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Effects of thermosonication on apple and guava juices quality

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ABSTRACT

Apple and guava juice samples were sonicated with processing variables of amplitude levels (21, 30 and 40%), temperature (20, 35 and 50 °C) at a constant frequency of 20 kHz, sonication times (2, 5 and 10 min) and pulse duration of 5 s on and 5 s off. The effects of sonication on the quality of apple and guava juice were studied for selecting physicochemical properties such as Hunter colour values (L*, a* and b*), turbidity, pH, titratable acidity, total soluble solids, and ascorbic acid content. Hunter colour values (L*, a* and b*), pH, °Brix, titratable acidity, cloud value and browning index were measured. Ascorbic acid content was found to be lower in samples treated with sonication than in the control. Retention of quality parameters was observed at the maximum treatment conditions of 40% amplitude level for 10 min, indicating stability of colour during sonication. Colour changes observed during sonication were subtle (TCD from 0 to 6.88 in apple juice and from 0 to 1.68 in guava juice). Colour values (L*, a*, b*), total colour difference (TCD), chroma, hue angle, browning index, ascorbic acid and turbidity were influenced by both ultrasonic amplitude level and treatment time, with the effects observed being either individual or interactive. A sonication treatment was demonstrated to be an effective technique to investigate the influence of sonication on colour and quality retention. Sonication could be employed as a preservation technique for apple and guava juice processing where colour and quality retention is desired.

Key words: apple, guava, juice, sonication, colour, turbidity, quality, vitamin C.

Introduction

Ultrasound is defined as sound waves having frequency that exceeds the hearing limit of the human ear (~20 kHz). Some animals utilize ultrasound for navigation (dolphins) or hunting (bats) using the information carried by back-scattering sound waves. Ultrasound is one of the emerging technologies that were developed to minimize processing, maximize quality and ensure the safety of food products. Ultrasound is applied to impart positive effects in food processing such as improvement in mass transfer, food preservation, assistance of thermal treatments and manipulation of texture and food analysis (Knorr *et al.*, 2011).

Apple, guava and their products are sensitive to high temperature treatments, such as thermal sterilization. The thermal processing of apple juice results in off-flavor formation, colour, vitamins and aromatic compound degradation (Hayashi, 1996). The introduction of new technologies in food industry might reduce the processing time and improve the industrial operating conditions, resulting in high quality products that preserve the natural characteristics of the food (Butz and Tauscher, 2002; Cárcel *et al.*, 2011). The ultrasound processing (US), also called sonication, is a non-thermal technology that has been effective in the inactivation of microorganisms and enzymes related to degradation of fruit juices allowing the treatment of thermo-sensitive food (Rawson *et al.*, 2011). Despite the fact that some authors reported some negative impacts on juice quality due to ultrasound processing, such as: a slight deterioration of sensory quality in calcium added orange juice (Gómez-López *et al.*, 2010) and some off-flavor formation in orange juice (Wong *et al.*, 2010), according to a recent review published by O'Donnell *et al.*, (2010). Fruit juices treated with ultrasound suffered minimal effects on the quality of final product and, because of that, this technology has been studied for several applications in food processing.

Ultrasound produces repeated cycles of compression and decompression called acoustic cavitation, which is the process of nucleation, growth and collapse of bubbles in liquids exposed to ultrasonic waves at low frequency (20 kHz–100 kHz) and high power (10–1000 W/ cm²). The collapse of the bubbles generates high local temperatures (5000 K) and high pressures (1000 atm) resulting in high shear rates and generating strong micro-streaming, which can contribute for enzyme and microbial inactivation (Apfel, 1981; Mason, 1991). The enzyme and microbial inactivations due to ultrasound have been reported as dependent on the nature of the enzyme; the process variables (ultrasound power intensity, ultrasound frequency, temperature or pressure); the

characteristics of the medium (viscosity, food matrix composition) as well as on the type of connection and chemical reactions that they establish with other molecules (Cárcel *et al.*, 2011 and Chemat *et al.*, 2011).

Among the food enzymes, peroxidase (POD) and polyphenol oxidase (PPO) are frequently involved in multiple deteriorative changes, such as enzymatic browning, with consequent loss of sensorial and nutritional properties of fruit and vegetables (Oms-Oliu *et al.*, 2008). These enzymes are usually inactivated by thermal treatments, which demand large amount of energy besides imparting several quality losses (Pereira and Vicente, 2010).

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. 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 result in regions of high localized temperatures up to 5000 K and 50,000 kPa, and high shearing effects (Piyasena *et al.*, 2003 and Zenker *et al.*, 2003). Consequently, the intense local energy and high pressure bring about a localized sterilization effect.

Power ultrasound is an emerging and promising alternative technology to heat treatment for food processing (Mason *et al.*, 2005). Power ultrasound has been previously reported to be effective against food borne pathogens in orange juice (Valero *et al.*, 2007), apple cider and milk (Dennis *et al.*, 2006) and guava juice in combination with carbonation (Cheng *et al.*, 2007). Ultrasound has been identified as a potential technology to meet the FDA requirement of a 5 log reduction in pertinent microorganisms found in fruit juices (Salleh-Mack and Roberts, 2007).

Juice colour is a key factor in influencing consumer sensory acceptance. For a fruit juice, colour is typically measured by light absorbance at a specific wavelength of 420 nm, (Wong & Stanton, 1992), total colour difference (TCD) or Hunter colour parameters (L, a, b) (Ahmed *et al.*, 2002; and Chutintrasria and Noomhorm, 2007). Differences in perceivable colour can be analytically classified as very distinct (TCD > 3), distinct (1.5 < TCD < 3) and small difference (TCD < 1.5) (DrLange, 1999). L indicates lightness, a indicates chromaticity on a blue (-) to yellow (+) axis and b indicates chromaticity on a green (-) to red (+) axis (Rocha and Morais, 2003). A decline in lightness (L value) has been associated with browning in fruit (Labuza *et al.*, 1992). To minimize colour degradation and browning in juice, the optimization of non-thermal processing parameters is required.

