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Effects of Continuous High-frequency Ultrasound on Selected Characteristics of Orange Juice

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Abstract: Ultrasound has been reported as a possible alternative to disinfect water. However, few studies have been conducted to establish application of high-frequency ultrasound on liquid foods. Processing of orange juice for 30 min using continuous high-frequency ultrasound (CHFU) increased temperature up to 53 °C. Ultrasonic power was determined by calorimetry method. The increased temperature could be a critical parameter to decide whether some effects to orange juice of CHFU are due to heat generated during CHFU processing. Changes in pH, titratable acidity, and browning index were not significant while 'L', 'a', 'b', 'SI', and Hue values increased. Ascorbic acid degradation and some inactivation of pectin methylesterase (PME) were observed over time. An approximate 5 log reduction in yeast counts was obtained by application of CHFU for 30 min.

Key words: Ascorbic acid, orange juice, pectin methylesterase, ultrasound, yeast

Introduction

Applications of ultrasound can be divided into two categories. First, mechanical effects which include crystallization, degassing, destruction of foams, extraction of flavors, mixing, homogenization, and tenderization of meat. Second, chemical and biochemical effects that include bactericidal action, effluent treatment, modification of growth of living cells, oxidation, and sterilization of equipment (Mason, 1998). Applications of ultrasound to food processing include treatment of orange juice, extraction of apple juice using frequencies in the order of 20-300 kHz, treatment of grapes to produce wine (800 kHz) for 5-20 minutes, and clarification of wine to precipitate tartrates (Chendke and Fogler, 1975). Ultrasound disrupts biological cell walls destroying bacteria, but the intensity required is extremely high to achieve 100% destruction (Mason, 1993). The use of hydrodynamic cavitation to disrupt yeast cells has also been reported (Save *et al*., 1994) as well as the inactivatation of lipoxygenase in whole soy flour (Thakur and Nelson, 1997). Exposure to 20 kHz generator for three hours at pH 4.0 decreased the activity of lipoxygenase by 75-80%. This may provide a good potential basis for development of new processes for food preservation or product modifications. Orange juice is a candidate for application of this technology since its fresh flavor characteristics are degraded by thermal treatments such as traditional pasteurization, 98°C for 10 s or 75°C for 10 s (Parish, 1998).

The objectives of this research were to evaluate the effect of continuous high-frequency ultrasound (CHFU) on physical and chemical properties of reconstituted orange juice and to evaluate the effect of CHFU on pectin methylesterase activity and yeast counts of fresh orange juice.

Materials and Methods

Continuous High-frequency Ultrasound (CHFU) Treatments: Valencia fresh and concentrated orange juice was obtained from a commercial source in Florida and kept frozen until tested for analyses. CHFU treatments were performed using an ultrasonic K80 generator (Meinhardt, Leipzig, Germany), with 100 W maximum power output and 10, 20, and 30 min process time. Frequency was held constant at 850 kHz and intensity at 580 mV. The ultrasonic chamber had a useful inner diameter of 70 mm, and 346 mL useful volume. Volume of 40 mL juice at 5 ^{B}C was used for all analyses.

Ultrasonic Power Determination: Many authors have suggested that the thermal effect of ultrasound can be used as a means of obtaining the effective ultrasonic powder. The most common method to estimate the amount of ultrasonic power entered into a sonochemical reaction is calorimery, which involves measurement of the initial rate of a temperature rise produced when a system is irradiated by power ultrasound. Power dissipated (W) into 40 mL of fresh orange juice at each processing time and temperature was calculated (Table 1) according to Kimura *et al* (1996):

$$P_d = mCp\ (dT/dt) \quad (1)$$

Where:
P_d = power dissipated
Cp = specific heat (3.822 kJ/kg K) (Singh and Heldman, 2001)

m = mass of orange juice (kg)
T = Temperature (^{B}C)
t = time of reaction (sec)

