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ScienceDirect
Food Chemistry 104 (2007) 1396–1401
Food Chemistry
www.elsevier.com/locate/foodchem

Effects of sonication and carbonation on guava juice quality

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Received 1 June 2006; received in revised form 4 January 2007; accepted 5 February 2007

Abstract

The effects of carbonation and sonication on the quality of guava juice were studied for selected physicochemical properties such as colour, cloud stability, pH, acidity, total soluble solids, polyphenoloxidase (PPO) activity, ascorbic acid content and microbial stability. Ascorbic acid content was found to be significantly ($P < 0.05$) higher in samples treated with carbonation and sonication than in the control. It is suggested that carbonation provides more nuclei for cavitations that permit the elimination of dissolved oxygen in the juice. In addition, such a treatment also gave rise to a greater cloudiness and PPO activity, which could be attributed to the production of a stabilized colloid system due to smaller particle size and higher phenolic compounds availability, respectively. Nevertheless, sonication, coupled with carbonation, was not an efficient treatment for microbial inactivation at room temperature.

Keywords: Juice; Sonication; Carbonation; Ultrasound; Guava

1. Introduction

As consumers are becoming more health conscious, demands for natural and fresh-like foods have increased interest in using non-thermal technologies in food processing to produce foods with minimal damage to nutritional and sensory properties. Several technologies are being explored as a potential alternative to thermal processing, among which are membrane filtration, osmotic dehydration, pulse electric field, ultrasound, irradiation and high pressure. Research in this area is currently underway worldwide. However, the economic viability of these emerging technologies still needs to be determined before use of such technologies becomes commonplace.

Sonication is seen to be useful for minimal processing, due to the fact that transferring of acoustic energy to food is instantaneous and throughout the whole product. This means to say that sonication could affect a process with reduced processing time, higher throughput and lower energy consumption (Mason, Paniwnyk, & Lorimer, 1996; Piyasena, Mohareb, & McKellar, 2003; Zenker, Hienz, & Knorr, 2003). When high power ultrasound propagates in a liquid, cavitation bubbles will be generated due to pressure changes. These micro bubbles will collapse violently in the succeeding compression cycles of a propagated sonic wave. This results in regions of high localized temperatures up to 5000 K and 50,000 kPa, and high shearing effects (Mason, 1991; Piyasena et al., 2003). Consequently, the intense local energy and high pressure bring about a localized sterilization effect.

The lethal effects of ultrasonic waves have been proven by many research groups. The lethal effect of ultrasound is reported to be very much dependent on type of microorganism and processing parameters and medium. It is found that ultrasound treatment is not so effective on small and round cells and spores (Ahmed & Russell, 1975; Allinger, 1975; Boucher & Lechowich, 1979; Ordonez, Sanz, Hernandez, & Loper-Lorenzo, 1984; Seymour, Burfoot, Smith, Cox, & Lockwood, 2002; Suslik, 1988) On the other hand, sonication, when applied together with heat, appeared to have a synergistic effect in killing microorganisms (Ciccolini, Taillandier, Wilhem, Delmas, & Strehaiano, 1997; Garcia, Burgos, Sanz, & Ordonez, 1989; Sala, Bur-

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doi:10.1016/j.foodchem.2007.02.001

gos, Condon, Lopez, & Raso, 1995). Zenker et al. (2003) also reported that thermosonic (heat plus sonication) treatments of liquid foods required a lower processing temperature than did treatment with conventional thermal processing to achieve equivalent degrees of bacterial inactivation.

In the present work, the effect of sonication, coupled with carbonation, on quality of guava juice was studied. Carbonation is, overall, referred to a process whereby carbon dioxide is applied to a medium. The use of carbon dioxide as a preservative against microbial spoilage is of minor significance. However, when dissolved in liquid it is believed to reduce surface tension and to create more nuclei for cavitation. Hence, its effects, in enhancing the sonication process, on guava juice quality are a subject of interest in this study.

