THE Society for Food Science & Technology

Effect on Orange Juice of Batch Pasteurization in an Improved Pilot-Scale Microwave Oven

L. CINQUANTA, D. ALBANESE, G. CUCCURULLO, AND M. DI MATTEO

ABSTRACT: The effects on orange juice batch pasteurization in an improved pilot-scale microwave (MW) oven was evaluated by monitoring pectin methyl-esterase (PME) activity, color, carotenoid compounds and vitamin C content. Trials were performed on stirred orange juice heated at different temperatures (60, 70, 75, and 85 °C) during batch process. MW pilot plant allowed real-time temperature control of samples using proportional integrative derivative (PID) techniques based on the infrared thermography temperature read-out. The inactivation of heat sensitive fraction of PME, that verifies orange juice pasteurization, showed a *z*-value of 22.1 °C. Carotenoid content, responsible for sensorial and nutritional quality in fresh juices, decreased by about 13% after MW pasteurization at 70 °C for 1 min. Total of 7 carotenoid compounds were quantified during MW heating: zeaxanthin and β-carotene content decreased by about 26%, while no differences ($P < 0.05$) were found for β-cryptoxanthin in the same trial. A slight decrease in vitamin C content was monitored after MW heating. Results showed that MW heating with a fine temperature control could result in promising stabilization treatments.

Keywords: carotenoids; microwave; orange juice; pasteurization; pectin-methyl-esterase

Introduction

Because of its pleasant taste, fresh flavor, and nutritional value, orange juice is the most common juice manufactured by the beverage processing industry: more than 2.3 millions metric tons (65 degrees brix) of orange juice were produced around the world during marketing year 2007/08 (USDA-FAS 2008). Due to the low pH of orange juice (pH < 4), growth of pathogenic microorganisms is suppressed; while pectin methyl-esterase (PME; EC 3.1.1.11) activity is important because this enzyme is responsible for the hydrolysis of the pectin present in citrus fruit juices that causes loss of fresh juice cloudiness. Heat pasteurization of orange juice is designed to inactivate PME, which is more thermal resistant than spoilage microorganisms (yeasts and lactic acid bacteria), whose thermal death curves during microwave (MW) pasteurization were previously studied (Cuccurullo and others 2007). Conventional thermal processing often leads to detrimental changes in the sensory and nutritional qualities of such product. Colour of orange juice is mainly due to carotenoid pigments (Lee and others 2001; Vikram and others 2005; Meléndez-Martínez and others 2007), and is bears a relation with the technological treatments: a relatively large loss of provitamin A (β-carotene, α-carotene, and β-cryptoxanthin), and carotenoids was found after thermal processing in orange juice (Lessin and others 1997). Vitamin C is another key parameter to evaluate orange juice quality and large differences in kinetic parameters at elevated temperatures have been observed (Fennema 1996). MW heating seems a very attractive technique, owing to its well-known advantages over conventional heating. Microwaves heat the addressed target directly with no need for intermediate fluid, thus allowing for faster, more effective, and economical treatment. Notwithstanding the above, nonuniform heating patterns in the food may be expected as a consequence of unevenness of the electric field in the cavity (Oliveira and Franca 2002). A further complication concerns the dependence of heat generation inside the food on dielectric properties, geometry, and food location inside the cavity (Datta and Anantheswaran 2001). Finally, strong difficulties in controlling the heating process have been encountered due to the ineffectiveness of metal probes for measuring temperature in the electromagnetic cavity. For instance, orange juice pasteurization through continuous MW heating was investigated employing a device measuring temperature of the fluid exiting the loaded cavity independently of the temperature distribution inside the oven (Nikdel and Mackellar 1992; Villamiel and others 1998; Vikram and others 2005). As a consequence of the inability to directly monitor the proper temperature, a higher loss in orange juice vitamin C was found after MW heating processes than in conventional ones, On the other hand, it must be noted that smaller ascorbic-acid losses and browning were found after batch MW treatment of orange juice than in batch conventional heating (Abd-El-Al and others 1994). The aim of this research was to evaluate MW orange juice pasteurization, using a pilot plant endowed with a real time temperature control system. The effect of MW batch pasteurization at different temperatures was evaluated by monitoring PME activity, color, carotenoid compounds, and vitamin C changes. A temperature control system was implemented on sample surface by means of Infrared Thermography System (IRT) with direct digital Proportional Integrative Derivative (PID) control. The system allowed to adjust the magnetron released power to quickly reach and keep the target temperatures of orange juice samples undergoing pasteurization treatment. A magnetic stirrer provided for

