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비가열, 재래식 및 통전가열한 오디주스의 품질 평가

양지원[1] · 한대석[1] · 이창호[1] · 박성진[2] · 김영언[1,†]

[1]한국식품연구원, [2]한림성심대학교 관광외식조리과

Evaluation on the Quality of Fresh, Conventionally Heated and Ohmically Heated Mulberry Fruit Juice

Ji-Won Yang[1], Dae-Seok Han[1], Chang-Ho Lee[1], Sung-Jin Park[2] and Young-Eon Kim[1,†]

[1]Division of Metabolism and Functionality Research, Korea Food Research Institute, Seongnam 463-746, Korea
[2]Dept. of Tourism Food Service Cuisine, Hallym Polytechnic University, Chuncheon 200-711, Korea

Abstracts

The aim of this study was to establish the superiority of ohmic heating over conventional heating for the sterilization of mulberry juice. Heat treatment of fresh juice significantly reduced the concentration of soluble solids, lowered the pH, and lowered the reducing sugar content ($p<0.01$). Color measurements showed decreases in the *L* and *a* values and increases in the *b*, H and C values after heat treatment, although the total color differences were smaller after ohmic heating than after conventional heating of fresh juice. The antioxidant capacities, such as reducing power, FRAP, and DPPH, decreased in the order of fresh juice, ohmically heated juice and conventionally heated juice. Furthermore, the anthocyanin, flovonoid, and total antioxidant capacities of the juices significantly decreased in the same order. Sensory evaluations showed no difference between fresh and ohmically heated mulberry fruit juice excluding off-flavor, whereas conventionally heated juice received significantly lower evaluations. The microbial counts were zero in the juice after either heat treatment. Thus, ohmic heat treatment can be effectively used to sterilize fresh mulberry juice to obtain good shelf life with minimal physicochemical, color, antioxidant and sensory deterioration.

Key words: Mulberry fruits, juice, ohmic heating, sterilization

Introduction

To achieve efficient production of high-quality fruit juice, it is necessary to inactivate the juice by applying high-temperature heating for a very short duration to avoid microbial spoilage. The major drawbacks of conventional heating are its low energy efficiency and long drying times. For these reasons, Halleux *et al* (2005) examined ohmic heating, where the electrical resistance of the food itself generates heat as current is passed through it, and found that it provided 82.97% energy saving while reducing the heating time by 90.95% as compared to conventional heating. Ohmic heating avoids excessive thermal damage to labile substances and thus preserves the nutritional value of the food, flavor compounds and pigments (Palaniappan & Sastry 1991). It also achieves faster inactivation of lipoxygenase, polyphenol oxidase (Vikram *et al* 2005, Nolsoe & Undeland 2009, Sagar & Kumar 2010, Ghnimi *et al* 2008, Zareifard *et al* 2003, Castro *et al* 2004) and microbial and pectin esterase (Leizerson & Shimoni 2005a).

In addition, many reactions affecting the color of foods can take place during thermal processing (Barreiro *et al* 1997, Suh *et al* 2003), such as degradation of carotenoids (lycopene, xanthophylls, etc.), anthocyanins, and chlorophylls and browning reactions (Barreiro *et al* 1997, Lozano & Ibarz 1997, Ibarz *et al* 1999). Thus, color can be used as a quality indicator to evaluate the extent of deterioration due to thermal processing (Avila & Silva 1999) and it is also an important attribute itself, because color is usually the first property the consumer observes (Saenz *et al* 1993). Therefore, in addition to changes in the total amount of phenolic compounds, color changes have an important role in explaining the changes occurring in food structures. Of particular concern regarding damage due to heat treatment are deep-colored fruits and vegetables rich in phenols including flavonoids, anthocyanins, and carotenoids, which have significant antioxidant capacities and thus play a poten-

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[†] **Corresponding author** : Young-Eon Kim, Tel : +82-31-780-9073, Fax : +82-31-701-9876, E-mail : radog@kfri.re.kr

tially important role in human health by reducing the risk of cancer, cardiovascular disease, and other pathologies (Bravo 1998, Konczak & Zhang 2004, Sass-Kiss *et al* 2005, Cieslik *et al* 2006).

One such fruit is the mulberry (*Morus alba* L.), a kind of deep-colored berry and a traditional Korean edible fruit that can be eaten fresh. Traditionally mulberry fruit has also been used in Korean folk medicines to effectively treat fever, strengthen joints, protect the liver from damage, lower blood pressure, and facilitate discharge of urine. Among berries, mulberries are particularly rich in phenolic compounds and anthocyanins (Ercisli & Orhan 2007, Bae & Suh 2007, Tsai *et al* 2005). Thus, in recent years, mulberries have been widely converted to juice for use in the production of wine, fruit juice, jam and canned food (Lee & Choi 2011).