Quality Determinations: Soluble solid of orange juice was determined using a refractrometer (Bausch and Lomb, Rochester, NY). Results were reported as Brix. For titratable acidity, 10 ml samples were accurately measured into an Erlenmeyer flask and 10 mL of distilled water was added to each sample. The resulting mixture was titrated with 0.1 NaOH to pH 8.1. The results were expressed as % citric acid (AOAC, 1999). The pH of samples was determined before titration. Browning index was measured with a spectrophotometer (Perkin Elmer UV-V, Norwalk, CT) at 420 nm. Juice samples were centrifuged for 10 min at 326 x g in a clinical centrifuge (Sorvall, Dupont Instruments, Newton, CT) to remove sinking pulp. The supernatant was diluted 1:1 with absolute ethanol and allowed to stand one hour. An additional centrifugation at 326 x g for 10 min completed clarification. Absorbance of the supernatant was measured at 420 nm (Johnson *et al.*, 1995). Color of orange juice was measured in a cylindrical sample cup, 5 cm diam x 2 cm high, filled to the half, using a LABSCAN Model 6000 0/45° spectrocolorimeter (Hunter Associates Laboratory, Fairfax, VA) and results expressed as 'L', 'a', 'b' values. Hue ($\tan^{-1}(b/a)$) and Saturation Index ($(a^2+b^2)^{1/2}$) were calculated. The test kit (Boehringer Mannheim GmbH, Mannheim, Germany) by colorimetric method for the determination of ascorbic acid content was used and results expressed as mg ascorbic acid L^{-1}.

Pectin Methylesterase (PME) Analysis: Assay of PME in fresh orange juice was conducted by titrating the liberated carboxyl group at pH 7.5 (25°C) using the modified method (Tajchakavit and Ramaswamy, 1997). The activity was expressed as PME unit mL^{-1} which represented the μequivalents of acid liberatd per min per mL at pH 7.5 and 25°C. The procedure consisted of adding 2.0 mL juice sample to 50 mL of pectin substrate (previously adjusted to pH 7.5) with constant stirring and quickly adjusting pH to 7.5 with 0.2 mol L^{-1} NaOH. The reaction was initiated as soon as the pH was adjusted to 7.5. The amount of 0.02 mol L^{-1} NaOH used was recorded during the reaction period of 30 min.

$$\text{PME units } mL^{-1} = \frac{(\text{mL 0.02 mol } L^{-1} \text{ NaOH})\,(\text{mol } L^{-1} \text{ of NaOH})\,(10^3)}{(\text{mL sample})\,(\text{time(min)})} \qquad (2)$$

Yeast Counts: Potato dextrose agar (Fisher Scientific, Houston, TX) acidified with sterile 10% tartaric acid was used to isolate a yeast from fresh orange juice. Direct examination of a representative colony under the microscope was achieved to verify that one is actually dealing with yeast (Samson *et al.*, 2000). The isolated yeast was grown in tryptic soy broth (Fisher Scientific, Houston, TX) in order to inoculate fresh juice at a density of 8.08 log CFU mL^{-1} (APHA, 1992). Results were expressed as Log CFU ml^{-1} after treatment.

Statistical Design: A completely randomized design with replications per treatment was used for this study. The data were analyzed using the ANOVA procedure (SAS, 1997). When significant, means were separated using the Fisher's protected LSD (Steele and Torrie, 1980).

Results and Discussion

Calorimetry and Quality Determinations: The initial temperature and raised temperature were recorded against time (*t*) every 10 min using a thermometer placed in the reaction chamber containing orange juice (Table 1). Calorimetric power measurements (Table 1) were achieved under the conditions of 5 ^{B}C initial temperature, 40 mL volume of orange juice in the cylindrical chamber.

Table 1: Calorimetrical powder of CHFU treated orange juice at different time and temperature

Processing	Time temperature	Powder dissipated	Total heat
(sec.)	(°C)	(W)	(KJ)
0	600	1200	1800
5	36	46	53
0	7.9	644	4.08
0	4.74	7.73	9.54

Kimura *et al.* (1996) and Ratoarinoro *et al.* (1994) determined that the calorimetric method was evaluated as a means to standardize the ultrasonic power of individual ultrasonic devices. They described that the calorimetrically determined ultrasonic power was independent of the volume and shape of a vessel, even if ultrasound was introduced using different devices. They also mentioned that although the efficiency of the use of acoustic energy is dependent on the reaction system, it should be clearly determined how much ultrasonic energy is absorbed by the system if the reaction conditions such as temperature, time, solvent present and so one are well defined. Hoffman *et al.* (1996) and Lauterborn and Ohl (1997) demonstrated that thermal effect of ultrasound by ultrasound was due to the combination by an instantaneous source of heat by the collapsed bubbles and dissociation of water and consequent production of hydrogen peroxide.