MS 20090483 Submitted 5/29/2009, Accepted 09/26/2009. *Author Cinquanta is with Dipt. di Scienze e Tecnologie Agro-Alimentari Ambientali e Microbiologiche, Univ. del Molise, Via F. De Sanctis, 86100 Campobasso, Italy. Authors Albanese and Di Matteo are with Dipt. di Ingegneria Chimica Alimentare and author Cuccurullo is with Dipt. di Ingegneria Meccanica, Univ. di Salerno, Via Ponte Don Melillo, 84084 Fisciano (SA), Italy. Direct inquiries to author Cinquanta (E-mail: cinquant@unimol.it).*

© 2009 Institute of Food Technologists®
doi: 10.1111/j.1750-3841.2009.01412.x
Further reproduction without permission is prohibited

Journal of Food Science
A Publication of the Institute of Food Technologists

sample intensive mixing to achieve temperature uniformity while processing.

Materials and Methods

Microwave heating system

The pilot-scale microwave oven was composed of a Hitachi magnetron rated with 3 kW absorbed electrical power and operating at standard frequency of 2.45 GHz, illuminating an aluminum cubic chamber (1 × 1 × 1 m). The system was completed by an infrared-thermography equipment: a FLIR shortwave system Thermacam mod. P65, Stirling cooled, allowing Computer Aided Thermography at 12 bit, 50Hz (Figure 1). The IRT exhibited a spatial resolution at the operating distance of 1.75 mm/pixel (approximately 4.5 dpi), due to the 240 × 240 pixel matrix and optics in use. Square windows (5 × 5 cm^2) where realized on the top and side walls of the oven to allow the IR camera to look inside the cavity through a suitable metallic grid. The IRT detected temperatures were transferred to a suitably written code, based on the Labview® architecture, which was able to realize the closed loop control by checking the difference between the actual sample surface temperature and the target setpoint. In particular, the code was responsible for adjusting magnetron delivered power by controlling its duty cycle with a PID procedure tuned with an open loop experiment according to the classical Ziegler–Nichols method. A magnetic stirrer suitably shielded was placed inside the chamber to flatten temperature inside the sample. Temperature oscillations read-out on the sample surface were contained within ± 0.6 °C.

Sample preparation

The fresh sweet orange (*Citrus sinensis* Osbeck) fruits were purchased from the local fruit market. Freshly squeezed juice was prepared in laboratory, using a domestic juicer Braun Citromatic MPZ2 (Braun Italiana, Milano, Italy). The extracted juice was centrifuged and the liquid phase frozen at –18 °C. Orange juice samples were placed at the middle of the chamber in cylindrical beakers of 7 cm diameter, each filled with 250 mL sample. The bottom of the beaker was in contact with a magnetic stirrer suitably shielded and able to realize 40 rpm. Stirred orange juice was batch pasteurised into MW oven for various time/temperature combination treatments: 60 °C for 2 and 5 min; 70 °C for 1, 2.5, and 5 min; 75 °C for 1, 2.5 and 5 min; 85 °C for 0.25, 0.5, 1, and 2 min. Temperature increase of the samples exhibited an essentially linear come-up (33 °C/min) from the switching-on of the magnetron up to the time when target temperature was first attained; for instance, the set point of 70 °C was reached in about 1.5 min. Linear behavior was expected since the come-up time interval was really small when measured in the proper time scale. Time/temperature treatments conventionally started at the instant the juice reached the target temperature as indicated by IRT. While the permanence time elapsed, the PID control acted to keep the target temperature fixed within ± 0.5 °C. After thermal treatment, the samples were immediately frozen.