In the present study, the physicochemical properties, color, antioxidant activity, total amount of phenolic compounds, and shelf life of mulberry fruit (*Morus alba*) juices were investigated in order to examine and compare the qualities of ohmically heated and conventionally heated juices after the same heating temperatures and times. This research will be valuable for both industrial applications and academic research in which the ohmic heating technique is used for thermal processing of beverages, concentrated juice and puree products.

Materials and Methods

1. Preparation and Processing of Mulberry Fruit Juice

Mulberry fruit of full maturity was provided by the Yecheon Red Pepper Powder Farm Corporation. The berries were selected for uniformity of weight (approx. 3 g) and color (purple red), and fruit with apparent injuries were removed. For the experiments, 250 g of the stored frozen mulberry fruit and 2,250 mL of water were mixed and homogenized for 5 min. The mixture was then centrifuged at 7,500 rpm and 4℃ for 30 min. The supernatant was filtered through a filter paper (Whatman No. 41, Florida, USA). As a food additive, 1% calcium lactate was added to the raw juices. A 2,000 mL sample of fresh mulberry fruit juice was immediately transferred to the cell, and then the sterilization procedure was carried out.

2. Heating Methodology

Samples in a polycarbonate container were inserted into a water bath (C-SKW1, Chang Shin Science Co., Seoul, Korea) heated to 75℃ and held at this temperature for 20 min. Electronic temperature sensors were inserted in the center point of the glass container. The temperature differences among different locations were within approximately 1℃ during the heating. A schematic diagram of this ohmic heating circuit is presented in Fig. 1. The mulberry fruit juice was sterilized by ohmic heating in a parallelepipedic cell (14.5×6.6×8 cm) with two planar electrodes (14.5×8 cm) connected to an ac generator (50 Hz; voltage and intensity up to 200 V and 7.5 A; EPI-MODEL 24, Frontier Engineering Co., Osaka, Japan). The juice was then directly placed in the parallelepipedic cell between the electrodes. A Teflon-coated electronic temperature sensor with a compression fitting was used to measure the temperature in different sections of the sample in the test cell. The selected range of temperature was 50 to 120±2℃, and the heating time was varied from 0 to 300 min using a temperature control system. The sample was ohmically heated from 20℃ to 75℃, and the temperature was kept constant at 75±1℃ for 20 min by adjusting the power on and off during the ohmic heating. The temperature of each sample was assumed to be uniform in the cell, since the maximum difference among the temperatures measured at different locations was approximately within 1℃. The experiments were replicated three times.

3. Determination of Total Soluble Solid Content, Titratable Acidity, pH, and Reducing Sugar Content

The total soluble solid (TSS) content, expressed as °Bx, was measured with a portable refractometer (PR-201α, Atago Co.,

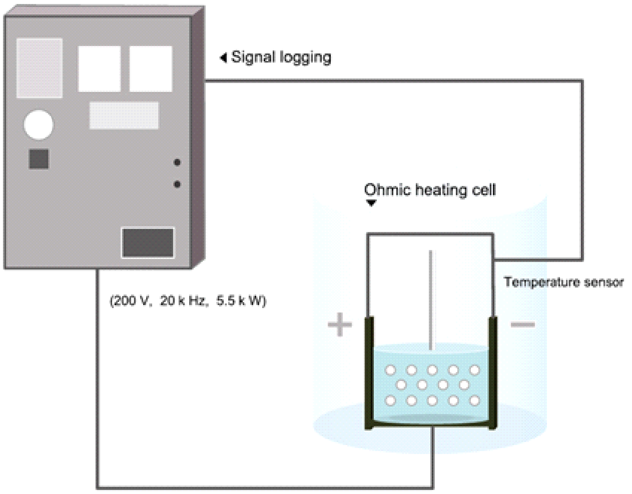

Fig. 1. Schematic diagram of the ohmic heating system.

Ltd., Tokyo, Japan) and corrected for a temperature of 20℃ (Section 22.024, AOAC, 1984). The titratable acidity(TA) was determined with 0.1 M NaOH up to pH 8.3, and the results are expressed as percentage of citric acid as determined by standard procedures (AOAC, 1984). The pH values for the sample juice were measured with a digital pH meter (Model 320, Thermo Orion, Massachusetts, USA). The 3,5-dinitrosalicylic acid (DNS) method (Miller 1959) was employed as a standard protocol to determine the amount of reducing sugar. All analyses were carried out in triplicate.

