Manothermosonication of Foods and Food-Resembling Systems: Effect on Nutrient Content and Nonenzymatic Browning

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The effect of manothermosonication (MTS), an emergent technology for food preservation, on thiamin, riboflavin, carotenoids, and ascorbic acid was evaluated in milk and orange juice. The effect of both heat treatment and MTS on several compounds produced in nonenzymatic browning in model systems was also studied. MTS does not affect significantly the nutrient content studied. However, it changes the behavior of nonenzymatic browning. No formation of 5-(hydroxymethyl)-2-furfuraldehyde (HMF) was detected in fruit juice model systems after heat and MTS treatments at the experimental conditions used. In a milk-resembling system, free HMF formation by MTS is higher compared to that by heat treatment. As the MTS temperature increases, free HMF production by both treatments equaled on another. For bound HMF the production rate is lower by MTS than by heat treatment under the experimental conditions used. Formation kinetics of brown pigments and that of fluorescent compounds are different for both treatments. Fluorescence and brown pigment production are faster in MTS.

Keywords: *Vitamins; nonenzymatic browning; ultrasound; heat treatment; manothermosonication*

INTRODUCTION

Food preservation requires the destruction of microorganisms and enzymes. Heat treatment is the most widely method used to achieve this goal, but heat can also have a negative impact on nutritive value and organoleptic properties of foods. For this reason, there is an increased interest in new procedures able to inactivate enzymes and destroy microorganisms with little or no heat. Some of these methods combine heat with other physical or chemical agents to potentiate the effects of heat, so that the intensity of heat treatment applied could be reduced. Manothermosonication (MTS), which consists of the simultaneous application of heat and ultrasound under moderate pressure (100−700 kPa), is one of these new technologies (*1*−*3*).

As MTS is only suitable for treatment of liquid foods, two of the potential products to which MTS could be applied are fruit juices and milk. MTS is an efficient tool to inactivate enzymes from psychrotrophic bacteria (*4*), which are responsible for some quality problems of milk and some dairy products (*5*), and to inactivate thermoresistant pectin methylesterase in orange juice (*6*) and pectic enzymes from tomato paste (*7*). Moreover, ultrasonic waves and heat combine additively to inactive pathogenic microorganisms (*8*) and synergistically to destroy spores (*9*). Therefore, MTS could be a promising alternative method to treat milk and also to preserve fruit juices.

Although the effect of MTS on several enzymes and microorganisms has been studied, there is a lack of knowledge about its effect on other food components, reactions, and properties. Some aspects about ultrasound processing on the quality of dairy products have been recently reviewed (*10*), and the effects on milk of a combined treatment of heat and ultrasound in continuous flow have also been studied (*11*). These authors studied the effect on fat globule and particle distribution and detected a synergistic effect on denaturation of α-lactalbumin and *β*-lactoglobulin, but no effect was observed on casein. Despite all of these works, nothing is known about the effect of ultrasound on other important aspects of food such as vitamins and nonenzymatic browning reactions (NEB). Before MTS can be applied in milk or orange juice processing, an evaluation of the impact of MTS treatments on their nutritive value and organoleptic properties is needed.

Milk is a good source of thiamin and riboflavin, and orange juice is a good source of ascorbic acid and carotenoids. Thiamin is relatively unaffected by light and stable to oxidation, but it is among the least stable vitamins in solutions at neutral pH such as milk (*12*, *13*). Riboflavin is a heat stable vitamin, but it is very sensitive to oxidation and mainly to degradation by light (*13*−*15*). Its oxidation causes not only a loss in the nutritive value of dairy products but off-flavors, too (*13*, *14*). Vitamin C is quite resistant to heat treatment at low pH values, but it is sensitive to oxidation (*12*, *13*). Carotenoids composition in orange juice is complex (*16*), and their stability is similar to that of vitamin A in that they are sensitive to oxygen, light, and acid media (*12*, *14*).