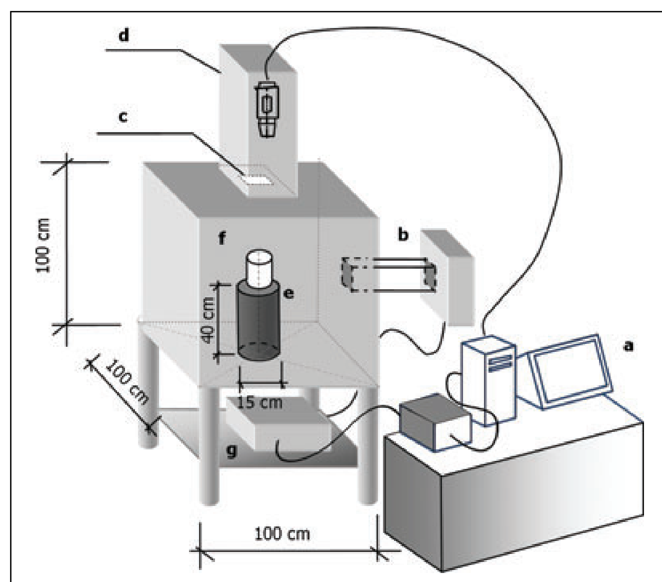

Figure 1 — Microwave pilot plant heating system. (a) Computer for aided thermography and PID control; (b) magnetron and standard rectangular WR-340 waveguide with 2 : 1 aspect ratio; (c) hole with grid to look inside the chamber; (d) IR camera room; (e) magnetic stirrer; (f) sample; (g) power board and aux.

Analytical measurements

Total soluble solid (°Brix) content was determined at 20 °C using a digital refractometer (PR1, Atago, Japan); pH was determined using an electronic pH meter (Crison, model Micro pH 2002, S.A., Barcelona, Spain), and acidity by titration with NaOH 0.1N.

Microbial examination

A total of 10 mL of orange juice were mixed with 90 mL of Ringer solution (Oxoid, U.K.). Serial dilutions were carried out using Ringer solution, and the microbial status was evaluated by means of plate counts. Total plate count was determined on pour plates of Standard Plate Count Agar, PCA (Oxoid, U.K.), which were incubated at 32 °C for 24 h. *Lactobacillus* spp. were enumerated in pour plates of de Man, Rogosa, and Sharpe (MRS) agar (Oxoid, U.K.). The plates were incubated at 37 °C for 48 h under anaerobic conditions using anaerobic systems. Yeasts and moulds were enumerated using Rose–Bengal Chloramphenicol Agar (Oxoid, U.K.) and incubated at 25 °C for 5 d.

Pectin methyl-esterase activity

PME activity was determined by titrating the liberated carboxyl group at pH 7.5 (30 °C) (Tajchakavit and Ramaswamy 1997). The activity was expressed as PME unit per milliliter (PMEU), representing the μequivalents per milliliter × minute of acid liberated at pH 7.5 and 30 °C. The procedure consisted of adding 2 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 NaOH. The reaction was initiated as soon as the pH was adjusted to 7.5. The amount of 0.02 mol/L NaOH used was recorded during the reaction period of 30 min. During the entire titration, a constant temperature of 30 °C was held. Thermal inactivation of PME was assumed to follow a 1st-order rate indicating a logarithmic order of inactivation, mathematically expressed as:

$$\ln\left(\frac{A}{A_0}\right) = -kt$$

where A represented residual enzyme activity (PME unit per milliliter), Ao represented initial enzyme activity (PME unit per milliliter), t and k represented time (s) and reaction rate constant (1/s) at a particular temperature, respectively. The decimal reduction time (D values), the time required to reduce the PME activity by 90%, was related to reaction rate constants by $D = \frac{2.303}{k}$

The D values were then described as a direct exponential function of temperature:

$$Ln\left(\frac{D_1}{D_2}\right) = \frac{T_2 - T_1}{z}$$

where D_1 and D_2 are decimal reduction times at temperatures T_1 and T_2, respectively, with z representing the temperature required for 1 log cycle reduction in D value.

Color

Color was measured using a CR-300 Minolta Chroma meter (Minolta, Milano, Italy) with an 8 mm measuring area. A Minolta standard white reflector plate was used to standardize the instrument under Commission Intl. de l'Eclairage (CIE), illuminant C conditions. Samples of orange juice were filled into 25 mm glass petri dishes and CIE L^*, a^*, b^* values were determined. Chroma $\sqrt{(a^{*2} + b^{*2})}$, that quantifies color intensity, and total color differences $\Delta E = \Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}$, which indicate the magnitude of the color difference between juices at initial time and after the thermal treatment, were also determined.