The changes in temperature followed an exponential shape. This means that if liquid like water or orange juice is sonicated for a longer time, the temperature will tend to stay equilibrate. This happens because after a certain time the liquid will be degassed and there will be no more nuclei to form cavities and therefore the cavitation phenomenon will no longer take place (Entezari and Kruus, 1996). The TA, pH, Brix, browning index were not affected by CHFU or raised temperature during process time (Table 2). In general, Hunter color 'L', 'a', and 'b' values increased after 10 min and remained constant thereafter. This result may be due to flocculation of orange juice particles as visually observed after heat treatment with ultrasound.

Ascorbic Acid Loss: Ascorbic acid decreased rapidly from 470 mg L^{-1} to 65 mg L^{-1} with increased processing time and temperature (Fig. 1). When ascorbic acid concentration was measured as a function of heating, linear regression analysis showed r^2 of 0.9812. Johson *et al.*, (1995) described that degradation of ascorbic acid is

Table 2: pH, titratable acidity (TA), ° Brix, and browning index for continuous high-frequency ultrasound treated orange juice

Process time (min.)	pH	TA[1]	°Brix	Browning index[2]
0	3.79NS	0.81NS	11NS	0.08NS
10	3.77	0.83	11	0.08
20	3.77	0.85	11	0.08
30	3.78	0.86	11	0.09
LSD (0.05)	0.23	0.22	1.96	0.03

[1]ml citric acid/100 mL O.J.
[2]light absorbance at 420 nm
NS: Non Significant

Table 3:Hunter color values for continuous high-frequency ultrasound treated orange juice

	Color values				
Process time (min.)	'L'	'a'	'b'	'Hue'	'SI'
0	46.1b	-6.6a	21.4b	105.8c	21.4d
10	48.9ab	-6.6b	23.5a	106.2a	23.7c
20	51.7a	-6.3ab	24.2a	104.6d	25.0a
30	50.7a	-6.7b	23.3ab	106.0b	24.2b
LSD (0.05)	3.07	0.42	1.93	0.09	0.1

ab: means in a column not followed by the same letter differ ($p < 0.05$)

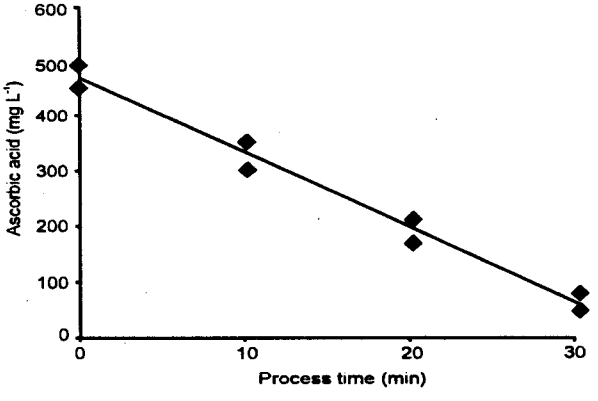

Fig. 1: Ascorbic acid content of continuous high-frequency ultrasound treated orange juice as affected by process time

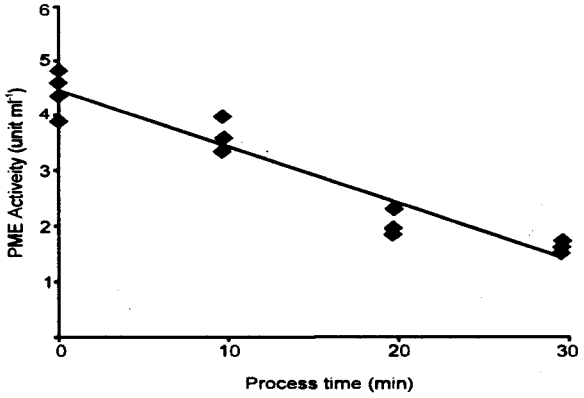

Fig. 2: Pectin methylesterase (PME) activity in continuous high-frequency ultrasound treated orange juice using as affected by process time