Cheng *et al.*, (2007) found that the ascorbic acid content was higher in samples treated guava juice with carbonation and/or 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. 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.

Despite the several works that have been published on ultrasound processing of fruit juices, few are addressed to enzyme inactivation and the effects of sonication on apple juice quality parameters have not been reported elsewhere. Therefore, the purpose of the present work was to find optimum ultrasound operating conditions for the processing of apple juice and to evaluate the effects of US processing on juice quality parameters.

The objective of this work was to investigate the effect of sonication on changes in colour, browning and cloud value of apple and guava juice as a function of sonication time and amplitude level using response surface methodology.

Materials and methods

2.1. Preparation of Apple and Guava Juices samples:

Plant Material: Apples (Anna delicious) and Guava (*Psidium guajava*) were obtained from the store of the Ministry of Agriculture, Cairo, Egypt, and were kept in cold conditions (4 °C) until needed. The apple cultivar was chosen because of its wide popularity as a food and rapid browning of the slices after preparation. Fruits were washed before the treatment. Apple and guava samples representing common cultivars were obtained from local food stores during the fall and winter of 2012 and stored briefly at 4 °C until needed. One hour prior to use, fruits were removed from the refrigerator and equilibrated to room temperature. Apple and guava fruits were rinsed with water, sectioned to longitudinal slices. Apple and Guava Juices samples were prepared from

individual apples with a juicerator and guava juice with drained steel. Juice was collected in a beaker containing 5mg ascorbic acid / 100ml juice with stirring. The amount of ascorbic acid used was not enough to prevent browning for more than 1 hr. However, the ascorbic acid was used to prevent instantaneous browning thereby providing a short lag time to allow test experiment to be sonicate treated and processed (Sapers and Douglas, 1987).

2.2. Ultrasound treatment:

A 1500-W ultrasonic processor (DAIGGER, 281 of -1, ILL 6006 I, Sonics and Materials Inc., USA) with model GEX 750 and serial No. 50292T was used for sonication. Samples were processed at a constant frequency of 20 kHz with pulse duration of 5s on and 5s off. The energy input was controlled by setting the amplitude of the sonicator probe. Extrinsic parameters of amplitude (21, 30 and 40%), temperature (20, 35 and 50 °C) and time (2, 5 and 10 min) were varied with pulse durations of 5 s on and 5 s off, according to Rawson *et al.*, (2011). Twenty milliliters of apple and guava juice samples were used. The ultrasound probe was submerged to a depth of 2 cm in the sample. All treatments were carried out in triplicates.

2.3. Methods of analysis:

2.3.1. Turbidity:

Cloud value of samples was measured spectrophotometrically as absorbance at 660 nm using 4054 - UV/Visible spectrophotometer, (LKB-Biochrom Comp., London, England), with distilled water serving as a blank (Tung-Sun., *et al* 1995).

2.3.2. pH determination:

The pH of apple and guava juice samples was measured using a digital pH-meter (HANNA, HI 902 meter, Germany).

2.3.3. Total Soluble Solids (^oBrix):

The percent of Total Soluble Solids (TSS), expressed as ^oBrix (0-32), was determined with a Hand refractometer (ATAGO, Japan). Measurements were performed at 20.0 ±0.5°C. The refractometer prism was cleaned with distilled water after each analysis.

2.3.4. Titratable acidity (TA):

Sample (1 g) was titrated with 0.1 N sodium hydroxide (NaOH) to the end-point in the presence of phenolphthalein indicator (1%), according to the method of Tung-Sun., *et al* (1995). Total acidity was calculated with reference to malic acid in apple juice and citric acid for guava juice:

2.3.5. Ascorbic acid or Vitamin C content determination:

Vitamin C was analyzed using the AOAC (2006) method. The titrant was prepared with 50 mg of 2, 6-dichloroindophenol Na salt and 42 mg of sodium bicarbonate in 50 mL of water. The solution was diluted to 200 mL with distilled water. A 100 mL aliquot of apple and guava juice was added to 100 mL of the extracting solution and then filtered using a No.1 filter paper (Whatman, Maidstone, England). The solution was then titrated with the titrant until the solution turned bright pink for at least 5 s. A standard curve was created using pure ascorbic acid (Sigma Aldrich, St. Louis, MO). Vitamin C retention was calculated using equation (2).

$$\text{Retention (\%)} = \frac{\text{mg ascorbic acid/100 mL juice after treatment}}{\text{mg ascorbic acid/100 mL juice before treatment}} \times 100 \quad (1)$$

2.3.6. Non-enzymatic browning determination:

Non-enzymatic browning was measured spectrophotometrically by 4054 - UV/Visible spectrophotometer, (LKB-Biochrom Comp., London, England), as absorbance at 420nm using ethanol as blank according to the method of Birk *et al* (1998).

2.3.6. Non-enzymatic browning (A_{420nm}):

The browning index was measured using the method of Meydav *et al.*, (1977). A 10-ml apple and guava juice sample was centrifuged (10 min, 3000 rpm) (Hanil Union 32R, 32GRh, Korea) to remove coarse particles from the sample. Five milliliters of ethyl alcohol (95%, Sigmae Aldrich, Dublin, Ireland) was added to 5 ml of juice supernatant and centrifugation was repeated. The absorbance of the supernatant was measured spectrophotometrically at 420 nm using 4054 - UV/Visible spectrophotometer, (LKB-Biochrom Comp., London, England).

