Analysis Of Variance (ANOVA) was performed to determine changes in the quality of concentrated cornelian cherry juice through processes. Significant differences between means were determined by Duncan's multiple range test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Required Time for Concentration

The final juice concentration of 42° Brix was achieved in 137, 125 and 93 minutes at 100, 38.5 and 12 kPa respectively, by conventional heating. Applying microwave energy decreased required times to 115, 90 and 75 minutes at 100, 38.5 and 12 kPa, respectively.

The results of required times' measurements are shown in Table 1. Results show that the elapsed time to reach the final concentration (42° Brix) for microwave-heated products is evidently shorter than conventionally heated ones. Also the elapsed time to reach the final concentration is shorter for the samples processed at lower operational pressures.

Outlet Temperature

Table 1 shows the outlet temperature of the concentrated samples. Applying higher pressures led to increasing time and temperature of concentrating for both conventional and microwave heating methods. This result shows that the operational time can be reduced depending on pressure. At operational pressures of 12, 38 and 100 k Pa, boiling points of the solutions were 50, 75 and 100°C respectively. During the concentration boiling point of the solution

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Table 1. Comparison of elapsed time for microwave and conventional- heated products ($P \leq 0.05$).

Heating method	Pressure (kPa)	Time (min)	Outlet temperature (°C)
control	100	152	95
Rotary evaporator	12	93	49
	38.5	125	76
	100	137	97
Microwave	12	75	60
	38.5	90	82
	100	115	102

increased by increasing time, which can be explained by an increase in the soluble solid concentration. In the microwave, increasing the boiling point is more evident because of the superheating phenomenon. Superheating phenomenon is described as a heated liquid at a temperature more than its boiling point, without boiling (Fazaeli *et al*., 2013).

Tannin Content

During thermal treatment of plants, complex phenomena happen and new compounds are produced, the most important reactions are hydrolysis, oxidation, polymerization, interaction of components and thermal decomposition (Djordjevic, 1995).

Results showed that heating method affects tannin content of concentrates as shown in Table 2. Tannin content of microwave-heated products is evidently higher than conventionally heated ones. Applying higher pressures led to increase of time and temperature of process and the decrease in tannin content of Cornelian cherry juice for both heating methods. The loss of tannins at high temperature may be due to the degradation or interaction with other components of juice to form insoluble complexes (Embaby, 2010).

Rakic *et al*, (2004) investigated the effect of thermal treatment on tannin content and antioxidation properties of oak acorn Quercus cerris extract. They reported that thermal treatment in 200 °C for 15 minutes. resulted in a decrease of 3.14 % in tannin content.

Total Anthocyanin Content

Thermal treatment as the most common processing unit has influenced the stability of anthocyanins significantly. Therefore,

Table 2. Comparison of the tannin content in cornelian cherry juice concentrated with microwave and rotary evaporator ($P \leq 0.05$).

Heating method	Pressure (kPa)	Time (min)	Tannin content (mg/100g)
control	100	152	45.18± 0.2702
Rotary evaporator	12	93	64.81± 1.4174
	38.5	125	53.97± 3.07313
	100	137	44.51± 3.0375
Microwave	12	75	69.04±0.6179
	38.5	90	65.31±0.6726
	100	115	59.39±0.1307

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thermal degradation of anthocyanins has been studied widely in many fruits and their products (Kirca and Cemeroglu, 2003; Ahmed *et al*., 2004; Wang and Xu, 2007).

Figure 3 shows that the microwave at 12 kPa pressure led to better preservation of anthocyanin compounds in the concentrate. Applying microwave instead of conventional heating method can decrease the time of process. Assawarachan and Noomhorm (2011) showed that microwave compared to conventional heating proved to be more efficient for the anthocyanin preservation present in the cornelian cherry juice. This may be described by the fact that in microwave heating, the generated heat was absorbed by the material directly, which resulted in faster heating rates, in comparison with conventional heating in which heat is usually transferred from the surface to the interior.

Oliveira *et al*. (2010) observed a 12 to 42% reduction of anthocyanins in cooked blueberries during progressive heating from 12 to 99ºC for 60 minutes. They suggested that anthocyanins are naturally unstable and degradation is primarily caused by oxidation.

This instability had been previously noted by Sadilova *et al*. (2006) in strawberry, elderberry and black carrot concentrates. They suggested that the degradation mechanism is due to hydrolysis of the anthocyanin sugar moiety which leads to formation of a phenolic non bioactive aglycone.