4. Determination of Color

Color (CIE L, a, b) measurements of the juice samples were carried out using a spectrophotometer (Color-Eye 3100, Kollmorgen Instruments Corp., New York, USA). These L, a and b CIE color values are also used to calculate the hue angle ($\tan^{-1}(b/a)$), chroma $\{(a^2+b^2)^{1/2}\}$ and total color differences $\{(L^2+a^2+b^2)^{1/2}\}$ before and after pasteurization (Lee & Coates 1999).

5. Determination of the Antioxidant Compound Contents

The analysis was carried out in triplicate using the pH differential method (Giusti & Wrolstad 2001) with two buffer systems: 0.025 M potassium chloride (KCl) buffer at pH 1.0 and 0.4 M sodium acetate ($NaC_2H_3O_2$) buffer at pH 4.5. For each analysis, 200 μL of the anthocyanin sample was mixed separately with 1.8 mL of potassium chloride and sodiumacetate buffer and the absorbances at 520 nm and 700 nm were determined. The difference in absorbance of the samples at different pH was determined as follows:

$$\text{Abs} = (A_{510\text{ nm}} - A_{700\text{ nm}}) \text{ at pH } 1.0 - (A_{510\text{ nm}} - A_{700\text{ nm}}) \text{ at pH } 4.5$$

The monomeric anthocyanin pigment concentration (mg/L) in the original sample was determined using the following formula: monomeric anthocyanins pigment (mg/L) = $(A\times MW\times DF\times 1000)/(\varepsilon\times 1)$, where A is the absorbance difference between the two pH ranges, ε is the cyanidin-3-glucoside molar absorbance (26,900), MW is the molecular weight of anthocyanin (449.2), and DF is the dilution factor.

The total phenolic content was determined by using Folin-Ciocalteu's reagent (Lim *et al* 2007). For these experiments, 0.3 mL of sample was placed in each test tube, followed by 1.5 mL of Folin-Ciocalteu's reagent (1:10 dilution with distilled water) and 1.2 mL of sodium carbonate (7.5% w/v). The tubes were vortexed, covered with parafilm, and allowed to stand for 30 min at room temperature. The absorption at 765 nm was measured. The total phenolic contents were expressed in gallic acid equivalents (mg per 100 g fresh fruit). The total flavonoid contents were estimated according to the method previously described (Liu *et al* 2008). A 250 μL aliquot of extract was mixed with 1.25 mL of distilled water and 75 μL of 5% $NaNO_2$. After 6 min, 150 μL of 10% $AlCl_3$ was added. Finally, 500 μL of 1 M NaOH was added and the total volume was increased to 2.5 mL with distilled water. The absorbance was measured at 510 nm. The results were expressed in catechin equivalents (mg per 100 g fresh fruit).

6. Determination of the Antioxidant Activity

The DPPH free radical-scavenging activity of the juices was measured using the method described by Gorinstein *et al* (2004). A 0.1 mM solution of DPPH in methanol was prepared. An 0.2 mL aliquot of sample was added to 2.8 mL of this solution and kept in the dark for 30 min. The ability of the sample to scavenge the DPPH radical was calculated with the following equation:

$$\%\text{ inhibition} = \{(A_0 - A_1)/A_0\}\times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the sample. The reducing power of the extracts was determined as described previously (Oyaizu 1986). A 1 mL aliquot of extract (10% v/v) was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The mixture was incubated at 50℃ for 20 min, then mixed with 2.5 mL of 10% trichloroacetic acid by vortexing. The mixture was centrifuged at 1,000 g for 10 min, and then 2.5 mL aliquot of the supernatant was mixed with an equal amount of milli Q water and 0.5 mL of 0.1% $FeCl_3$. The absorbance was measured at 700 nm using a spectrophotometer. All assays were performed in triplicate. L-Ascorbic acid(0~0.1 mg/mL) was used as reference standard, and the results are expressed in L-ascorbic acid equivalents (mg per 100 g fresh fruit). The FRAP assay (Benzie & Strain 1996) is based on the ability of phenolic compounds to reduce Fe^{3+} to Fe^{2+}. To prepare the FRAP reagent, 0.1 M acetate buffer (pH 3.6), 10 mM TPTZ, and 20 mM ferric chloride

(10:01:01, v/v/v) were mixed. Then, 20 μL of the previously diluted extract was added to 150 μL of the reagent. The absorbance was measured at 593 nm using a microplate spectrophotometer (170-6930, Benchmark Plus, USA). The analysis was performed in triplicate using an aqueous trolox solution as standard, and the results are expressed in L-ascorbic acid equivalents (mg per 100 g fresh fruit).