Nonenzymatic browning is one of the most important and complex reactions that occur in foods. It affects both nutritional value and organoleptic properties. One of the main pathways is Maillard reaction, but ascorbic acid and sugars can also undergo browning reaction in the absence of amino acids, that is, caramelization and degradation of sugars. The Maillard reaction has been traditionally divided, although some recent publications

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(*17*) suggest new ways of considering the Maillard reaction, in three stages (*18*, *19*). The initial stage is the combination of the carbonyl group of the reducing sugars with a free amino group to give a Schiff base followed by Amadori′ rearrangement. Sugar dehydration, sugar fragmentation, and amino acid degradation, with concomitant yellow color formation and a strong absorption in the near-UV, are typical processes of the intermediate stage. In the final stage aldol condensation and aldehyde−amine polymerization are the main reactions involved, and this is when most of the color is produced. It is a very complex and not well understood reaction. However, some indicators have been proposed for monitoring each stage: furosine, carboxymethyllysine, lactulosyl-lysine, 5-(hydroxymethyl)-2-furfuraldehyde (HMF), lysylpyrraline, brown color, fluorescence, and others (*18*, *19*). Nevertheless, results in the literature are difficult to compare because treatment conditions and monitored compounds are different and because of the complexity of the reaction itself, even in the simplest model systems (*18*). Both milk and orange juice, which are much more complex than model systems, are quite sensitive to NEB reactions. In milk, lactose can isomerize via the Lobrey de Bruyn-Alberda van Ekenstein transformation followed by degradation to acids and other sugars. Alternatively, lactose may also react with the amino groups of lysine of caseins and whey proteins to form an Amadori product, lactulosyllysine (*20*). These are the main pathways that lead to HMF formation in dairy products. In fruit juice, browning has been linked mainly to ascorbic acid and sugar caramelization (*21*, *22*), although browning during storage in dehydrated orange juice has been related to the Maillard reaction (*23*, *24*).

The aim of this work is to explore the effect of MTS as a new technology on some nutrient contents and on the formation of several compounds produced during nonenzymatic browning reactions.

MATERIALS AND METHODS

MTS and Heat Treatments. Heat and MTS treatments were performed in triplicate in a continuous (*7*) or in a batch system (*1*, *8*) already described. Most experiments were performed in the continuous system; only those on the milk-resembling system were done in the batch system. Sonication conditions were 117 μm of amplitude, 20 kHz, and 200 kPa gauge pressure. Thiamin and riboflavin were studied in commercial ultrahigh-temperature-treated (UHT) milk without any modification. Carotenoids and ascorbic acid were studied in fresh orange juice obtained by squeezing navel oranges purchased in a local market and also treated in the continuous system. Browning compounds were determined in two model systems: a milk-resembling system, which consisted of 0.1 mol/L phosphate buffer, pH 6.6, with 3% (w/v) sodium caseinate and 2% (w/v) glucose, and a fruit juice-resembling system, which consisted of a 0.1 mol/L citrate buffer, pH 3.5, with 12% (w/v) glucose.

Nonenzymatic Browning Compound Determination. In the study of the impact of heat and MTS treatment on nonenzymatic browning reaction several compounds have been investigated: bound HMF (B-HMF), free HMF (F-HMF), brown pigments (BP, and fluorescent intermediary compounds (FIC).

B-HMF was determined as described by Morales et al. (*25*) with some modifications. Sugars and low molecular weight compounds were eliminated after thermal or MTS treatments using a 7 mL column of Bio-gel P-4 (Bio-Rad). Protein-containing fractions were treated with 0.5 mL of 0.3 N oxalic acid solution for 1 h at 100 °C. After cooling, the mixtures were deproteinized by adding 0.5 mL of 40% trichloroacetic acid and centrifuged at 10000g for 15 min. The supernatant was filtered [using a syringe filter 4 mm Millex-HV (Millipore)] and injected in the HPLC column.