2.3.7. Colour determination:

Colour of Egyptian sonicated and non-sonicated apple and guava juice was measured using spectro-colourimeter (Tristimulus Colour Machine) with the CIE lab colour scale (International Commission on Illumination) as mentioned by Hunter (1975) and Sapers and Douglas, (1987). Colour of unsonicated and sonicated apple and guava juice samples was measured using a HunterLab colourimeter Hunter a*, b* and L*. Parameters were measured with a colour difference meter using a spectro-colourimeter (Tristimulus Colour Machine) with the CIE lab colour scale (Hunter, Lab Scan XE - Reston VA, USA) in the reflection mode. The instrument was standardized each time with white tile of Hunter Lab Colour Standard (LX No.16379): X= 72.26, Y= 81.94 and Z= 88.14 (L*= 92.46; a*= -0.86; b*= -0.16). The instrument (65°/0° geometry, D25 optical sensor, 10° observer) was calibrated using white and black reference tiles. The colour values were expressed as L* (lightness or brightness/ darkness), a* (redness/greenness) and b* (yellowness/blueness). The Hue (H)*, Chroma (C)* and Browning Index (BI) was calculated according to the method of Palou *et al.* (1999) as follows:

$$H^* = \tan^{-1} [b^*/a^*] \quad (2)$$

$$C^* = \text{square root of } [a^{2*} + b^{2*}] \quad (3)$$

$$BI = [100 (x-0.31)] 10.72 \quad (4)$$

Where:- $X = (a^* + 1.75L^*) / (5.645L^* + a^* - 3.012b^*)$

Total colour difference (TCD) was determined using Eq. (5) which indicates the magnitude of the colour change after treatment. Colour measurements were taken in triplicate.

$$TCD = \sqrt{(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2} \quad (5)$$

where L_0 is initial value of L^* , a_0 is initial value of a^* , and b_0 is initial value of b^* . L^* , a^* and b^* values were recorded as the mean of triplicate readings.

2.3.8. Statistical analysis:

Mean values from the three separate experiments or replicate analysis were reported. The obtained results were analyzed statistically using the analysis of variance (ANOVA with two ways) and the Least Significant Difference (LSD) as described by Richard and Gouri, (1987).

Results and Discussion

3.1. Effect of sonication on photochemistry of apple and guava juices:

The values obtained for titratable acidity, pH and °Brix of sonicated and unsonicated samples are shown in Tables 1 and 2. Sonication of freshly apple and guava juice with ultrasound irrespective of ultrasonic amplitude level (%) and time did not cause significant difference ($p < 0.05$) in these parameters. Titratable acidity, pH and °Brix for control samples were 3.57 g of malic acid / 100ml of apple juice, 0.502 and 11 and 3.64 g of citric acid/100 ml of guava juice, 0.58 and 10, respectively. The mean values for pH, °Brix (total soluble solids) and titratable acidity of control and sonicated apple and guava juice samples under the maximum treatment conditions (61 lm; 10 min) are shown in Tables 1 and 2. Sonication of apple and guava juice with ultrasound, irrespective of amplitude level (lm) or treatment time, did not cause significant differences ($p < 0.05$) in these parameters. These results are in agreement with those obtained by Cheng *et al.*, (2007) for sonicated guava juice and Ugarte-Romero *et al.* (2006) for apple cider.

The analysis results of sonication treatments (Tables 1 and 2) show that no significant differences were observed for pH, percent acidity or total soluble solids among the apple and guava juice samples studied. The determined pH values were decreased by increased of pulser from 21 to 40 % ranged from 3.52 to 3.33, when the determined acidity value were increasing from 0.6 to 0.74 in apple juice. Similar results were found in guava juice, as seen in Table 2. On the other hand, total acidity did not vary upon sonication which may be explained by the buffering effect of components present in the fruit juice (Rodrigo *et al.*, 2003; Watson, 2004). Sugars

were 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: Effect of thermosonication (time, temp., and pulser) on physico-chem properties of apple juice.

samples	Time (min.)	Temp. (°C)	Pulser (%)	pH	TSS (°Brix)	ACIDITY (%)
Con.	0	0	0	3.57±0.014	11	0.502±0.04
A1	2 min.	20	21%	3.435±0.007	11	0.642±0.04
A2			30%	3.415±0.007	11	0.667±0.04
A3			40%	3.413±0.056	11	0.669±0.04
B1		35°c	21%	3.525±0.007	11	0.603±0.0
B2			30%	3.395±0.021	11	0.67±0.0
B3			40%	3.33±0.014	11	0.737±0.0
C1		50°c	21%	3.525±0.007	11	0.603±0.0
C2			30%	3.48±0.014	11	0.653±0.09
C3			40%	3.43±0.014	11	0.636±0.0
Con.	0	0	0	3.49±0.002	11	0.703±0.04
A1	5 min.	20°c	21%	3.445±0.007	11	0.736±0.04
A2			30%	3.425±0.007	11	0.746±0.0
A3			40%	3.405±0.007	11	0.763±0.0
B1		35°c	21%	3.53±0.014	11	0.569±0.04
B2			30%	3.47±0.014	11	0.703±0.04
B3			40%	3.405±0.007	11	0.743±0.04
C1		50°c	21%	3.465±0.007	11	0.737±0.0
C2			30%	3.445±0.021	11	0.746±0.0
C3			40%	3.41±0.014	11	0.756±0.0
Con.	0	0	0	3.815±0.035	11	0.502±0.04
A1	10min.	20°c	21%	3.755±0.148	11	0.542±0.04
A2			30%	3.735±0.036	11	0.569±0.04
A3			40%	3.54±0	11	0.589±0.0
B1		35°c	21%	3.63±0.014	11	0.603±0.0
B2			30%	3.55±0.028	11	0.670±0.0
B3			40%	3.48±0.014	11	0.737±0.0
C1		50°c	21%	3.45±0.014	11	0.743±0.0
C2			30%	3.43±0.042	11	0.745±0.09
C3			50%	3.422±0.035	11	0.756±0.0