7. Microbiological Analysis

Aerobic bacteria, coliform bacteria and yeast and mold count were measured with Petrifilm Count Plates (3MTM PetrifilmTM Aerobic Count Plate, 25℃, 5 day; 3MTM Petrifilm TM Yeast & Mold Count Plate, 25℃, 5 day; 3MTM PetrifilmTM and Coliform Count Plate, 37℃, 48 hr) according to the AOAC(1984) official methods 990.12, 991.14 and 997.02.

8. Sensory Evaluation

To evaluate sensory acceptability of the microfiltered and pasteurized juice, an effective test using a nine-point hedonic scale was carried out according to the procedure outlined by Meilgaard *et al* (1999). On this scale, nine is equivalent to “like extremely,” five is equivalent to “neither like nor dislike,” and one “dislike extremely.” The acceptance test was performed by twenty untrained panelists, all sugar cane juice consumers. The test was performed in individual rooms, where the juice was served in 50 mL disposable cups under white light at a temperature of 15±1℃, always 2 hr after a meal. The samples were served individually.

9. Statistical Analysis

All experiments were carried out in triplicate and data were expressed as mean±standard deviation (S.D.) using SPSS version 11.5.0 (SPSS Inst.,). One-way analysis of variance (ANOVA) and Duncan’s multiple-range test were used to determine the significance of the difference among samples with a significance level of 0.01.

Results and Discussion

The temperature changes in the mulberry fruit juice during sterilization using conventional and ohmic heating are shown in Fig. 2. To reach 75℃, the conventional heating process takes 2,995 sec and the ohmic heating takes 205 sec, ohmic heating is about five times faster. The total soluble solid (TSS) content, titratable acidity (TA), pH of fresh, conventionally heated and ohmically heated mulberry juice are listed in Table 1. First, the TSS of fresh, ohmically heated, and conventionally heated mulberry fruit juice are 2.71, 2.76, and 2.81. TSS was the highest for conventionally heated juice and significantly lower for the fresh juice, which showed the lowest value. These results are similar to those obtained in a study of ohmic heating and conventional heating of pomegranate juice (Yildiz *et al* 2009). However, the TSS was not significantly different after sterilization of apple juice using an ultra- high-pressure treatment (Suárez-Jacoboa *et al* 2011). The pH value of fresh, ohmically heated, and conventionally heated mulberry fruit

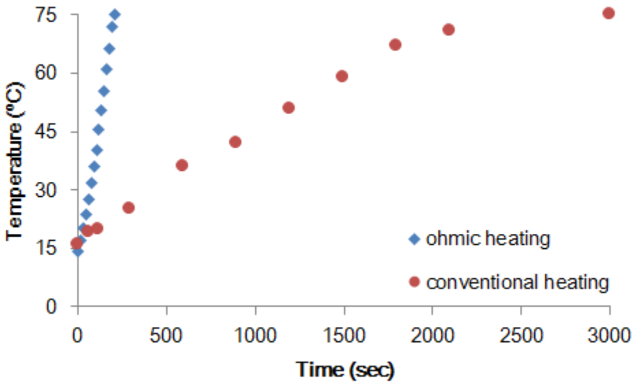

Fig. 2. Heating curve of mulberry fruit juice.

Table 1. Physicochemical properties of fresh, conventionally heated and ohmically heated mulberry juice

Processing process	Total soluble solid (°Bx)	pH	Titratable acidity (g citric acid 100 mL^{-1})	Reducing sugar (g glucose 100 mL^{-1})
Fresh juice	2.71±0.02^{c}	5.38±0.01^{b}	0.43±0.04^{a}	6.42±0.00^{c}
Conventionally heated juice	2.81±0.01^{a}	5.47±0.01^{a}	0.36±0.01^{b}	9.13±0.01^{a}
Ohmically heated juice	2.76±0.01^{b}	5.41±0.01^{a}	0.37±0.00^{ab}	7.20±0.00^{b}

Values are means±standard deviations(S.D.) of triplicate analysis from 3 different productions.
Values in the same column with different superscripts differ significantly(p<0.01).

juice are 5.38, 5.41, and 5.47. As for pH, increases in pH are directly related to decreases in TA. The pH value of the fresh juice was significantly lower than those of the heated juices, because increases in temperature lead to the evaporation of organic acids. However, ohmic heating and conventional heating did not produce significantly different pH changes. Finally, the TA of fresh, ohmically heated, and conventionally heated mulberry fruit juice are 0.43, 0.37, and 0.36. The TA of fresh juice was significantly higher than that of the conventionally heated juice. These TA results are also similar those obtained in the aforementioned study of ohmic heating and conventional heating of pomegranate juice (Yildiz *et al* 2009).