F-HMF was determined as described by Morales et al. (*25*) with some modifications. After treatments, samples were deproteinized by adding 0.5 mL of 40% trichloroacetic acid and centrifuged at 10000*g* for 15 min. After the filtration [using a syringe filter 4 mm Millex-HV (Millipore)], the sample was ready for HPLC analysis.

HPLC analysis of HMF was performed in an analytical liquid chromatographic system from Pharmacia LKB (Uppsala, Sweden) in isocratic conditions. The column was a reversed-phase Superpac Spherisorb ODS, 2.5 μm, 4 × 250 mm (Pharmacia). The flow rate was 1 mL/min, the mobile phase was 80 mmol/L acetate buffer, pH 3.9, and HMF was detected by measuring the absorbance at 280 nm. A calibration regression line was obtained from standards in a range between 0 and 80 μmol/L HMF (Sigma, St. Louis, MO) in Millipore-filtered water.

FIC were determined by fluorescence spectrophotometry as described by Morales et al. (*26*) using a Hitachi F-1200 fluorometer (Tokyo, Japan). Excitation and emission wavelengths were set at 345 and 415 nm, respectively.

BP were determined as described by Del Castillo et al. (*23*) by reading the absorbance at 420 nm using a Unicam UV 500 spectrophotometer with Vision 32 software (Cambridge, U.K.).

Riboflavin Determination. Riboflavin was determined fluorometrically according to the method described by Rashid and Potts (*27*). Twenty milliliters of milk was pipetted in an Erlenmeyer flask, and 2 mL of 10% (w/v) lead acetate solution acidified to pH 3.2 with glacial acetic acid was added to the 20 mL of milk. After mixing, it was filtered through a No. 42 ashless Whatman filter paper. Fluorescence was measured in the filtrate (excitation and emission wavelengths were set at 440 and 530 nm, respectively). A calibration curve was obtained using riboflavin supplied by Sigma Chemical Co.

Thiamin Determination. Thiamin was determined with a fluorometric method, after acid and enzymatic hydrolysis, filtration, and oxidation to thiochrome in a basic medium with potassium ferricyanide (*28*). Twenty milliliters of milk was adjusted to pH 3.5 and treated at 120 °C for 15 min. After cooling, the pH was adjusted to 6.6 and 500 μL of 1% (w/v) papain (Sigma Chemical Co.) solution and 500 μL of 10% (w/v) takadiastase (Fluka) solution were added. The mixture was incubated at 37 °C during 12 h. Samples were then filtered through Whatman No. 1 paper, and the filtrate was used for thiamin analysis. Five hundred microliters of the filtrate were pipetted in test tubes containing 0.5 g of NaCl, and 500 μL of an oxidizing reagent [0.04% ferricyanide potassium in 15% (w/v) NaOH] were added and rapidly mixed. After this step, 5 mL of isobutanol was added and the tubes were shaken vigorously and centrifuged at 1000*g* during 10 min. Thiochrome was measured fluorometrically in the supernatant (excitation and emission wavelengths were set at 365 and 435 nm, respectively). Blanks were treated the same way except that the oxidizing reagent was substituted by 15% (w/v) NaOH. A calibration curve was obtained using thiamin supplied by Sigma Chemical Co.

Ascorbic Acid Determination. The method used is based upon the reduction of the dye 2,6-dichlorophenolindophenol (DCPIP) with ascorbic acid in an acid solution (*29*). One milliliter of orange juice was diluted to 10 mL with metaphosphoric acid−acetic acid solution. This solution was titrated rapidly with a DCPIP solution (50 mg of DCPIP sodium salt in 50 mL of water in which 42 mg of $NaHCO_3$ had been previously dissolved). A calibration curve was obtained using ascorbic acid from Sigma Chemical Co.