2. Materials and methods

2.1. Preparation of guava juice

Guava (*Psidium guajava*) fruits were purchased in bulk from a local wet market in Penang, Malaysia. The fruits were screened, washed, pitted and cut into slices prior to juice extraction by using a home juicer. Juice was then filtered with sterilized muslin cloth to remove coarse particles.

2.2. Experimental design and statistical analysis

A completely randomized design, with a 2×2 factorial set of carbonation (with and without) and sonication (with and without), was adopted. Where necessary, general linear ANOVA and least significant difference multiple comparison were carried out on the experimental data obtained by using SPSS Version 12.00 (SPSS Inc., Chicago, IL, USA) at a probability level of 5%.

2.3. Carbonation and sonication treatments

Treatments were performed immediately after fresh guava juice was prepared. Juice collected (~1 l) was divided into two halves. The first half was subjected to carbonation by adding 250 g of dry ice purchased from Tokai Marine & Trading Sdn. Bhd., Malaysia. The juice was then subdivided into two halves (~250 ml each) after warming (~15 min) to room temperature (~20 °C), which would serve as a carbonated sample and be subjected to sonication at 35 kHz in an Elma® cleaning bath (Model T700h W/Acc, Singen, Germany) for 30 min, respectively. The latter was a combination sample which was treated with both carbonation and sonication.

On the other hand, the second half of the juice collected was subdivided into two parts to serve as a control sample and as a sonicated sample, respectively. The sonicated sample was treated under the conditions as described earlier. All control and treated samples were treated in sterilized media bottles and stored at 4 °C before being analyzed within 24 h. Sample preparation and treatments were carried out in duplicate. Temperature of all samples was controlled in the range of 15–20 °C.

2.4. Method of analysis

2.4.1. pH and total soluble solids

The pH and total soluble solids were determined with a Hanna pH meter (pH 210, Bedford, UK), and a hand refractometer (Atago, Model HSR-500, Tokyo, Japan), respectively. Temperature compensation for refractometry was determined.

2.4.2. Titratable acidity

Sample (1 g) was titrated with 0.1 N sodium hydroxide (NaOH) to the end-point in the presence of phenolphtalein indicator (1%). Total acidity was calculated with the following equation, with reference to malic acid:

$$\%\text{acid} = \left[\frac{\text{ml} \times (0.1\ \text{N NaOH}) \times (0.067) \times (100)}{\text{grammes of sample}}\right]$$

2.4.3. Ascorbic acid determination

Ascorbic acid content was determined according to the AOAC 967.21 standard procedure (AOAC, 1995). Samples of 10 ml each were diluted to 100 ml with 3% metaphosphoric acid and then filtered through Whatman (no. 4) filter paper. An aliquot of 5 ml filtrate was titrated with 2,6-dichlorophenol iodophenol (DCPIP) indicator to the end point. Ascorbic acid content was calculated as milligrammes ascorbic acid per 100 ml sample juice.

2.4.4. Colour analysis

Colour measurements were made using a Minolta colorimeter (Model CM-3500d, Osaka, Japan). CIE $L^*a^*b^*$ values were measured and total colour difference was calculated as $\{(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}\}$.

2.4.5. Clarity

To measure clarity, a sample was centrifuged at 2500*g* for 10 min; the supernatant was taken, and the percent transmittance was measured from 400 to 800 nm with a Shimadzu UV–vis spectrophotometer (Model 1601, Tokyo, Japan). Distilled water was used as a reference. High percent transmittance corresponds to high clarity.

2.4.6. Assay for polyphenoloxidase (PPO) activity

PPO activity of treated samples was conducted, following Augustin, Ghazali, and Hashim (1985). Enzyme activity was determined by measuring the rate of increase in absorbance at 410 nm with a Shimadzu UV–vis spectrophotometer (Model 1601, Tokyo, Japan) at room temperature. The reaction mixture contained 0.5 ml 0.05 M catechol, 1.5 ml centrifuged sample and 0.2 M potassium phosphate buffer (pH 6.8) in a final volume of 5 ml. The rate of reaction was calculated from the initial linear slopes

of activity curves. The enzyme unit (U) was defined as the change in absorbance of 0.001 min^{-1} under the conditions of the assay.