For the most part, these results are consistent with previous reports. It has been found that fresh apple juice has a lower pH value than pasteurized juice (Charles-Rodríguez *et al* 2007) and that microwave treatment decreases the pH of blackberry juice and increases its TA (Ghazaleh *et al* 2011). It was determined that the pH and TA of apple juice subjected to ultrahigh-pressure sterilization are not significantly different from those of fresh juice (Suárez-Jacoboa *et al* 2011), and the pH and TA of grape juice did not vary significantly for different sterilization methods (Garde-Cerdán *et al* 2007).

The higher sterilization times involved in conventional heating increased the reducing sugar content significantly. The reducing sugar content of fresh, ohmically heated and conventionally heated mulberry fruit juice are 6.42, 7.20 and 9.13. This is consistent with several previous reports. For example, mulberry fruit extract (Suh *et al* 2003) and grape juice (Bozkurt *et al* 1999) treated at high temperatures had significantly higher reducing sugar contents. The high-temperature sterilization of apple juice (Lee *et al* 2012) produced higher reducing sugar contents. However, the reducing sugar content of grape juice was not significantly different for different sterilization methods (Garde-Cerdán *et al* 2007).

The color values of raw, conventionally heated and ohmically heated mulberry fruit juice are listed in Table 2. The color changes occurring in processed fruit juices are complex and cannot be explained by a single processing factor. Such changes can be caused by various combinations of enzymatic and nonenzymatic reactions occurring in this temperature range that turn the juice brown when the juice takes up oxygen. Color deterioration in fresh and processed fruits is usually related to enzymatic browning reactions (Giner *et al* 2001, Van Loey *et al* 2002), although nonenzymatic Maillard-type reactions can also cause color changes (Moyer & Aitken 1980).

In this study, the lightness L of mulberry juices decreased significantly with increasing sterilization time. As expected, ohmic heating of mulberry fruit juice also browns the juice. The L values of fresh, ohmically heated, and conventionally heated mulberry fruit juice are 47.90, 33.80 and 29.60, respectively. Because a decrease in the L value is directly indicative of browning (Petriella *et al* 1985, Sapers *et al* 1989), these observations indicate that the browning reactions are initiated by the thermal treatment and are more severe for conventional heating than for ohmic heating. These color changes can probably be attributed to partial precipitation of unstable particles suspended in the juice, as described by Genovese *et al* (1997).

The a and b values indicate the redness and yellowness, respectively. The a values of fresh, ohmically heated and conventionally heated mulberry fruit juice are 19.45, 15.82 and 15.28. The b values of fresh, ohmically heated and conventionally heated mulberry fruit juice are 21.92, 26.34 and 33.59. After sterilization treatment, the b values shifted significantly in the positive direction, especially for conventional heating. In contrast, the a values shifted significantly in the negative direction after sterilization treatment, again especially for conventional heating. Thus, the juice became more yellow and less red after longer sterilization times.

Table 2. Changes in color values of fresh, conventionally heated and ohmically heated mulberry juice

Processing process	Color			Hue angle(°)	Chroma	Total color differences
	L	a	b			
Fresh juice	47.90 ± 0.10^{a}	19.45 ± 0.04^{a}	21.92 ± 0.01^{c}	0.96 ± 0.00^{c}	26.72 ± 0.01^{c}	-
Conventionally heated juice	29.60 ± 0.03^{c}	15.28 ± 0.02^{c}	33.59 ± 0.04^{a}	1.05 ± 0.00^{a}	38.82 ± 0.05^{a}	22.10
Ohmically heated Juice	33.80 ± 0.03^{b}	15.82 ± 0.02^{b}	26.34 ± 0.60^{b}	1.03 ± 0.01^{b}	30.72 ± 0.50^{b}	15.22

Values are means±standard deviations(S.D.) of triplicate analysis from 3 different productions.
Values in the same column with different superscripts differ significantly($p<0.01$).

In recent years, the hue angle has been used to characterize the color changes in some foods. The mean hue angle for fresh juice is 0.96, while those for the heat-treated juices were higher, probably because of changes in the pigment profile. Lower hue angle ratios indicate that there exist phenolic compounds that strongly influence the juice color and that less browning has occurred. The hue angle ratios of the ohmically heated samples were found to be lower than those of the conventionally heated samples, since less browning occurred during the heat treatment (p<0.01).

Finally, the chroma value C, which represents the color intensity, was lower for fresh juice than for sterilized juice, and increased for longer sterilization times. The final C was higher for conventionally heated juice than for ohmically heated juice, which is consistent with the color trends noted above.