Carotenoids

For carotenoid analysis, 25 mL of samples were diluted in 50 mL exane–acetone–ethanol solution (50 : 25 : 25), and centrifuged for 5 min at 6500 rpm at 5 °C. The surface layer with pigments was recovered and dried. Subsequently, the residue was redissolved in 2 mL of a methyl tert-butyl ether (MTBE) and 2 mL methanol–KOH (90 : 10) solution was added, for saponification. The sample was shaken and wrapped in aluminum foil to protect it from light. Prior to capping, the sample was gently blanketed with nitrogen, before being closed and stored overnight in the dark at room temperature. A solution of 5 mL water–butyl-hydroxyl-toluene (BHT) (99.9 : 0.1) was added to the sample for 3 times, then water-soluble extracts were removed by adding sodium sulfate anhydrous. The extracts were concentrated at less than 35 °C in a rotary evaporator and dried under nitrogen, added with 1 mL methanol–acetone (2 : 1) and filtered (Millipore FHLC, 0.5 μm), before injection. An aliquot (20 μL) was separated by a reverse-phase high-performance liquid chromatography (HPLC) system (Waters 600 controller pump), fitted with a YMC (Hampsted, N.C., U.S.A.) stainless steel column (250 × 4.6 mm i.d.), packed with 5 μm silica spheres that were chemically bonded with C_{30} material, at a flow rate of 1 mL/min. The mobile phase was constituted by methanol : MTBE : water with the following gradient: 0 to 12 min (90 : 5 : 5), 25 min (11 : 89 : 0), 40 min (25 : 75 : 0), 60 min (50 : 50 : 0). The eluent was monitored by means of a photo-diode array detector (Waters 996), set at 350, 430, and 486 nm; chromatographic data and UV–visible spectra were handled by a Millenium driver station. Solvents were of HPLC grade and commercial standards used for determination were purchased from Extrasynthese (Genay, France). Total carotenoids were calculated by considering the response factor of lutein for unidentified peaks.

Vitamin C

The vitamin C analysis was carried out by HPLC (P 680, Dionex, Sunnyvale, Calif., U.S.A.) connected to a Dionex Model 170 UV-Vis spectrophotometer using a C_{18} (ODS) column (5 μm × 4.6 × 30 cm, Waters) with an UV detector set at 254 nm. Meta-phosphoric acid solution (0.08 M) was used as the mobile phase at a flow rate of 0.5 mL/min. Solvents were of HPLC grade and commercial standards used for determination were purchased from Extrasynthese (Genay, France).

Statistical analysis of results. Experiments were performed in triplicate. Data reported are the mean and standard deviation values calculated from the replicates. The results were analyzed using one-way analyses of variance. Differences ($P < 0.05$) between means were studied with the Student–Newman–Keuls test.

Results and Discussion

Pectin methyl-esterase inactivation

The purpose of orange juice pasteurization is to inactivate PME, which causes cloud loss, since the thermal resistance of PME has been recognized to be greater than that of common bacteria and yeasts present in juice. In our trials, unprocessed orange juice presented a total bacterial count of 2.6×10^3 CFU/mL (mainly *Lactobacillus* spp. 3×10^2), yeast and moulds count of 2.9×10^3 CFU/mL and a PME activity equal to 1.40×10^{-4} PMEU/mL, according to Kim and others (1999) and Collet and others (2005). The maturity index (°Brix/total titratable acidity) was 9.3, with a solids content of 8.4 °Brix and pH value was 3.6; the latter were very important factors that influenced the enzymatic orange juice stability. The PME activity, monitored during MW heating at 70 °C highlighted a curvilinear decrease that could be resolved into 2 first-order rate curves (Figure 2), according to Tajchakavit and Ramaswamy (1997). The net result was the identification of 2 fractions of PME, confirming the presence of several isoenzymes (Versteeg and others 1980) that can be separated in 2 groups: 1 more thermal resistant than the other and both thermally inactivated according to 1st-order decay kinetics. The pasteurization conditions are generally based on inactivation of the heat sensitive fraction of PME: Cameron and others (1994) reported that incubation of orange juice at 80 °C for 2 min with conventional heating was required to inactivate the most active form of PME. In instances of juice receiving light pasteurization treatment (66 °C for 10 s), a 16% residual PME activity was observed, while full pasteurization treatment (90 °C for 60 s), directed at the heat stable isoenzyme of pectinesterase, reduced PME activity by 99.9% (Sadler and others 1992). In our experimental conditions, the heat sensitive fraction of PME was inactivated after about 1 min at 70 °C of MW heating (Figure 2). This point, corresponding to 5.5×10^{-5} PMEU, was lower than values recommended for orange juice pasteurized (Kimbal 1991). The MW thermal behavior of PME in orange juice was estimated at different times and temperatures (60, 70, 75, and 85 °C), to calculate the decimal reduction time (D) and z-values. The D values of PME were: $D_{60°C} = 23.2'$, $D_{70°C} = 10.6'$, $D_{75°C} = 2.9'$, and $D_{85°C} = 1.7'$, with a z-value equal to 22.1 °C. Estimated data were lower than those reported by Tajchakavit and Ramaswamy (1997) for MW heating: $D_{70} = 20.0'$ ($z = 31.1$ °C); and by Ingallinera and others (2005) for conventional heating on laboratory scale: $D_{75} = 14.0'$ ($z = 21.5$ °C). Different results achieved by employing traditional techniques can be related to different temperature/time history of orange juice samples. Different D values were due to differences in heating mode, heating power, and mass of the