3.2. Effect of sonication on ascorbic acid content (mg/100ml) of apple and guava juices:

The level values of ascorbic acid in fresh juice were 7.06 and 23.01 mg/100 mL of apple and guava juice respectively which is slightly higher than the values reported by (Cheng *et al.*, (2007) and Ugarte-Romero *et al.* (2006) which may be due to a difference in the guava and apple cultivars investigated, as seen in Table 3 and 4. Ascorbic acid degradation of guava juice samples observed as processing time was increased from 0 to 10 min for an amplitude level of 22.55 mg/100 mL at 20 °C and 6.58 in apple juice samples, as seen in Tables 3 and 4. For guava juice samples processed at 35 °C and an amplitude level of 22.44 mg/100 mL, but for apple juice samples processed at 35 °C and an amplitude level of 5.82 mg/100 mL, % retention of ascorbic acid reduced.

This effect was more pronounced at higher holding times. The retention of AA was 90.22%, 95.47% and 93.2% in apple juice samples and 99.26%, 98.87% and 97.96% in guava juice samples for 2, 5 and 10 min processing times respectively. However when sonication was carried out at a higher temperature (50 °C), degradation of ascorbic acid increased. At the maximum amplitude and longest processing time, % ascorbic acid retention reduced to 80.28 in apple juice samples and 92.70 in guava juice samples% (pb0.05), as seen in Tables 3 and 4. However prolonged processing at higher power levels may induce chemical decomposition of ascorbic acid. Reported degradation of ascorbic acid, during sonication may result from the extreme physical conditions which occur within the bubbles during cavitation collapse at micro-scale (Tiwari *et al.*, 2008a,b) and several sonochemical reactions occurring simultaneously or in isolation. Cavities formed by sonication may be filled with water vapour and gases dissolved in the juice, such as O₂ and N₂ (Kom *et al.*, 2002). The ascorbic acid degradation at higher amplitude and maximum holding times during ultrasonic processing could be related to oxidation reactions, promoted by the interaction of free radicals formed during sonication (Portenlager and Heusinger, 1992). Ascorbic acid is an unstable compound, which under less desirable conditions decomposes

easily; thus the milder the processing, the higher the vitamin C retention in juices (Rawson *et al.*, 2011) (Odriozola-Serrano *et al.*, 2008).

Table 3 and 4 show that the ascorbic acid content in all treated samples was significantly ($P < 0.05$) lower than in the control sample. Samples treated with sonication showed the lowest ascorbic acid contents. This could be attributed to the higher temperature and cavitations effects caused by sonication. This higher temperature could have disfavoured ascorbic acid degradation, because the rate of ascorbic acid degradation is temperature-dependent (Al-Zubaidy and Khalil, 2007 and Burdurlu *et al.*, 2006). On the other hand, employment of sonication resulted in lower ascorbic acid content, the most likely reason being the elimination of dissolved oxygen that is essential for ascorbic acid degradation during cavitation.

Tables 3 and 4, show the effect of amplitude level and treatment time on ascorbic acid content (mg/100ml) in sonicated apple and guava juice. Ascorbic acid degradation is mainly due to sonochemical reactions and the extreme physical conditions which occur during sonication. It is known that hydrogen ions (H^+), free radicals ($O^{\cdot-}$, OH^{\cdot} , HO_2^{\cdot}) and hydrogen peroxide (H_2O_2) are formed during the sonolysis of water molecules (Adekunte *et al.*, 2010) present in juice samples. The ascorbic acid degradation during ultrasonic processing could be related to oxidation reactions, promoted by the interaction with free radicals formed during sonication (Hart and Henglein, 1985 and Adekunte *et al.*, 2010). Hydroxyl radicals produced by cavitation may be involved in the degradation of ascorbic acid. Sonication can be related to advanced oxidative processes, since both pathways are associated with the production and use of hydroxyl radicals.

Table 2: Effect of thermosonication (time, temp., and pulser) on physico-chem properties of guava juice.

samples	Time (min.)	Temp. (°C)	Pulser (%)	pH	TSS (°Brix)	ACIDITY (%)
Con.	0	0	0	3.64±0.034	10	0.576±0.034
A1	2 min.	20°C	21%	3.64±0.051	10	0.595±0.041
A2			30%	3.63±0.051	10	0.608±0.051
A3			40%	3.62±0.051	10	0.627±0.050
B1		35°C	21%	3.62±0.040	10	0.654±0.040
B2			30%	3.61±0.051	10	0.675±0.052
B3			40%	3.59±0.040	10	0.695±0.040
C1		50°C	21%	3.58±0.051	10	0.703±0.051
C2			30%	3.56±0.051	10	0.735±0.040
C3			40%	3.53±0.040	10	0.786±0.051
Con.	0	0	0	3.66±0.003	10	0.565±0.034
A1	5 min.	20°C	21%	3.62±0.040	10	0.609±0.040
A2			30%	3.62±0.034	10	0.678±0.034
A3			40%	3.59±0.051	10	0.748±0.040
B1		35°C	21%	3.57±0.040	10	0.768±0.040
B2			30%	3.57±0.040	10	0.789±0.051
B3			40%	3.55±0.051	10	0.803±0.051
C1		50°C	21%	3.55±0.040	10	0.828±0.040
C2			30%	3.54±0.052	10	0.854±0.040
C3			40%	3.54±0.040	10	0.873±0.051
Con.	0	0	0	3.68±0.034	10	0.583±0.034
A1	10min.	20°C	21%	3.65±0.0404	10	0.672±0.0404
A2			30%	3.61±0.034	10	0.576±0.034
A3			40%	3.55±0.034	10	0.768±0.043
B1		35°C	21%	3.48±0.404	10	0.764±0.051
B2			30%	3.48±0.051	10	0.864±0.051
B3			40%	3.49±0.051	10	0.864±0.043
C1		50°C	21%	3.57±0.040	10	0.874±0.051
C2			30%	3.54±0.051	10	0.863±0.043
C3			50%	3.53±0.034	10	0.874±0.051