Carotenoid Determination. A quick method for a rapid evaluation of total carotenoids has been used. The method is based on the high correlation existing between total carotenoids concentration in orange juice and absorbance at 450 nm of an alcoholic extract (*30*). Five milliliters of orange juice was diluted to 50 mL with absolute ethanol. The solution was kept in the dark until a precipitate was formed (∼30 min) and

Table 1. Residual Thiamin and Riboflavin in Commercial UHT Milk after MTS in a Continuous Equipment at 200 kPa and 117 μm Treatment at Different Temperatures and Residence Times (Mean and Standard Deviation)

MTS temp/time	% residual thiamin	% residual riboflavin
55 °C/12 s	100.4 ± 0.90	99.7 ± 0.76
55 °C/25 s	100 ± 0.85	98.5 ± 0.55
65 °C/12 s	98.8 ± 0.82	99.2 ± 0.44
65 °C/25 s	97.7 ±0.83	100.2 ± 0.78
75 °C/12 s	96.7 ± 1.10	98.3 ± 0.72
75 °C/25 s	99.5 ± 0.99	100.2 ± 0.50
85 °C/12 s	98.3 ± 0.78	97.5 ± 0.43
85 °C/25 s	98.7 ± 0.58	99.3 ± 0.90

Table 2. Residual Ascorbic Acid and Carotenoids in Fresh Orange Juice after Heat Treatments (HT) and MTS at 117 μm and 200 kPa

treatment	% residual ascorbic acid	% residual carotenoids
MTS 62 °C/ 30 s	90 ± 1.1	88.3 ± 1.02
MTS 62 °C/ 15 s	90.8 ± 1.20	92 ± 1.12
HT 62 °C/ 30 s	99 ± 1.0	100 ± 0.71
HT 62 °C/ 15 s	99 ± 1.10	100 ± 0.78

centrifuged at 2500*g* during 10 min. Absorbance of the supernatant between 390 and 590 nm was measured with a Unicam UV 500 spectrophotometer with Vision 32 software.

Statistics. All treatments were performed in triplicate, and all of the parameters studied were also determined per triplicate for each treatment. The Statview program (Abacus Concepts Inc., Berkeley, CA) was used for statistical analysis.

RESULTS

Effect of MTS on Thiamin and Riboflavin Contents. Table 1 shows residual thiamin and riboflavin contents in commercial UHT milk after MTS treatments at different temperatures for 25 and 12 s. It is quite clear that MTS treatments at 55, 65, 75, and 85 °C have no effect on the contents of these vitamins in milk. Mean values calculated were 21 μg/100 mL for thiamin and 95 μg/100 mL of milk for riboflavin. The normal ranges for these vitamins in milk are 20−80 μg of thiamin/100 mL and 80−250 μg of riboflavin/100 mL (*31*, *32*), so the data found are among these values. There was no significant difference between values calculated for the different treatments.

Effect of MTS on Ascorbic Acid and Total Carotenoids Content. Table 2 shows the percentage of residual ascorbic acid and carotenoids content in fresh orange juice after heat treatments and MTS at 117 μm and 200 kPa at different conditions. Heat and MTS treatments at 62 °C during 15 and 30 s produce only little additional loss in these nutrients. The average content of ascorbic acid in orange juice was ∼67.7 mg/100 mL, which is within normal values (40−60 mg/100 g of orange juice; *31*). The main loss was ∼10% of ascorbic acid in MTS treatment at 62 °C during 30 s.

There is a slight difference among residual carotenoid contents calculated for the different MTS treatments. Average carotenoid content expressed as β-carotene was 1.2 mg/100 mL of juice (normal values of carotenoids are within 0.5−2.85 mg/100 mL of juice; *33*).

Effect of MTS on HMF. No formation of F-HMF was detected in fruit juice model systems after heat and MTS treatments at the experimental conditions used.