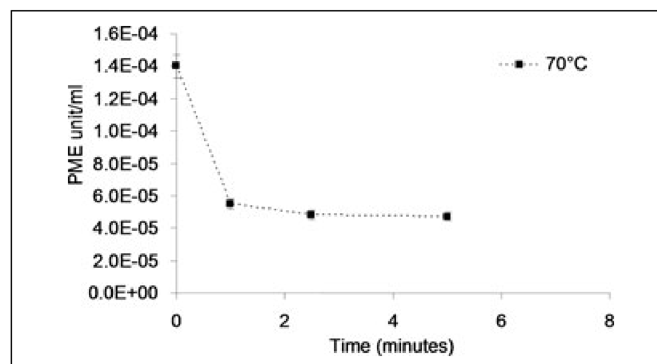

Figure 2 — Residual pectin methyl esterase activity (PME) in orange juices heated with microwave at 70 °C.

sample under test: in particular, in the cited paper by Ingallinera and others (2005), very small orange juice samples (100 μL) were considered. The different values between MW experiments can be explained by the effectiveness of the system adopted for temperature control. In our trials, the set point temperature was closely recovered due to the combination of IRT readout and stirring mode.

Color

Food color affects the perception of orange juice quality and is an important attribute in orienting consumers' preference. Fresh orange juice samples presented the following Hunter values: $L^* = 54.24 \pm 0.2$; $a^* = -1.26 \pm 0.10$, and $b^* = 11.38 \pm 0.04$. Chroma, a parameter that quantifies the intensity of color in orange juice, did not change significantly ($P < 0.05$) after MW heating at 70 °C until 2.5 min, while it increased significantly ($P < 0.05$) from 9.93 to 12.25 in instances of orange juice undergoing MW heating at 75 °C for 2 min and to 13.97 after MW heating at 85 °C for 1 min. On the other hand, it was noted that chroma did not change significantly in orange juice after 5 min of MW heating at 60 °C. Total color differences (ΔE) indicate the magnitude of the color difference between fresh orange juices and pasteurized juice at different time (Figure 3). ΔE rose to 0.77 after MW heating for 1 min at 70 °C and to 5.73 after 2.5 min at 70 °C. After 1 min of MW heating at 75 °C ΔE increased to 8.3 while after 1 min at 85 °C the value set to 11.2. It has been considered that a ΔE of 2 would be a noticeable visual difference for a number of situation (Francis and Clydesdale 1975). MW treatment at 60 °C for 5 min preserves the color parameters of unprocessed orange juice with respect to higher temperatures tested that caused ΔE values higher than 2. As expected, the samples processed at 75 °C and 85 °C showed higher colour differences than those heated at 60 °C and 70 °C.