Table 3: Effect of thermosonication (time, temp. and pulser) on ascorbic acid (V.C) content of apple juice.

samples	Time (min.)	Temp. (°C)	Pulser (%)	V.C. content.
Con.	0	0	0	7.056±0.040
A1	2 min.	20°C	21%	6.972±0.002
A2			30%	6.312±0.004
A3			40%	6.953±0.001
B1		35°C	21%	6.864±0.005
B2			30%	6.487±0.005
B3			40%	6.644±0.003
C1		50°C	21%	5.994±0.040
C2			30%	5.669±0.005
C3			40%	5.522±0.001
Con.	0	0	0	6.686±0.003
A1	5 min.	20°C	21%	6.737±0.040
A2			30%	6.588±0.005
A3			40%	6.760±0.001
B1		35°C	21%	6.014±0.002
B2			30%	5.988±0.006
B3			40%	6.089±0.003
C1		50°C	21%	5.886±0.004
C2			30%	5.783±0.052
C3			40%	5.685±0.043
Con.	0	0	0	6.946±0.004
A1	10min.	20°C	21%	6.580±0.051
A2			30%	6.029±0.051
A3			40%	6.151±0.002
B1		35°C	21%	5.816±0.003
B2			30%	6.331±0.040
B3			40%	6.21±0.051
C1		50°C	21%	6.005±0.051
C2			30%	6.129±0.040
C3			50%	5.667±0.002

Table 4: Effect of thermosonication (time, temp. and pulser) on ascorbic acid (V.C) content of guava juice.

samples	Time (min.)	Temp. (°C)	Pulser (%)	V.C. content.
Con.	0	0	0	23.014±0.001
A1	2 min.	20°C	21%	22.849±0.003
A2			30%	22.398±0.003
A3			40%	22.029±0.002
B1		35°C	21%	22.562±0.004
B2			30%	22.231±0.001
B3			40%	22.021±0.004
C1		50°C	21%	22.443±0.005
C2			30%	22.212±0.003
C3			40%	22.221±0.004
Con.	0	0	0	23.542±0.001
A1	5 min.	20°C	21%	22.752±0.001
A2			30%	22.251±0.005
A3			40%	22.254±0.003
B1		35°C	21%	22.232±0.005
B2			30%	22.214±0.001
B3			40%	22.012±0.004
C1		50°C	21%	22.437±0.004
C2			30%	22.415±0.001
C3			40%	22.005±0.002
Con.	0	0	0	23.453±0.001
A1	10min.	20°C	21%	22.546±0.003
A2			30%	21.334±0.001
A3			40%	22.553±0.004
B1		35°C	21%	22.446±0.003
B2			30%	22.408±0.005

B3			40%	22.591±0.001
C1		50°C	21%	22.406±0.002
C2			30%	21.541±0.004
C3			50%	21.333±0.001

3.3. Effect of sonication on turbidity in apple and guava juices:

Turbidity of guava juice samples observed as processing time was decreased from 0 to 10 min and 21% pulser for an amplitude level of 2.85 at 20 °C and 2.19 in apple juice samples, as seen in Tables 5 and 6. For guava juice samples were processed at 35 °C and an amplitude level of 22.44 mg/100 mL, while for apple juice samples processed at 35 °C and an amplitude level of 5.82 mg/100 mL, % retention of ascorbic acid was reduced. A decrease in turbidity was observed for the apple and guava juice treated with sonication 10min at 50 °C and 50% pulser to 1.93 and 2.76, respectively. Turbidity in apple and guava juice was decreased by increasing pulser, temperature and sonication time (Tables 5 and 6). Results indicated that the apple and guava juices have been reported to show a decrease in turbidity when treated with sonication. However, the cloud value of the guava juice improved due to the ultrasound treatment. The same results were found by Marowski *et al.*, (2009) who reported that the cloud value of the melon juice was improved due to the ultrasound treatment. In all cases, juice turbidity values fulfilled the requirements given by Dietrich *et al.* (1996) for cloudy juices. The most important parameter, differentiation cloudy guava juice from clear apple juice, was turbidity. Cloudy juices were characterised by having an average total turbidity of 2.85 and 2.36 in fresh guava and apple juice respectively. The same result has been obtained by Marowski *et al.*, (2009).

The turbidity of fruit juices may be due to finely divided particles of pectin, cellulose, hemicellulose, proteins and lipids in suspension (Irwe and Olsson, 1994 and Klavons *et al.*, 1994). The cloud stability is a desirable feature in fruit juices because it favorably affects the flavor and colour of the juice. Cloud values are presented in Tables 5 and 6. It can be seen, for assays 2, 5 and 10min. sonication the cloud value was much lower than the control (non-sonicated juice) at the end of the tested period (10min.). The pattern of increase in turbidity of all juice samples appeared to be the same. The results revealed three distinct phases during haze formation, which may be termed as lag phase, growth phase, and terminal phase. This was in agreement with previous studies, which have shown a similar two-staged pattern of haze formation in packaged beer (McMurrou'gh *et al.*, 1992) and apple juice (Tajchakavit *et al.*, 2001).