Total Phenolic Content

Results (Figure 4) show minimum amount of phenolic compounds obtained by conventional heating at 100 kPa pressure and using higher pressures led to increase in time and temperature and decrease in total phenolic content of cornelian cherry juice. Since the time required to achieve the final pomegranate juice concentration of 42° Brix by conventional heating at 100 kPa pressure was higher than that of the other methods, these results seem reasonable. Rajauria *et al*. (2010) reported that, polyphenolics are vulnerable to heat damage and therefore they are lost during different processing operations. Reducing the phenol content of cornelian cherry concentrate by increasing time could be due to thermal degradation or

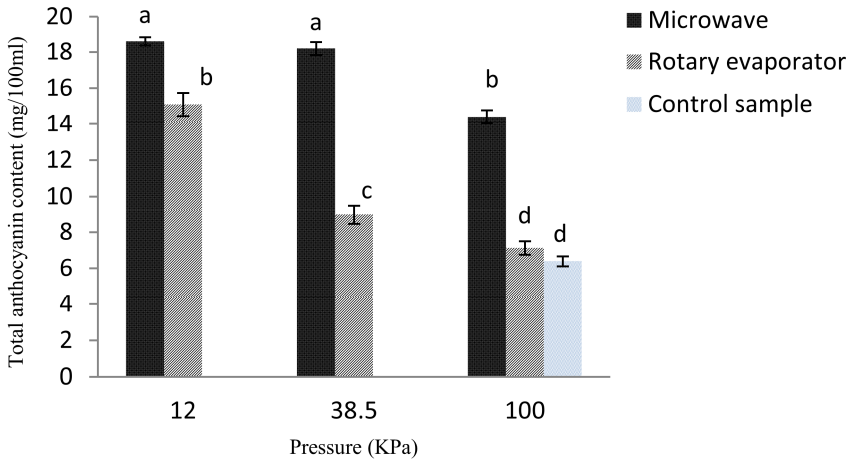

Figure 3. Variation of total anthocyanin from the cornelian cherry juice concentrated with microwave and rotary evaporator ($P \leq 0.05$).

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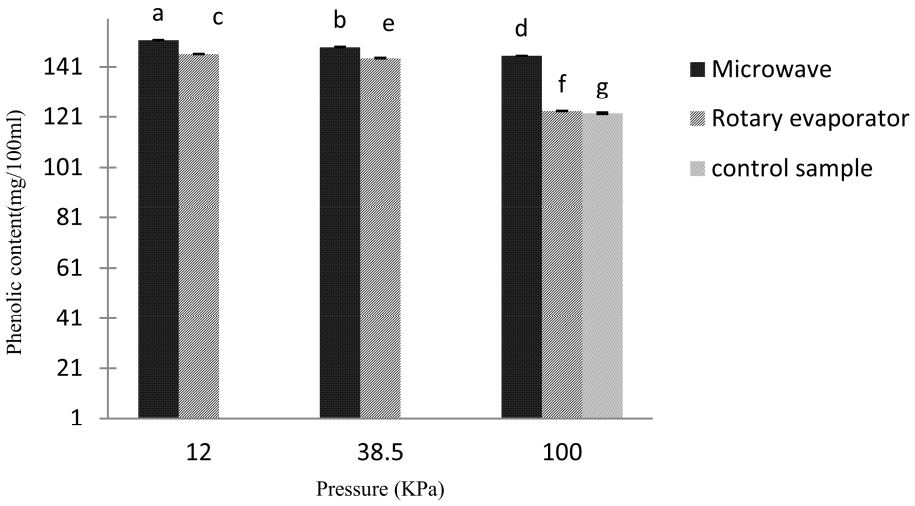

Figure 4. Variation of the phenolic compounds from the cornelian cherry juice concentrated with microwave and rotary evaporator ($P \leq 0.05$).

polymerization reaction of phenolic compounds and anthocyanins (Tsanova-Savova *et al*., 2002).

Paul and Ghosh (2012) showed that total phenolic content in pomegranate juice decreases as the temperature and time of heat treatment increase.

Determination of Antioxidant Activity

Natural antioxidants are significantly lost as a consequence of processing (Rababah *et al*., 2011). Several researchers came to the conclusion that thermal treatments are the main reason for the depletion of natural antioxidants (Jonsson, 1991).