Figures 1 and 2 show the formation of F-HMF and B-HMF by heat and MTS treatments at 92, 102, and 111 °C in a milk-resembling system. At 92 °C, F-HMF

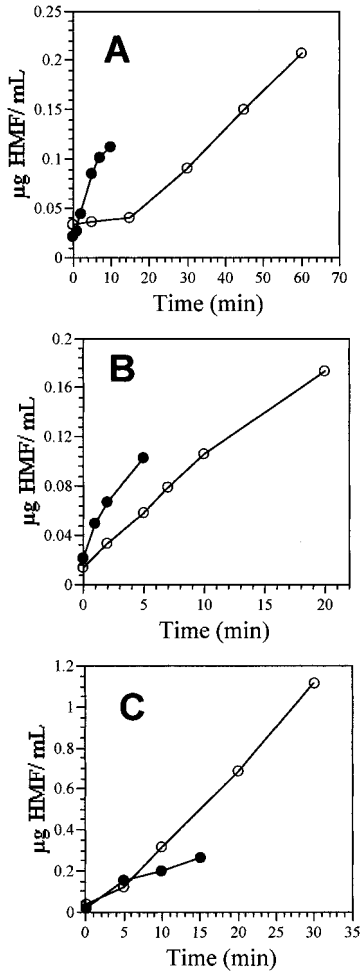

Figure 1. Formation of F-HMF by heat (○) and MTS (●) at 200 kPa and 117 μm at different temperatures: (A) 92 °C; (B) 102 °C; (C) 111 °C.

formation by MTS is higher compared to that by heat treatment, but as the MTS temperature increases, the difference between the two treatments becomes less. Even at 111 °C, the formation rate of F-HMF by heat treatment is higher than by MTS. B-HMF production occurs more rapidly in heat than in MTS treatment under the experimental conditions used. This is specially clear at 111 °C, where its formation by heat treatment is higher than by MTS.

Effect of MTS on Fluorescent Compounds and Brown Pigments. Figure 3 shows the formation of BP and FIC by heat and MTS treatments at 90 °C in a milk-resembling model system. The formation kinetics of the two groups of compounds are different for both treatments, although they are similar for both compounds in the same treatment. Increases in fluorescence and absorbance are faster when ultrasounds are applied combined with heat.

The evolution of FIC and BP in a fruit juice-resembling system by MTS at different temperatures is shown in Figure 4. Their formation seems to be temperature independent in the temperature range studied. Moreover, the evolutions of both groups of compounds (FIC and BP) are parallel. Figure 4 also compares the evolution of FIC and BP produced by heat and MTS treatment at 75 °C. It shows clearly that heat treatment at 75 °C during the same time scale (1 min) used by MTS has almost no effect on BP or FIC formation, whereas there is a clear increase in both

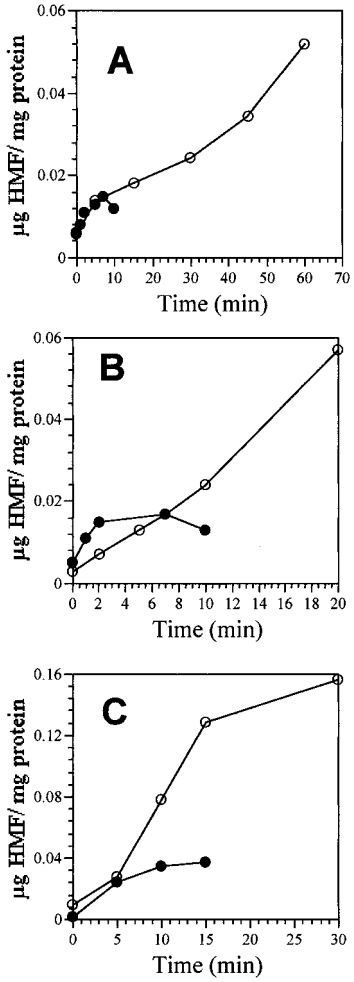

Figure 2. Formation of B-HMF by heat (○) and MTS (●) at 200 kPa and 117 μm at different temperatures: (A) 92 °C; (B) 102 °C; (C) 111 °C.