Table 5: Effect of therosonication (time, temp., and pulser) on turbidity of apple juice.

samples	Time (min.)	Temp. (°C)	Pulser (%)	Turbidity (A660nm)
Con.	0	0	0	2.365±0.001
A1	2 min.	20°C	21%	2.322±0.001
A2			30%	2.241±0.001
A3			40%	2.3295±0.001
B1		35°C	21%	2.229±0.001
B2			30%	2.275±0.001
B3			40%	2.335±0.01
C1		50°C	21%	2.264±0.001
C2			30%	2.323±0.001
C3			40%	2.332±0.001
Con.	0	0	0	2.405±0.0
A1	5 min.	20°	21%	2.083±0.001
A2			30%	2.095±0.001
A3			40%	2.046±0.0014
B1		35°C	21%	2.269±0.001
B2			30%	2.212±0.001
B3			40%	2.133±0.001
C1		50°C	21%	2.199±0.001
C2			30%	2.245±0.001
C3			40%	2.27±0.0
Con.	0	0	0	2.367±0.001
A1	10min.	20°C	21%	2.190±0.001
A2			30%	1.962±0.001
A3			40%	1.919±0.0
B1		35°C	21%	2.235±0.001
B2			30%	2.033±0.0014
B3			40%	1.943±0.003
C1		50°C	21%	2.356±0.004
C2			30%	2.02±0.004
C3			50%	1.931±0.001

Table 6: Effect of thermosonication (time, temp., and pulser) on turbidity of guava juice.

samples	Time (min.)	Temp. (°C)	Pulser (%)	Turbidity (A660nm)
Con.	0	0	0	2.849±0.005
A1	2 min.	20°C	21%	2.846±0.003
A2			30%	2.843±0.004
A3			40%	2.841±0.001
B1		35°C	21%	2.839±0.003
B2			30%	2.837±0.001
B3			40%	2.834±0.005
C1		50°C	21%	2.811±0.001
C2			30%	2.809±0.004
C3			40%	2.804±0.005
Con.	0	0	0	2.854±0.005
A1	5 min.	20°C	21%	2.838±0.002
A2			30%	2.836±0.005
A3			40%	2.829±0.040
B1		35°C	21%	2.827±0.003
B2			30%	2.824±0.002
B3			40%	2.821±0.004
C1		50°C	21%	2.811±0.003
C2			30%	2.807±0.002
C3			40%	2.802±0.001
Con.	0	0	0	2.845±0.005
A1	10min.	20°C	21%	2.841±0.002
A2			30%	2.84±0.0
A3			40%	2.839±0.002
B1		35°C	21%	2.831±0.004
B2			30%	2.83±0.012
B3			40%	2.82±0.0
C1		50°C	21%	2.791±0.001
C2			30%	2.773±0.002
C3			50%	2.757±0.004

The ultrasound processing reduces the size of the suspended particles in a liquid providing better uniformity and stability. This size reduction increases the number of individual particles leading to a reduction of average distance and an increase in the total surface area of the particles (Rao, 1999). The results of this study are consistent with the results published by Tiwari *et al.*, (2009), who observed greater stability of orange juice subjected to sonication. The authors attribute the greater stability of particulate matter from the juice to the particle reduction after ultrasound treatment. Moreover, they suggest that the activity of the enzyme pectin methylesterase (PMEs) and its interactions with its substrate (pectin) had a great impact on the stability of orange juice. Conversely, Wu *et al.*, (2008) observed that reducing the size of the particles in sonicated tomato juice proved to be more dependent on the US amplitude than the inactivation of PME.

3.4. Effect of sonication on colour characteristics and degradation in apple and guava juices:

L*, a* and b*, total colour difference (TCD), hue angle (H*), chroma (C*), cloud and browning index values were influenced by the two factors investigated, i.e. ultrasound amplitude level and sonication time and their effects were either individual or interactive. Instrumental colour was monitored and modeled as it is a key quality index influencing consumer acceptance of juice products. Colour values for raw unprocessed apple and guava juice were 44.15, -0.5, 13.4 and 63.61, -1.85, 13.68 for L*, a*, b*, respectively. During sonication treatments, it was observed that the lightness (L*) values of juice were significantly lower at 20 °C and lower amplitude levels compared to raw unprocessed samples. The largest increase was observed at higher processing times and higher amplitude level (10 min). At higher temperatures (35 and 50 °C) and amplitude levels, b* and a* increased significantly (Table 7 and 8). An increase in lightness value is attributed to the partial precipitation of unstable suspended particles followed by a decrease due to oxidative darkening. However, Tiwari *et al.* (2008a, b) observed an increase in cloud value due to the homogenization effect of sonication which may explain the observed increase in L* value. These colour changes may be due to independent or interaction effects of the extrinsic control variables of amplitude level or processing time (min). Differences in visual colour can be classified based on total colour difference (TCD). Choi *et al.*, (2002) reported that TCD values were corresponded to the noticeable differences in the visual perception of products. In the present study TCD was observed to be very distinct for the maximum treatment conditions investigated. It should be noted that changes in colour values may be regarded as a negative sensory impact of processing. A correlation between different parameters investigated is shown in Tables 7 and 8.

Sonication resulted in a decrease in a*, b*, C* and an increase in L* value, H*, BI and TCD in apple and guava juice (Tables 7 and 8). TCD values increased from 0 to 6.88 in apple juice and from 0 to 1.68 in guava juice respectively, indicating visual colour differences. Differences in perceivable colour can be analytically

classified as very distinct ($TCD > 3$), distinct ($1.5 < TCD < 3$) and small difference ($TCD < 1.5$). Then, the obtained results indicated that the differences in perceivable colour can be analytically classified as very distinct in apple juice and small distinct in guava juice. Choi *et al.*, (2002) indicated that a $TCD > 2$ corresponds to noticeable differences in the visual perception of many products. Similarly, Tiwari *et al.*, (2008a) reported significant differences in perceivable colour during sonication of orange juice. Results representing the linear and quadratic effects of the independent variables for the colour parameters (L^* , a^* , b^* , TCD, Chroma and Hue angle), are presented in tables 7 and 8.