Results (Figure 5) expressed that IC_{50} value (lower values indicate more powerful antioxidant activity) showed that using higher pressures led to increase in time and temperature and decrease in antioxidant activity of cornelian cherry juice by both conventional and microwave heating methods. Although the processing temperature due to superheating in the

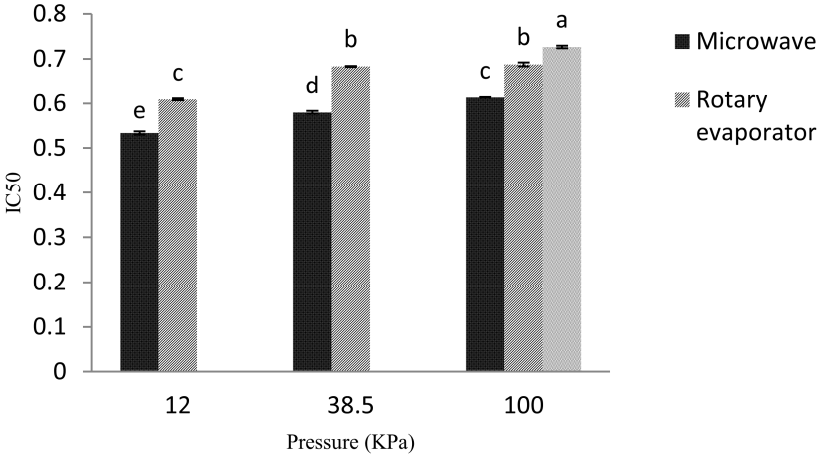

Figure 5. Comparison of the IC_{50} values microwave and rotary concentration methods at different pressures ($P \leq 0.05$).

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microwave was more than the evaporator, results showed that antioxidant compounds in the microwave heating method can be better preserved, which is due to the short processing time.

Study on the effect of heating on the antioxidant activity revealed that using microwave instead of conventional heating method under low pressure could better conserve the antioxidant activity of juices. It is due to the enhanced penetration of heat that provides a constant internal temperature that lasts until the final concentration is reached (Oliveria and Francis, 2002). These results are in agreement with those reported by Fazaeli *et al.* (2013).

The results of a previous research showed that antioxidant activity is directly related to anthocyanin and phenolic contents (Camire *et al*., 2002; Moyer *et al*., 2002).

Cornelian cherry has the highest ascorbic acid, anthocyanin, phenolic tannin and antioxidant activity among the many other fruits (Seeram *et al*., 2002; Pawlowska *et al*., 2010). Therefore, by reducing the amount of these compounds over time during thermal processing, reduction of the amount of antioxidant activity by increasing the time seems reasonable.

CONCLUSIONS

The results showed that the heating method and conditions (temperature and pressure) affect the tannin content, total anthocyanin content, total phenol and antioxidant activity of the samples. The lowest time was obtained when microwave heating at low pressure was applied. Thus microwave application at low pressure instead of conventional heating method can better conserve phytochemicals in cornelian cherry juice. Due to advantages of microwave processing and the increase in domestic and industrial application of this equipment, substituting this heating method instead of the traditional one should be considered.

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مقایسه مایکروویو و گرمادهی معمول بر روی ترکیبات فیتوکمیکال کنسانتره زغال اخته (*Cornas mass* L.)

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چکیده

در این مطالعه اثر گرمادهی توسط انرژی مایکروویو و گرمادهی معمول بر روی محتوای تانن، آنتوسیانین کل، محتوای فنول کل و فعالیت آنتی اکسیدانی آب زغال اخته طی حرارت دهی در فشارهای عملیاتی مختلف (۱۰۰، ۳۸.۵ و ۱۲ کیلوپاسکال) بررسی شد. کنسانتره زغال اخته با بریکس نهایی ۴۲ در زمان های ۱۳۷، ۱۲۵ و ۹۳ دقیقه توسط گرمادهی معمول به ترتیب در فشارهای ۱۰۰، ۳۸.۵ و ۱۲ کیلوپاسکال بدست آمد. به کارگیری انرژی مایکروویو به جای گرمادهی معمول زمان مورد نیاز برای تغلیظ آبمیوه را به ۱۱۵، ۹۰ و ۷۵ دقیقه به ترتیب در فشارهای ۱۰۰، ۳۸.۵ و ۱۲ کیلوپاسکال کاهش داد. نتایج بدست آمده از تغلیظ آبمیوه زغال اخته نشان داد که فراوری گرمایی توسط انرژی مایکروویو در مقایسه با گرمادهی معمول در فشار پایین (۱۲ کیلو پاسکال) باعث کاهش کمتر مقدار ترکیبات فیتوکمیکال (محتوای تانن، آنتوسیانین کل، فنول کل و فعالیت آنتی) موجود در آب زغال اخته شد.

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