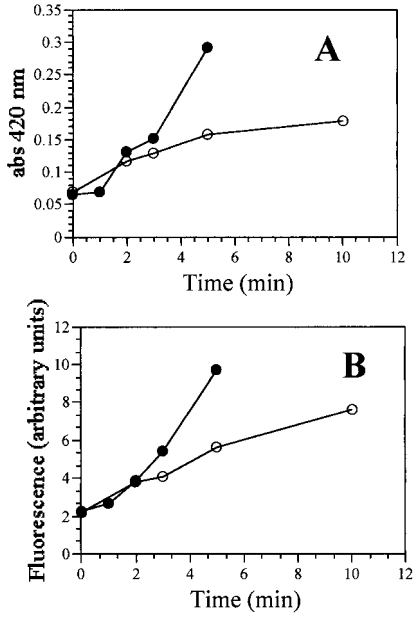

Figure 3. Production of BP (A) and FIC (B) by heat (○) and MTS (●) at 200 kPa and 117 μm at 90 °C in milk.

groups of compounds during MTS treatment. FIC and BP were also determined after heat and MTS treatments of fresh orange juice at 62 °C for 15 and 30 s,

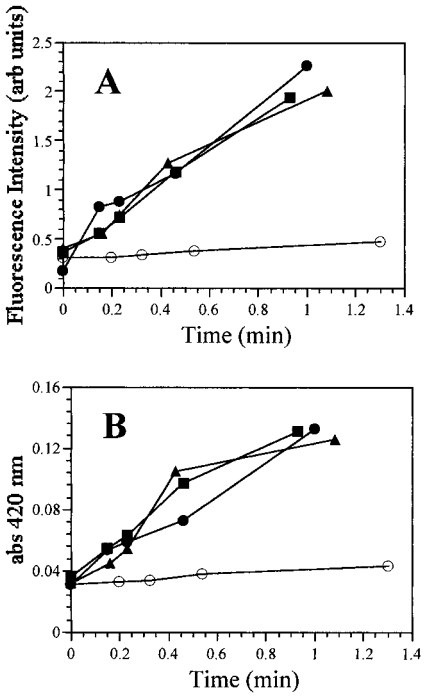

Figure 4. Production of FIC (A) and BP (B) in fruit juice resembling system by MTS at 200 kPa and 117 μm at 69 (■), 75 (●), and 82 °C (▲) and by simple heating at 75 °C (○).

and no significant differences were found for either FIC or BP, perhaps due to a high blank value (data not shown).

DISCUSSION

Effect on Vitamins. Several mechanisms can act when ultrasound is applied in a liquid medium: (1) purely thermal due to the high temperatures and pressures located in hot spots produced by bubble implosion, (2) mechanical stresses produced by microstreaming and by implosion shock waves, and (3) free radical production. Mechanical stresses affect only molecules with a high molecular weight (*34*, *35*), and although temperatures in hot spots can be as high as 5000 °C, the volume of liquid that reaches these temperatures is very small (*36*), so only a few molecules could be affected by this high temperature (5000 °C) during a short time (10 ns) treatment. At least in theory, free radical production seems to be the most probable mechanism by which ultrasound could destroy vitamins.

Vitamins studied in this work seem to be not especially affected by the combined action of heat and ultrasound under moderate pressure. In milk, both thiamin and riboflavin are quite resistant to MTS and to heat treatment. Several authors have reported heat inactivation kinetics of these vitamins but at higher temperatures and for longer periods of time (*37*). For thiamin, *D* values at 129 °C in model systems between 114 and 11.6 min have been reported, depending on several factors (*38*). In foods, thiamin resistance is higher, giving *D* values at 137 °C of ~60 min; an E_a of thermal inactivation was calculated to be 27 kcal/mol (*39*). Riboflavin is a heat resistant vitamin; one of the main causes of loss in milk and dairy products is photooxidation by exposure to light (*14*). Therefore, under the heating conditions used in this work, which are not intense, the lack of effect observed with these vitamins is expected.