2.4.7. Microorganism analysis

A series of decimal dilutions was prepared with 0.1% (w/v) peptone water. One millilitre of decimal dilutions of samples was pipetted into Petri dishes. Total plate counts were enumerated using the pour plate method. Plate count agar and plates were incubated at 37 °C for 48 h. Total yeast and moulds were enumerated on potato dextrose agar by the pouring plate technique, as well. Incubation for total yeast and moulds counts was done at 25 °C for 5 days. Each test was performed in duplicate and results were expressed as log colony-forming units (CFU) per millilitre.

3. Results and discussion

The analysis results of the carbonation and/or sonication treatments (Table 1) show that no significant differences were observed for pH, percent acidity or total soluble solids among the samples studied. The pH values determined ranged from 3.90 to 3.93, which were fairly consistent with the values reported by Yusof (2003), i.e. 3.2–4.1. On the other hand, total acidity did not vary upon carbonation which may be explained by the buffering effect of components present in the fruit juice (Rodrigo et al., 2003; Watson, 2004). Sugars are the major soluble solids in fruit juice. The data of total soluble solids measurement do not differ significantly from one another, because samples were freshly prepared, and it is believed that onset of microbial fermentation had not taken place in the samples prior to analysis.

Table 1 shows that the ascorbic acid content in all treated samples was significantly ($P < 0.05$) higher than in the control sample. Samples treated with both sonication and carbonation showed the highest ascorbic acid contents. This could be attributed to the lower temperature and cavitations effects caused by carbonation and sonication, respectively. When dry ice was incorporated during carbonation, sample temperature decreased substantially before it was warmed to room temperature. This reduced temperature could have disfavoured ascorbic acid degradation, because the rate of ascorbic acid degradation is temperature-dependent (Al-Zubaidy & Khalil, 2007; Burdurlu, Koca, & Karadeniz, 2006). On the other hand, employment of sonication resulted in higher ascorbic acid content, the most likely reason being the elimination of dissolved oxygen that is essential for ascorbic acid degradation during cavitation. This phenomenon is enhanced with carbonation due to the fact that dissolved carbon dioxide could have served as nuclei sites for cavitations (Mason, 1991). This explains the high ascorbic acid content observed in samples treated with both carbonation and sonication.

There were significant differences ($P < 0.05$) in all colour attributes among samples studied (Table 2). With respect to lightness (L^*), the lowest value corresponded to the combination sample. In addition, the same sample showed the highest red component ($+a^*$) and yellow component ($+b^*$). This subsequently results in the greatest colour difference observed in combination juice with reference to control sample. Nevertheless, this minute total colour difference can not be distinguished by the naked eye.

Fig. 1 illustrates that combination treatment was most effective in producing high clarity, with sonication treatment the next and carbonation the least effective. According to Balaban (2003), carbonation inactivates pectinesterase which is capable of destabilizing colloidal pectin molecules suspended in a juice. Hence, marginally

Table 1
Chemical analysis of guava juices

Samples	pH[A]	% Acidity[A]	Total soluble solids[A] (°Brix)	Ascorbic acid content[A] (mg/100 ml)
Control	3.93 ± 0.03^{a}	0.46 ± 0.02^{a}	8.20 ± 0.02^{a}	110 ± 0.5^{a}
Carbonated	3.91 ± 0.02^{a}	0.47 ± 0.01^{a}	8.32 ± 0.03^{a}	115 ± 0.8^{b}
Sonicated	3.92 ± 0.01^{a}	0.46 ± 0.01^{a}	8.16 ± 0.02^{a}	119 ± 0.8^{c}
Combination	3.90 ± 0.01^{a}	0.47 ± 0.04^{a}	8.28 ± 0.04^{a}	125 ± 1.1^{d}

[A] Means ± SD ($n = 4$), within a column with the same letter are not significantly different at the 5% probability level.