Amplitude level was significant for all the colour parameters except b^* , which was found to be insignificant. The observed colour changes in this study may be caused by cavitation, which governs various physical, chemical or biological reactions, such as accelerating chemical reactions, increasing diffusion rates, dispersing aggregates or breakdown of susceptible particles, such as enzymes and microorganisms (Sala *et al.*, 1995). The extreme physical conditions of temperature and pressure, which occur during sonication, lead to accelerated carotenoid isomerisation (Chen *et al.*, 1995). Studies show that C^* , and/or colour parameters, are indicative of pigments in fruits and vegetables.

There were significant differences ($P < 0.05$) in all colour attributes among samples studied (Table 7 and 8). With respect to lightness (L^*), the lowest value was corresponded to the apple juice sample. In addition, the same sample showed the lowest red component ($+a^*$) in apple juice and highest yellow component ($+b^*$) in guava juice. This subsequently result in the greatest colour difference was observed in apple and guava juice with reference to control sample. Nevertheless, this minute total colour difference could not be distinguished by the naked eye.

According to Balaban (2003), sonication with its high shearing effect occurring during cavitation, will not only denature pectinesterase but also will 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.

Results reported that the thermosonication treatment did not effect on non enzymatic browning with optical density (A_{420nm}). However, the values of non enzymatic browning (A_{420nm}) confirm the data obtained for sonication treatments by both ultrasonic amplitude level and treatment time showed very slight differences in sonicated apple juice, no differences in sonicated guava juice and lower compared to control (Tables 7 and 8). Colour values (L^* , a^* , b^*), total colour difference (TCD), chroma, hue angle, browning index, ascorbic acid and turbidity were influenced by both ultrasonic amplitude level and treatment time, with the effects observed being either individual or interactive.

Table 7: Effect of thermosonication (time, temp. and pulser) on colour characteristics in apple juice.

samples	Min., °C, Puls%	L^*	a^*	b^*	TCD	A_{420nm}	C^*	H^*	BI^*
Con.	0	44.2	-0.44	13.57	0	9.9	13.58	88.14	63.69
A1	2, 20, 21%	44.84	-0.77	13.11	0.39	10.6	13.13	86.64	58.98
A2	2, 20, 30%	47.35	-0.78	13.91	1.77	11.9	13.93	86.79	59.43
A3	2, 20, 40%	47.64	-1.55	12.55	1.14	13.1	12.65	82.97	49.74
B1	2, 35, 21%	42.65	-1.43	11.93	2.05	10.1	12.02	83.17	53.52
B2	2, 35, 30%	46.55	-1.43	12.69	0.70	12.2	12.82	83.57	52.2
B3	2, 35, 40%	49.63	-0.77	14.28	2.41	13.3	14.3	86.91	58.08
C1	2, 50, 21%	49.63	-0.93	12.96	2.08	10.1	13	85.89	51.16
C2	2, 50, 30%	47.22	-0.73	14.03	6.88	11.7	14.05	87.02	60.42
C3	2, 50, 40%	49.19	-0.82	14.28	2.306	13.0	14.31	86.71	58.54
Con.	0	43.75	-0.77	12.61	0	9.9	12.64	86.45	57.89
A1	5, 20, 21%	38.92	-0.91	12.06	2.35	7.2	12.1	85.71	62.51
A2	5, 20, 30%	46.75	-2.91	9.63	1.46	13.9	10.06	73.19	32.30
A3	5, 20, 40%	47.73	-2.27	11.02	0.94	14.3	11.25	78.37	40
B1	5, 35, 21%	35.02	-0.91	9.365	3.48	6.6	9.41	84.45	51.63
B2	5, 35, 30%	43.89	-1.5	9.58	1.90	12.1	9.7	81.13	39.03
B3	5, 35, 40%	46.26	-0.01	9.845	0.71	13.7	9.8	84.2	42.56
C1	5, 50, 21%	46.3	-2.82	9.465	1.62	14.1	9.87	73.42	32.14
C2	5, 50, 30%	43.99	-0.99	8.98	1.90	12.8	9.03	83.67	37.48
C3	5, 50, 40%	40.5	0.71	12.405	1.41	8.1	12.43	86.75	67.42
Con.	0	44.5	-0.3	14	0	9	14	88.78	66.08
A1	10, 20, 21%	47.8	-2.3	11.4	1.14	13.7	11.63	78.6	41.65
A2	10, 20, 30%	49	-2.3	11.2	0.55	13	11.43	78.4	39.54
A3	10, 20, 40%	48.4	-2.8	11	1.26	13.8	11.35	75.72	37.68
B1	10, 35, 21%	48.9	-1.7	12.5	1.22	13.7	12.62	82.25	47.56
B2	10, 35, 30%	49	-2	12	0.9	13.5	12.17	80.54	44.18
B3	10, 35, 40%	48.8	-2.3	11.8	0.32	13	12.02	78.97	42.56
C1	10, 50, 21%	47.2	-2.3	10.5	1.67	14	10.75	77.63	37.89
C2	10, 50, 30%	45.9	-3.1	9.5	2.43	13.7	10	71.9	31.74
C3	10, 50, 40%	48.7	-2.3	9.2	1.61	13.3	9.48	75.96	30.67

Table 8: Effect of thermosonication (time, temp. and pulser) on colour characteristics in guava juice.