Thermal conditions applied in the present study are not able to destroy ascorbic acid and carotenoids in a significant way (*12*, *37*, *40 41*). Precisely, this relatively low-temperature condition in our experimental conditions was chosen to have a reference with none of the negative consequences that high temperatures exert on orange juice.

Thiamin, riboflavin, ascorbic acid, and carotenoids are rather sensitive to oxidation, and perhaps this is because a higher destruction rate can be expected when ultrasound is applied. To our surprise, the effect of ultrasound on these compounds is not important. It is well-known that ultrasound can promote different chemical reactions (*36*) and produce free radicals under the experimental conditions used in the present work (*42*) and, as will be discussed later, affect the complex network of NEB reactions, so it is difficult to explain why only ascorbic acid and carotenoids seem to be slightly affected by ultrasonic waves and only in some concrete conditions. Perhaps an explanation could be based on the fact that reactions mediated by free radicals generated by ultrasound are restricted, or much more probable, to a small fraction of the irradiated volume. According to Riesz and Kondo (*43*), reactions mediated by free radicals generated by ultrasound can take place in three different regions: (1) inside the imploding bubble due to the high temperatures and pressures reached (reaction in this region would implicate the volatility of the compounds involved in the reaction); (2) in the interface between bubbles and bulk liquid where high temperature and pressure gradients exist (the effects produced depend on the hydrophobicity of compounds involved, which will allow a higher concentration of these molecules in this area); and (3) in the bulk liquid (in this region, free radicals generated in the bubble that have not been recombined or have not been scavenged in the interfacial region can react with compounds dissolved in the liquid). Free radicals are very unstable (hydroxyl radical half-life is 10^{-8} s), and they will react most probably not far away from the place where they are generated. According to this, the possibility of a nonvolatile molecule reacting with a free radical generated by ultrasound will depend on its concentration and its hydrophobicity. Increasing these parameters will increase the probability of reaction with free radicals generated during bubble implosion. Our results agree with this hypothesis: thiamin and riboflavin are water soluble vitamins, their concentration is not high [thiamin, 0.21 μg/mL (0.6 μmol/L); riboflavin, 0.75 μg/mL (2μmol/L)], and they are not affected by ultrasound. Perhaps their concentration is not high enough to allow their presence in the interfacial region, and this, combined with the fact that ultrasonic intensity applied is not extremely high, could explain why these substances are not affected by free radicals generated by ultrasonic waves under the experimental conditions. Carotenoids and ascorbic acid are slightly affected by MTS treatments contrary to what happens with thiamin and riboflavin, but carotenoids are present at higher concentration (18 μmol/L) and they are liposoluble so they can be easily present in the interface regions. On the contrary, ascorbic acid concentration is much higher (670 μg/mL, 3.5 mmol/L) than thiamin or riboflavin concentrations. Anyway, in the study of free radical production by MTS in treatment conditions similar to those used in this work, the terephthalate concentration (a quite hydrophobic anion) used was 12.5 mmol/L, >3-fold higher than the ascorbic acid concentration in this work. According to this hypothesis, higher ultrasound intensities or sonication of medium with higher vitamin concentrations would result in an increased loss of vitamins.

Effect on Browning Reactions. Experimental data obtained in this work show clearly that there are differences in browning reactions induced by heat and by MTS. Due to the complexity of NEB reactions and the nature of this work, it is outside the scope of this work to discuss formation mechanisms or kinetics of browning reactions. Recent reviews on this subject (*18*−20 *44*−*46*) emphasize the complexities of NEB reactions and note the lack of knowledge about the precise pathways, mechanisms, and kinetics of these reactions.