In summary, fresh juice clearly had the highest lightness and redness values, and because it required a shorter treatment time, ohmic heating yielded a significantly higher lightness value than conventional heating. The yellowness values showed the opposite trend. The hue angle was the lowest for fresh juice, higher for ohmically heated juice, and, highest for conventionally heated juice, as was the chroma value C. These results are consistent with those for blackcurrant syrups, grape juice, blood orange juice, purple carrots, oranges, pomegranate juice and apple juice (Skrede G 1985, Rhim *et al* 1989, Arena *et al* 2001, Uyan *et al* 2004, Leizerson & Shimoni 2005a, Maskan M 2006, Charles-Rodríguez *et al* 2007), which all had the highest lightness values when fresh. However, the lightness of red grapefruit showed the opposite trend (Lee & Coates 1999). Leizerson & Shimoni (2005a) reported that ohmic heating reduces the lightness of orange juice as the treatment time goes on. Similar observations of decreases in redness values during heating were reported by other researchers for red grapefruit juice (Lee & Coates 2003, Lee & Coates 1999), pomegranate juice (Alper *et al* 2005, Maskan M 2006), blackcurrant syrups (Skrede G 1985), grape juice (Rhim *et al* 1989), blood orange juice (Arena *et al* 2001), and purple carrots (Uyan *et al* 2004). However, the redness of apple juice showed the opposite trend (Charles-Rodríguez *et al* 2007), whereas its yellowness (Charles-Rodríguez *et al* 2007) showed a similar tendency to that observed in this study. Red grapefruit juice (Lee & Coates 2003, Lee & Coates 1999), pomegranate juice (Alper *et al* 2005, Maskan M 2006), blackcurrant syrups (Skrede G 1985), grape juice (Rhim *et al* 1989), blood orange juice (Arena *et al* 2001) and purple carrots (Uyan *et al* 2004), on the other hand, showed the opposite behavior of the yellowness value. Pea puree (Icier *et al* 2006), orange juice (Lee & Coates, 2003) and pomegranate juice (Yildiz *et al* 2009) had hue angle results similar to those observed in this study. Furthermore, similar increases in chroma due to the application of heat have been observed for the manufacture of tomato juice (Davis & Gould, 1955) and orange juice (Lee & Coates, 2003).

Beyond the color characteristics, the antioxidant capacities of raw, conventionally heated, and ohmically heated mulberry fruit juice measured using different antioxidant assays are listed in Tables 3, 4. In particular, the DPPH, reducing power, and ferric reducing antioxidant power were measured to evaluate the antioxidant activity, and the monomeric anthocyanin content, flavonoid contents and phenolic compound contents were measured to evaluate the antioxidant compound contents. The monomeric anthocyanin contents of fresh, ohmically heated, and conventionally heated mulberry fruit juice are 28.72, 24.61 and 19.69. The flavonoid contents of fresh, ohmically heated and conventionally heated mulberry fruit juice are 46.36, 42.90 and 39.12. The phenolic compound contents of fresh, ohmically heated and conventionally heated mulberry fruit juice are 67.13, 63.29 and 61.11. The antioxidant compound contents of the fresh juice was significantly higher than

Table 3. Changes in the antioxidant compound contents of fresh, conventionally heated and ohmically heated mulberry juice

Processing process	Monomeric anthocyanin contents (mg cyanidin-3 glucoside equivalents /g)	Total flavonids contents (mg catechin equivalents /g)	Total phenolic contents (mg gallic acid /g)
Fresh juice	28.72±0.30^{a}	46.36±0.43^{a}	67.13±0.10^{a}
Conventionally heated juice	19.69±0.51^{c}	39.12±0.74^{c}	61.11±0.70^{c}
Ohmically heated juice	24.61±0.51^{b}	42.90±0.28^{b}	63.29±0.41^{b}

Values are means±standard deviations(S.D.) of triplicate analysis from 3 different productions.
Values in the same column with different superscripts differ significantly(p<0.01).

those of the heated juices. The fresh mulberry juice sample had a significantly higher antioxidant capacity than the sterilized samples in all antioxidant assays. However, as compared to conventional heating, ohmic heating sterilization led to significantly increased antioxidant activity because of the reduced heating time. These results are consistent with the previously reported results that polyphenolic compounds are susceptible to heat damage and thus are lost during various processing operations (Skrede *et al* 2000, Rajauria *et al* 2010), whereas anthocyanins are stable during thermal processing (Kirca *et al* 2007, Wang & Xu 2007, Scalzo *et al* 2008). Furthermore, dehydration processes often deteriorate the color quality in foods, which reflects a loss of anthocyanins and other phytochemicals specific to fruits and vegetables (Krifi *et al* 2000, Kwok *et al* 2004).