Carotenoids

Carotenoids are quality indicators for orange juice as they contribute both to the color and to the nutritional value of the beverage. Oxidative degradation is the principal cause of extensive losses of carotenoids: such process depends on the availability of oxygen and is stimulated by heat, light, enzymes, metals (Rodriguez-Amaya 1999). Total carotenoid content, found to be 153.88 ± 4.5 μg/100g in fresh juices, decreased after MW pasteurization (70 °C for 1 min) to 133.90 ± 6.89 μg/100g, thus resulting in a 13% loss, while the decrease after heating at 60 °C for 2.5 min was 3%. More significant losses were recorded in orange juice processed at higher temperatures: the heat treatment caused losses ranging from 41% at 75 °C for 1 min, to 52% at 85 °C for 1 min. Of the 20 carotenoid peaks detected, 7 were quantified with reference to external standards, by taking into consideration, with the exception of zeaxanthin, the most important ones in terms of human health as well as the ones most commonly found in foods (Rodriguez-Amaya 1999). Since the optimal MW heating time/temperature combination needed to inactivate the heat sensitive fraction of PME, as reported above, has been reached at 70 °C for 1 min, it was therefore decided to show the chromatograms of the 7 carotenoids identified for fresh orange juice after MW heating at 70 °C for 1 min and after 2.5 min (Figure 4); the latter data, as well as the carotenoids at different temperatures, were discussed in a different paper. The major carotenoid detected in orange juice was β-cryptoxanthin: it accounted for about 64% of the total of carotenoids identified, and decreased by about 14% after MW pasteurization (70 °C for 1 min) (Figure 5). Structurally, cryptoxanthin is closely related to β-carotene; in the human body, cryptoxanthin is converted into vitamin A (retinol) and is, therefore, considered a provitamin A. Zeaxanthin represented 8.6% of total carotenoids in fresh orange juice and lutein content was 7.7%: after thermal treatment, the 1st decreased by 26%, while the latter increased by about 13%, thus confirming findings elsewhere reported (Lee and Coates 2003). Some carotenoids, such as zeaxanthin and β-carotene, are responsible for the visual color of fresh and pasteurized juices. After MW pasteurization, β-carotene decreased by about 27% and α-carotene by about 22%. Other authors reported a loss of 36% of provitamin A carotenoids (β-carotene, α-carotene and β-cryptoxanthin) in orange juice with thermal processing (Lessin and others 1997). In our trials, most pigment loss was caused by decrease in violaxanthin (53.4%), and antheraxanthin (31.0%). Such results were agreed with those by other authors (Lee and Coates 2003), that showed the high liability of 5,6-epoxide carotenoids such as violaxanthin and antheraxanthin, compared with other carotenoids present in processed fruit juices.

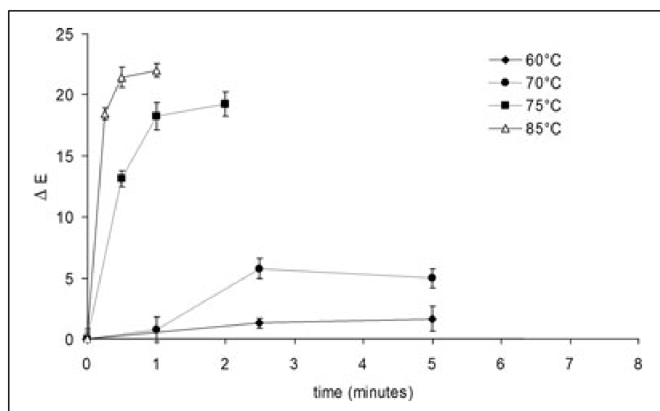

Figure 3—ΔE change of orange juice samples during microwave heating at different temperatures.

Vitamin C

One of the most important parameters of nutritional quality in orange juice is given by ascorbic acid content, whose properties as an antioxidant are associated with a reduced risk of cancer, neurological, and cardiovascular diseases (Byers and Perry 1992). Since vitamin C is heat labile, high processing temperatures can degrade this nutrient: several studies have been carried out to quantify the kinetic destruction of vitamin C at different temperatures

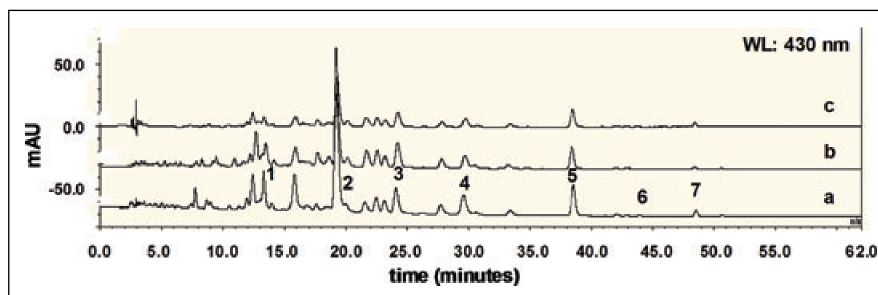

Figure 4—Carotenoids profiles in fresh orange juice (a), after microwave (MW) heating at 70 °C for 1 min (b) and after MW heating at 70 °C for 2.5 min (c). The identified peaks are: violaxanthin (1), antheraxanthin (2), lutein (3), zeaxanthin (4), β-cryptoxanthin (5), α-carotene (6), β-carotene (7).