samples	Min., °C, Puls%	L*	a*	b*	TCD	A _{420nm}	C*	H*	BI*
Con.	0	62.95	-1.99	13.75	0	20.5	62.98	1.72	646.5
A1	2, 20, 21%	62.57	-1.85	13.96	0.17	20.2	62.6	1.72	640.9
A2	2, 20, 30%	62.23	-1.76	14.09	0.39	20.1	62.35	1.72	639.5
A3	2, 20, 40%	62.04	-1.72	14.29	0.32	20	62.06	1.72	635.7
B1	2, 35, 21%	62.94	-1.87	13.76	0.35	20.1	62.98	1.72	640.6
B2	2, 35, 30%	62.56	-1.82	13.81	0.4	19.9	62.59	1.72	637.4
B3	2, 35, 40%	62.33	-1.71	13.86	0.48	19.5	62.35	1.72	636.6
C1	2, 50, 21%	62.55	-1.88	13.48	0.75	20.2	62.58	1.72	639.6
C2	2, 50, 30%	62.34	-1.74	13.75	0.6	20.1	62.36	1.72	640.5
C3	2, 50, 40%	61.6	-1.7	13.86	0.98	20	61.62	1.72	635.8
Con.	0	63.45	-1.96	13.87	0	20.8	63.48	1.72	648.4
A1	5, 20, 21%	62.93	-1.86	13.93	0.6	20.6	62.96	1.72	641
A2	5, 20, 30%	62.75	-1.79	14.02	0.62	20.2	62.78	1.72	639
A3	5, 20, 40%	62.34	-1.74	14.34	0.65	20	62.36	1.72	635.3
B1	5, 35, 21%	62.8	-1.97	13.56	0.98	20.1	62.83	1.72	636.2
B2	5, 35, 30%	62.55	-1.8	13.76	0.92	20	62.58	1.72	638.3
B3	5, 35, 40%	62.31	-1.65	13.98	0.85	19.7	62.33	1.72	639
C1	5, 50, 21%	63.55	-1.97	13.08	0.84	20.3	63.58	1.72	637.3
C2	5, 50, 30%	62.49	-1.79	13.25	1.19	20.1	62.52	1.72	638.6
C3	5, 50, 40%	61.5	-1.73	13.48	1.45	20	61.52	1.72	634.5
Con.	0	64.45	-1.61	13.42	0	21	64.47	1.14	664.7
A1	10, 20, 21%	63.93	-1.56	13.83	0.24	20.6	63.95	1.14	655
A2	10, 20, 30%	62.75	-1.51	14.22	0.9	20.1	62.77	1.14	650.9
A3	10, 20, 40%	64	-1.47	14.73	1	20	64.02	1.14	648.9
B1	10, 35, 21%	62.8	-1.97	13.24	1.48	20	62.83	1.72	636.2
B2	10, 35, 30%	64.55	-1.8	13.76	0.5	20	64.58	1.72	641.1
B3	10, 35, 40%	62.8	-1.65	14.29	0.91	19.1	62.82	1.72	639.8
C1	10, 50, 21%	64.55	-1.95	13.38	0.53	20.2	64.58	1.72	639.5
C2	10, 50, 30%	63.49	-1.75	13.45	1.03	20.1	63.51	1.58	641.7
C3	10, 50, 40%	61.3	-1.7	13.84	1.68	20	61.32		635.4

The chroma (C*), the hue angle (H*) and the browning index (BI) may improve the understanding of colour variations found in sonicated apple and guava juice. The C* indicates the degree of variation in the intensity of the chroma (a* and b*) of the sonicated treated sample with relation to fresh sample. The lower the value of C* shows the less the variation. Thus, the samples processed in sonication treatments at 0, 5 and 10min (the lowest C* and BI values) showed little difference of these samples compared to control. The values of hue angle (H*) confirm the data obtained for a* and b* since sonication treatments 0, 5 and 10min showed slight differences compared to control (Tables 7 and 8). On the other hand, the increase of C* showed the colour enhancement. Thus, samples submitted to the sonication treatment conditions of assays at 0, 5 and 10 with 20, 35 and 50 °C had their colour enhanced due to the decrease of redness and increase of yellowness. The juice colour was also intense in these assays because these samples presented closely same L* values in apple and guava juice when compared to the control. The hue angle (H*) showed variation of less than 5°, indicating that the characteristic colour of the apple and guava juice was maintained for all sonication treatments. Choi *et al.*, (2002) suggested that a TCD >2 corresponds to visually perceptible differences in various products. Thus, the colour of assays at 0, 5 and 10min resulted in juices that did not show visual difference compared to the control. For the other assays, a visual difference between the colour of sonicated juice and the control was observed. However, as previously commented, these differences are positive and a result of the colour enhancement. The condition of ultrasound that resulted in better retention of the colour of the apple and guava juice was the greatest intensity ultrasound treatment and the longest time (for 10 min). Tiwari *et al.*, (2009) and Thatyane *et al.*, (2012) obtained similar results with the sonication of blackberry and cantaloupe melon juice. The juice has retained a colour in extreme processing conditions (for 10min), indicating the stability of colour during sonication.

4. Conclusion:

The effects of ultrasound amplitude level and treatment time on apple and guava juices were investigated. No significant differences ($p < 0.05$) in pH, °Brix or TA were observed in sonicated samples. Colour values (L*, a*, b*), total colour difference (TCD), chroma, hue angle, browning index, ascorbic acid and turbidity were influenced by both ultrasonic amplitude level and treatment time, with the effects observed being either individual or interactive. However, sonication was found to have a significant effect on juice colour, ascorbic acid content and turbidity. The obtained results show that sonication treatments enhanced the manifestation of cavitation and subsequently produce juice with good ascorbic acid content, lower turbidity and maintaining colour. These sonication treatments were effective in reducing turbidity and browning. Sonication could be

employed as a preservation technique for apple and guava juice processing where quality and colour retention is desired.

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