Table 2
Effects of carbonation and/or sonication on colour attributes of guava juices

Samples	Colour attributes[A]			Total colour difference[A] ΔE^*
	L^*	a^*	b^*	
Control	94.00 ± 0.04^{a}	-0.01 ± 0.01^{a}	5.57 ± 0.03^{a}	NIL
Carbonated	94.02 ± 0.08^{a}	-0.03 ± 0.02^{b}	5.56 ± 0.08^{a}	0.08 ± 0.04^{a}
Sonicated	93.30 ± 0.06^{b}	0.06 ± 0.02^{c}	5.98 ± 0.05^{b}	0.84 ± 0.08^{b}
Combination	92.62 ± 0.09^{c}	0.16 ± 0.01^{d}	6.40 ± 0.05^{c}	1.62 ± 0.08^{c}

[A] Means ± SD ($n = 4$), within a column with the same letter are not significantly different at the 5% probability level.

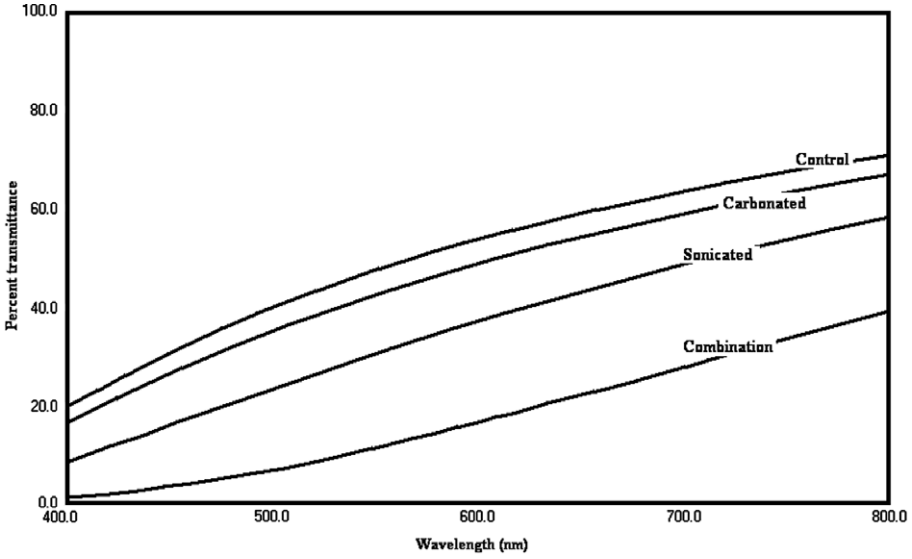

Fig. 1. Sample spectra illustrating cloud stability determined by percent transmittance.

decreased (Fig. 1) clarity was evident in the carbonated sample. On the other hand, sonication with its high shearing effect occurring during cavitation, will not only denature pectinesterase but also fragment colloidal pectin molecules into a smaller size which would be more stable in the colloid than the others. This particle size reduction explains why more fine particles were retained in the supernatant after centrifugation, resulting in a lower clarity. This effect is more prominent in the combination sample.

The results of carbonation and sonication effects on guava polyphenoloxidase (PPO) activity are shown in Fig. 2. It can be seen that treatments employed in this study were not effective in deactivating PPO; on the contrary, they enhance its activity. Values show in Table 3 summarize the PPO activity calculated for each treatment. No appreciable differences in PPO activity were evident for control and carbonated samples. However, sonicated and combination samples showed relatively higher PPO activity when compared to control and carbonated samples. In addition, it is interesting to note that neither additive nor synergistic benefit was demonstrated by the combination sample. One explanation for this experimental finding is that the use of power ultra-