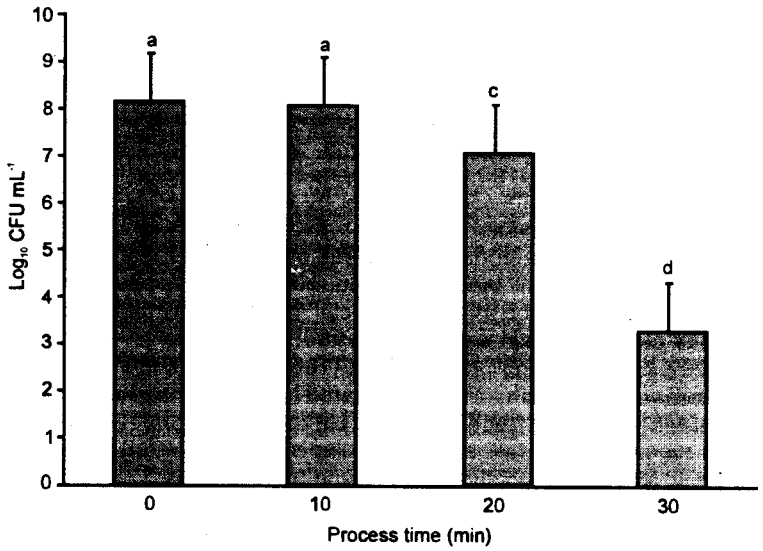

Fig. 3: Yeast counts (Log CFU ml^{-1}) in continuous high-frequency ultrasound treated orange juice using as affected by process time

known to occur by both oxidative and non-oxidative mechanisms and is generally characterized as a first-order reaction. They demonstrated that r^2 of ascorbic acid degradation in orange serum and in whole orange juice treated between 70.3 and 90.6°C ranged from 0.974 and 0.99. The ascorbic acid loss may be explained by three situations: 1) the loss by heat occurrence, 2) increased chemical reaction (oxidation of ascorbic acid) due to the hydrolysis of water to H^+ and OH^- by sonochemical oxidation (Entezari and Kruus, 1996) and hydrogen peroxide production (Lauterborn and Ohl, 1997), and 3) reaction with oxygen of the surface air due to the formation of bubbles that rise to the surface.

PME Analysis: After 30 min processing, PME in orange juice was partially inactivated (Fig.2). High solible solids of orange juice were considered a significant factor in contribution to the initial resistance against inactivation of the PME activation (Ogawa, *et al.*, 1990). Increased soluble solids protect PME against pressure as well as heat inactivation of the PME activation (Cano, *et al.*, 1997) at medium pressure. Tajhakai and Ramaswamy (1997) studied that thermal inactivation kinetics of PME in orange juice. Results indicated that D60 values of microwave and thermal inactivation of orange juice were 7.37 and 154 s respectively. They demonstrated temperature sensitivity plots of inactivation rates of both heat sensitive and heat resistant fractions of PME and also reported that the pasteurization conditions of orange juice is generally based on inactivation of the heat sensitive fraction of PME.

Microbial Analysis: An approximate 5 log reduction in yeast count was obtained by application of CHFU for 30 min (Fig. 3). DAKAKIBARA *et al* (1994) reported that ultrasonic irradiation increased the hydrolysis of lactose in milk but decreased the cell viability. They also reported that the viable cell count increased again when the ultrasound was stopped, because ultrasound did not destroy the ability for cell propagation. In case of pulsed electric fields, McDonald *et al.* (2000) reported that at 30 kV cm^{-1}, 5 to 6 log reduction of *E.coli* and *Listeria innocua* was achieved by 6 pulses per volume, with an outlet temperature of 54°C. They showed that for each of these organisms, less than 1 log inactivation is expected with a thermal treatment of 55°C or less. They also demonstrated that at 50 kV cm^{-1} and outlet temperatures near 60°C, reduction the inoculated bacterial species by 4 logs or more was due to the thermal inactivation. There are several published data of D values of thermal inactivation for yeasts to indicate that a one-log reduction in population would be expected every half minute to minute at temperatures above 55 °C. PUT and De Jong (1982) demonstrated that D_{55} and D_{60} values of vegetative cells of *Saccharomyces bailii* were 0.30 and 0.1 min respectively. Thermal death time curve at 60°C of *Saccharomyces cerevisiae* 175 which showed more than 5 log cycles for 10 min.

Conclusion

Ascorbic acid degradation, PME and yeast inactivation maybe due to the thermal effect of ultrasound. TA, pH, Brix,

and Browning index in orange juice were not affected significantly except for higher 'L', 'a', 'b' 'Hue', and 'SI'. However, This research confirmed that enzyme inactivation and decreased yeast cells by heat treatment could be important for achieving commercial stability of fruit-derived products with a nearly fresh quality.

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