Regarding the phenolic compounds, these secondary metabolites in plants are known to play an important role in the color and flavor of fruit juices. Phenols are also used as indicators of the physiological state of fruit products and of potential damage (Blanco *et al* 2001). In contrast to the deterioration of phenolic compounds during heat treatment observed in this study, the results obtained by Yildiz *et al* (2009) suggested that the total phenolic compound content of fresh juice was lower than that of the heat-treated juice and that conventional and ohmic heat treatments did not produce significant differences in phenol content. Mercali *et al* (2014) observed that the monomeric anthocyanin content in acerola pulp was higher after ohmic heating than after conventional heating. The phenol content of fresh apple juice was also observed to be higher than those of sterilized juices (Aguilar-Rosas *et al* 2007), because it did not undergo filtering. Thus, ohmic heating is comparable to other sterilization methods in terms of retained phenol content.

As for the sensory characteristics of the differently treated juices, Table 5 shows the results of sample quality tests conducted on the color, odor, flavor, taste and overall acceptance of the mulberry juice. It was observed that the different processes applied to the mulberry fruit juice had a statistically significant influence on its sensory acceptability in terms of all the different attributes evaluated, although some differences between fresh and heat-treated juices were not significant excluding off-flavor. The taste, color and overall acceptance of the mulberry fruit juice sterilized by ohmic heating were significantly better than those of the juice sterilized by conventional heating. Furthermore, although the odor and flavor of the fresh mulberry fruit juice were found to be better than those of the juices subjected to heat treatments, the differences between conventionally heated and ohmically heated juices were not significant. The conventionally heated mulberry fruit juice received the lowest grades, and it was the least preferred in terms of color (3.93), flavor (4.93), taste (4.13) and overall impression (4.20), probably because of its darker coloring

Table 4. Changes in the antioxidant activity of fresh, conventionally heated and ohmically heated mulberry juice

Processing process	Reducing power (mg ascorbic acid equivalents /g)	FRAP (mg ascorbic acid equivalnet /g)	DPPH radical scavenging activity (%)
Fresh juice	94.80±0.47^{a}	553.03±3.87^{a}	77.97±0.42^{a}
Conventionally heated juice	72.39±0.91^{c}	450.90±1.54^{c}	66.68±1.57^{c}
Ohmically heated juice	86.20±0.86^{b}	528.77±1.44^{b}	71.89±0.77^{b}

Values are means±standard deviations(S.D.) of triplicate analysis from 3 different productions.
Values in the same column with different superscripts differ significantly(p<0.01).

Table 5. Changes in the sensory evaluation of fresh, conventionally heated and ohmically heated mulberry juice

Processing process	Color	Off-odor	Flavor	Taste	Overall acceptance
Fresh juice	4.93±0.88^{ab}	5.93±1.16^{a}	5.60±1.30^{a}	5.80±1.27^{ab}	5.20±0.94^{ab}
Conventionally heated juice	3.93±1.22^{b}	4.67±0.82^{b}	4.93±1.49^{a}	4.13±0.92^{b}	4.20±1.32^{b}
Ohmically heated juice	5.07±1.03^{a}	3.73±1.16^{b}	5.73±1.34^{a}	6.53±1.06^{a}	6.47±0.90^{a}

Values are means±standard deviations(S.D.) of triplicate analysis from 3 different productions.
Values in the same column with different superscripts differ significantly(p<0.01).

caused by enzymatic activity (polyphenoloxidase and peroxidase), among other factors that can affect the taste. According to Souto *et al* (2004), enzymes can participate in a large number of oxidative and biodegradation reactions involving, for example, changes in color, chlorophyll degradation, and the oxidation of phenols, and many of these factors can also be associated with the taste, color, texture, and nutritional qualities of food. The ohmically heated juice received higher grades for color flavor, taste and overall acceptance, with an overall impression between “like very much” and “like extremely.”

These results are again consistent with previous investigations of ohmic heating. For example, fresh and ohmically heated orange juices were found exhibit similar flavor compound concentrations. Leizerson & Shimoni (2005b) performed a triangle sensory test to compare fresh, pasteurized and ohmically heated orange juices and found that the panelists could distinguish between fresh and conventionally pasteurized samples but not distinguish between fresh and ohmically heated samples. Tumpanuvatr & Jittanit (2012) indicated that the color and flavor of ohmically heated orange juice and pineapple juice were not significantly different from those of conventionally heated specimens. Thus, ohmic heating of mulberry juice produces similar results to those obtained for other fruit juices.