and processing, and large differences in kinetic parameters have been observed. These variations can be attributed to the fact that nutrient destruction is a complex function of many variables such as pH, oxygen, salt, sugar, presence of enzymes, amino acids and metal catalysis (Lee and Coates 1999). In our study, ascorbic-acid amount showed slight changes after the 1st minute of treatment at all temperatures: values of ascorbic acid retention were found ranging from 97% at 70 °C to 96.1% at 85 °C (Figure 6). Vitamin C retention decreased by increasing MW heating time, even at 60 and 70 °C. For the mentioned temperatures, vitamin C retention ranged from about 92% (after 2.5 min at 70 °C) to about 82% (after 5 min at 70 °C), while at 60 °C the values decreased from 97% (after 2.5 min), to about 88% after 5 min. The data reported in our study showed an higher ascorbic acid retention in comparison with vitamin C thermal degradation obtained in orange juice heated by ohmic and infrared methods (Vikram and others 2005).

Conclusions

Microwave processing, with the support of IRT temperature control, constitutes an effective technology to stabilize fresh orange juice trough reduction of PME activity. The heat sensitive fraction of PME in orange juice was inactivated by MW heating after about 1 min at 70 °C, with a z-value of 22.12 °C. An analysis of thermal treatment on different carotenoids' losses showed differences in sensitivity. In particular, carotenes with provitamin A activity (β-carotene, α-carotene, and β-cryptoxanthin) showed lower changes than xanthophylls after MW pasteurization at 70 °C for 1 min. A slight decrease in vitamin C content was monitored after MW heating.

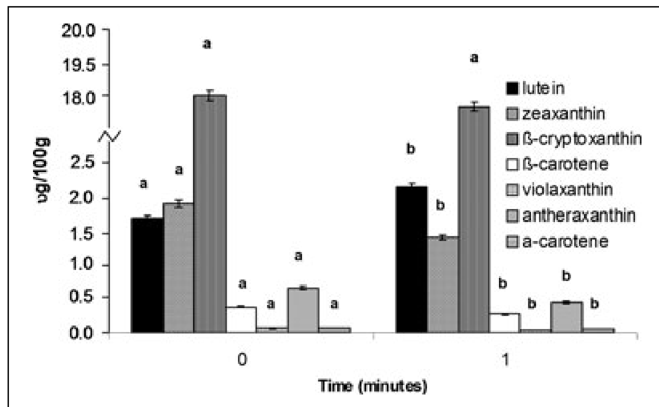

Figure 5—Change in carotenoids profiles in orange juice after microwave heating at 70 °C for 1 min. Different letters (a, b) indicate significant differences (P < 0.05) among carotenoid compounds.

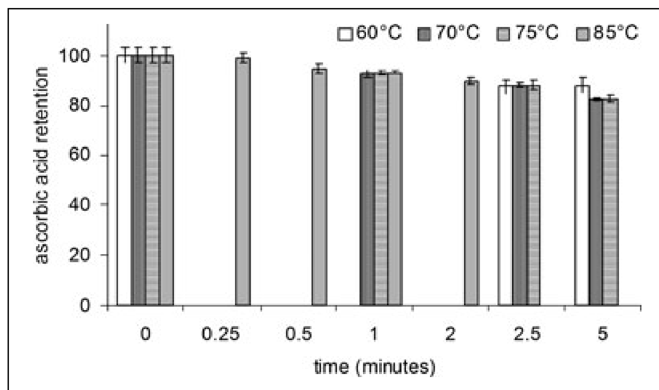

Figure 6—Vitamin C retention percent in orange juice samples during microwave heating at different temperatures.

References

Abd-El-Al MG, Abd-El-Fadeel MD, El-Samahy SK, Askar A. 1994. Application of microwave energy in the heat treatment of fruit juices, concentrates and pulps. Fruit Proc 4:307–12.