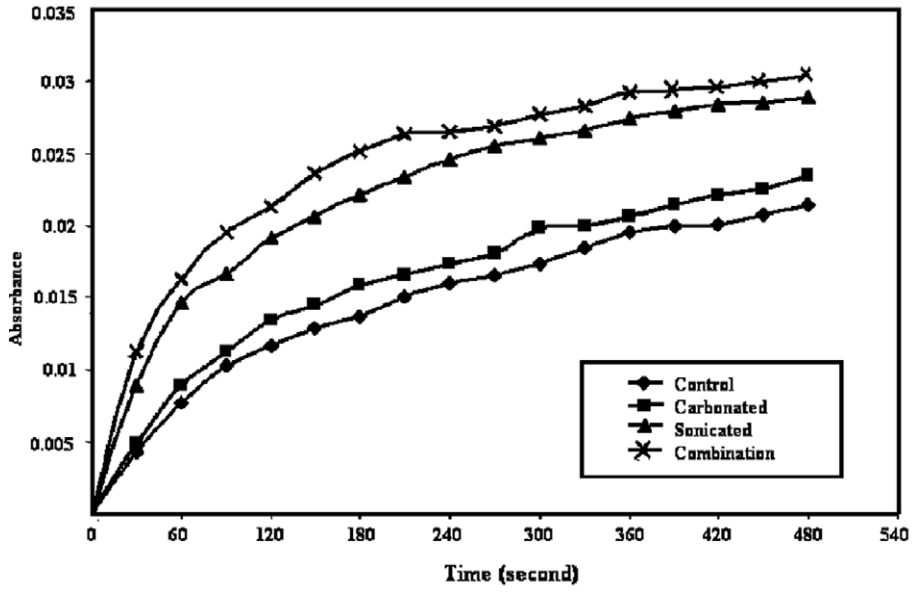

Fig. 2. Measurement of absorbance at 410 nm as a function of time for various treated samples.

Table 3
Guava polyphenoloxidase activity after each treatment

Samples	Polyphenoloxidase activity (U)[A]
Control	10.1 ± 0.60^{a}
Carbonated	11.0 ± 0.50^{a}
Sonicated	18.0 ± 1.5^{b}
Combination	20.1 ± 1.2^{b}

[A] Means ± SD ($n = 4$), with the same letter are not significantly different at the 5% probability level.

Table 4
Effects of carbonation and sonication treatments on the survival of microorganisms in guava juice[A]

Samples	Microbial survival (log CFU/ml)	Yeast and mould survival (log CFU/ml)
Control	4.05 ± 0.02^{a}	3.22 ± 0.21^{c}
Carbonated	3.86 ± 0.15^{bc}	3.33 ± 0.05^{b}
Sonicated	3.94 ± 0.10^{b}	3.06 ± 0.06^{d}
Combination	3.79 ± 0.09^{c}	3.47 ± 0.08^{a}

[A] Means ± SD ($n = 6$), within a column with the same letter are not significantly different at the 5% probability level.

sound enhanced the disruption of biological cell walls to facilitate the release of their contents (Mason et al., 1996). According to Escarpa and Gonzalez (2001), some phenolic compounds are bound to the cell wall; therefore, it is reasonable to suggest that these bound phenolic compounds could have been released into the juice upon sonication via cavitational collapse in the surroundings of colloidal particles, consequently giving rise to a higher PPO activity in both sonicated and combination samples.

Table 4 shows the results of standard aerobic plate count (APC) and yeast and moulds in treated samples. In general, a slight decrease in APC (<1-log cycle) was noted when the sample was treated with carbonation and/or sonication, whereas the destruction of yeast and moulds did not show a consistent trend. This implied that the microorganisms were resistant to the treatments, and corroborated Piyasena et al. (2003) who reported that sonication alone was not very effective in destroying microorganisms, except when coupled with pressure and/or heat. The mechanism of microbial inactivation is reported to be mainly due to thinning of cell membranes, localized heating and production of free radicals (Butz & Tauscher, 2002; Fellows, 2000). Hence, sonication, coupled with carbonation, was found to be of little value in killing microorganism at room temperature.

4. Conclusion

The combination effects of carbonation and sonication have been evaluated on guava juice. The obtained results show that carbonation enhanced the manifestation of cavitation and subsequently produces juice with higher ascorbic acid content, lower clarity and higher polyphenoloxidase activity. Unfortunately, these treatments do not have strong lethal effects on microorganisms.

Acknowledgement

Financial support from the Universiti Sains Malaysia Short Term Grant (304/PTEKIND/636011) is gratefully acknowledged by the authors.

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