As a final test, we compared the abilities of ohmic and conventional heating to inactivate the microorganisms in fresh mulberry juice to avoid microbial spoilage (Table 6). In this study, we attributed the inactivation of microorganisms to heat only. Both ohmic and conventional thermal treatments reduced the microbial counts to zero, possibly because there was very little bacterial contamination in the untreated product (Trouvé *et al* 1991). The microbiological viability of fresh and heat-treated juice in terms of the general bacteria counts, mold and yeast counts, and *E. coli* counts are shown in Table 6. The fresh juice had a general bacteria count of 3.70±0.25, a yeast and mold count of 1.30 ± 0.33, and an *E. coli* count of 1.00 ± 0.51 log CFU/g. These results are similar to the findings of Palaniapcpan *et al* (1992). Similarly, Rezzadori *et al* (2013) reported that heat-processed juices contained no detectable total plate appearances, yeast and molds, or *E. coli*. Furthermore, Leizerson & Shimoni (2005a) reported another similar result: ohmic and conventional heating sterilization of orange juice resulted in no detectable microorganisms. Rivas *et al* (2006) reported that pulsed electric field (PEF) and heat pasteurization treatment of carrot juice and oranges reduced the total plate count, mold count and yeast count.

Table 6. Changes in microbial counts of fresh, conventionally heated and ohmically heated mulberry juice

Processing process	General bacteria	Yeast molds	*E. coli*
Fresh juice	3.70±0.25	1.30±0.33	1.00±0.51
Conventionally heated juice	N.D	N.D	N.D
Ohmically heated juice	N.D	N.D	N.D

CFU : colony-forming units.
N.D. : no detect.
Values are means±standard deviations(S.D.) of triplicate analysis from 3 different productions.

In the present study, conventionally and ohmically heated mulberry fruit juices were compared to fresh juice to evaluate the impact of this technology on their quality characteristics. The physicochemical properties, TSS, pH, TA, and reducing power of the fresh juice were significantly different after the different heat treatments. The physicochemical properties were changed less by ohmic heating than by conventional heating. The L and a color values were decreased, and the b, H and C color values were increased by the heat treatments. The total color differences was lower for ohmic heating than for conventional heating, including, in particular, the change in H. This may be because the shorter heating time used for ohmic heating was not sufficient to inactivate the enzymes in mulberry fruit juice. The antioxidant capacity and antioxidant contents were the highest for fresh juice, lower after ohmic heating and even lower after conventional heating. Thus, the more rapid the process, the better the antioxidant capacity was preserved. Finally, sensory evaluation analysis showed no difference between fresh and ohmically heated mulberry fruit juice, while conventionally heated juice scored significantly lower. The general bacteria and yeast, mold and *E. coli* counts were all zero after both heat treatments.

While sterilization is important for ensuring the stability of fruit juice during transportation and storage, it may cause irreversible loss of nutritional quality and antioxidant activity and, in consequence, may affect the health-related properties of the juice. In conclusion, ohmic heating is a suitable technology for preserving bioactive nutrients in fruit juices that produces safe and fresh-seeming juice. Further studies are being conducted to explore the effect of this treatment on the long-term stability and shelf life of such products.

Summary

Ohmic heating is an alternative fast-heating method that is especially useful for the sterilization of liquid foods. The aim of this study was to compare the physicochemical, antioxidant, microbiological, and sensory characteristics of unheated, conventionally heated, and ohmically heated mulberry fruit juice to confirm the suitability of ohmic heating for practical applications. Heat treatment of fresh juice significantly reduced the concentration of soluble solids, lowered the pH, and lowered the reducing sugar content (p<0.01), and because of its long heating time, conventionally treated juice showed a significantly larger reduction in the amount of soluble solids and in the reducing sugar content (p<0.01). Color measurements showed decreases in the L and a values and increases in the b, H and C values after heat treatment, although the total color differences were smaller after ohmic heating than after conventional heating of fresh juice. Furthermore, the antioxidant capacities decreased from fresh, to ohmically heated, to conventionally heated mulberry fruit juices, as shown by reducing power, FRAP and DPPH assays and the anthocyanin, flavonoid and phenolic antioxidant contents of the juices also decreased significantly in the same order. Sensory evaluations showed no difference between fresh and ohmically heated mulberry fruit juice, whereas conventionally heated juice scored significantly worse. No microbial counts were detected in the juice after either heat treatment. Thus, ohmic heat treatment can be effectively used to sterilize fresh mulberry juice to obtain good shelf life safety with minimal physicochemical, color, antioxidant, and sensory deterioration.

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