Byers T, Perry G. 1992. Dietary carotenes, vitamin C and vitamin E as protective antioxidants in human cancers. Ann Rev Nutr 12:139–59.

Cameron RG, Niedz RP, Grohmann K. 1994. Variable heat stability for multiple forms of pectin methylesterase from citrus tissue culture cells. J Agric Food Chem 42:903–8

Collet LSFCA, Shigeoka DS, Badolato GG, Tadini CC. 2005. A kinetic study on pectinesterase inactivation during continuous pasteurization of orange juice. J Food Eng 69:125–9

Cuccurullo G, Cinquanta L, Sorrentino G. 2007. A procedure to achieve fine control in MW processing of foods. Infr Phys Technol 49:292–6

Datta AK, Anantheswaran RC. 2001. Handbook of microwave technology for food applications. New York: Marcel Dekker Press.

Fennema O. 1996. Food chemistry, 3rd ed. New York: Marcel Dekker Press.

Francis FJ, Clydesdale FM. 1975. Food colourimetry: theory and applications. Westport: AVI Publishing Co.

Ingallinera B, Barbagallo RN, Spagna G, Palmeri R, Todaro A. 2005. Effects of thermal treatments on pectinesterase activity determined in blood oranges juices. Enzyme Microb Technol 36:258–63

Kim H, Tadini C, Singh RK. 1999. Effect of different pasteurization conditions on enzyme inactivation of orange juice in pilot-scale experiments. J Food Proc Eng 22:395–403

Kimbal DA. 1991. Citrus processing: quality control and technology. New York: Van Nostrand Reinhold Press.

Lee HS, Coates GA. 1999. Vitamin C in frozen, freshly squeezed, unpasteurized, polyethylen-bottled orange juice: a storage study. Food Chem 65:165–8.

Lee HS, Coates GA. 2003. Effect of thermal pasteurization on Valencia orange juice colour and pigments. Lebensm Wiss Technol 36:153–6.

Lee HS, Castle WS, Coates G A. 2001. High-performance liquid chromatography for the characterization of carotenoids in the new sweet orange grown in Florida, USA. J Chrom A 913:371–7.

Lessin WJ, Catigani GL, Schwartz SJ. 1997. Quantification of cis–trans isomers of provitamin A carotenoids in fresh and processed fruits and vegetables. J Agric Food Chem 45:3728–32.

Meléndez-Martínez AJ, Vicario IM, Heredia FJ. 2007. Review: analysis of carotenoids in orange juice. J Food Comp Anal 20:638–49.

Nikdel S, Mackellar DG. 1992. A microwave system for continuous pasteurization of orange juice. Florida Agricultural Experimental Station Journal series no. 00725. Lake Alfred, Fla. Proceedings of the Florida State Horticultural Society 105:108–10.

Oliveira MEC, Franca AS. 2002. Microwave heating of foodstuffs. J Food Eng 53: 347–59.

Rodriguez-Amaya DB. 1999. A guide to carotenoid analysis in foods. Washington, D.C.: Intl. Life Sciences Inst. (ILSI) Press.

Sadler GD, Parish ME, Wicker L. 1992. Microbial, enzymatic, and chemical changes during storage of fresh and processed orange juice. J Food Sci 57:1187–97.

Tajchakavit S, Ramaswamy HS. 1997. Thermal vs. microwave inactivation kinetics of pectin methylesterase in orange juice under batch mode heating conditions. Lebensm Wiss Technol 30:85–93.

[USDA-FAS] United State Dept. of Agriculture, Foreign Agriculture Service. (2008) Available from: http://www.fas.usda.gov. Accessed Nov 18, 2008.

Versteeg C, Rombouts FM, Spaansen CH, Pilnik W. 1980. Thermostability and orange juice cloud destabilizing properties of multiple pectinesterases from orange. J Food Sci 45:969–71.

Vikram VB, Ramesh MN, Prapulla SG. 2005. Thermal degradation kinetics of nutrients in orange juice heated by electromagnetic and conventional methods. J Food Eng 69:31–40.

Villamiel M, del Castillo MD, San Martin C, Corzo N. 1998. Assessment of the thermal treatment of orange juice during continuous microwave and conventional heating. J Sci Food Agric 78:196–200.