Free HMF production by heat treatment in a milk-resembling system is similar to descriptions made in the literature (*47*). F-HMF production by MTS is a faster reaction at 92 °C and, as temperature increases, it approaches that of simple heating; even at 111 °C F-HMF production by heat is faster than by MTS. These rate differences could occur for very different reasons. Ultrasound can affect the F-HMF formation or destruction rate or even both. The temperature dependence of both reactions can be also very different, which together with the well-known fact that ultrasound intensity diminishes at higher temperatures, would explain the different F-HMF formation rates by heat and MTS treatments at different temperatures.

B-HMF is directly involved in reactions with proteins. B-HMF levels are lower after MTS treatments under all of the experimental conditions tested. This could be related to the well-known effect of MTS on proteins. Enzyme inactivation or protein degradation could diminish the availability of lysine groups of protein to react with glucose, reducing in this way B-HMF. Ultrasound could also promote reactions of sugars with other compounds, reducing also their availability to react with proteins. Ultrasound could also promote reactions of sugars and proteins without any B-HMF formation and, therefore, would not be detectable by our methods. Finally, ultrasound could destroy B-HMF after it is formed.

Although Villamiel and de Jong (*11*) have shown that caseins are not affected by a combined treatment of heat and ultrasound, their experiments were performed at much lower ultrasonic irradiation intensity (no external pressure and shorter times) and at lower temperatures, so from their data it is not possible to discard any of the possibilities mentioned before. Under our experimental conditions, it is possible that caseins have been affected by ultrasound.

In fruit juice-resembling systems, we did not observe formation of F-HMF. This is not surprising because treatment temperature/time combinations were not intense enough to yield these products, compared to those used in previous works about nonenzymatic browning in several media (*48*−*51*).

In milk-resembling systems, BP and FIC show a kinetic behavior, which also fits well to kinetic models used in the literature. The production of BP and that of FIC both by heat and by MTS show parallel behaviors. Overby and Frost (*52*) have observed a peak of fluorescence followed by an increase in brown pigments, which will indicate that formation of fluorescent compounds occurs prior to the development of brown pigments, and Cerruti et al. (*53*) concluded that FIC are

precursors of BP but different from them. Our results show, for both milk- and fruit juice-resembling systems, that the evolutions of FIC and BP are parallel for MTS treatments, but from our experimental data we cannot conclude if FIC are BP precursors, especially because without a suitable standard it is not possible to quantify compounds by fluorescence and therefore to compare them with BP measured by absorbance.

In fruit juice-resembling systems the most surprising results are BP and, mainly, FIC formations themselves. In previous works, FIC formation by heating in acidic media, which contained only citrate and glucose, was negligible (*53*). It has been stated that the formation of these compounds will require a first step with the reaction of an amine group (*54*) with a sugar, and this step would not be possible in a model system without amino groups, such as the one used in this work. Our heat treatment, but not MTS, results agree with this theory. Again, there could be different reasons for this behavior. First, MTS could accelerate reactions that take place in heat treatments and make them possible at temperatures lower than that necessary to observe the same phenomenon by simple heating. Morales et al. (*26*) have detected trace levels of fluorescent compounds in model systems containing only lactose but at higher pH values and after more intense heat treatments. The authors stated that the main way to produce FIC in milk is Maillard reaction, but they are also formed by Lobrey de Bruyn-Alberda van Ekenstein transformation. Although our model system is different, it is possible that ultrasound could promote this pathway, yielding a higher and faster production of FIC. Ultrasound could promote different reactions that could lead to the formation of both fluorescent and brown pigments. A previous work about the effect of ultrasound and γ-irradiation on glucose solutions has shown that ultrasonic waves are able to produce several glucosyl radicals and, in the presence of oxygen, polymers in the molecular weight range of 4000 Da (*55*), and perhaps this kind of compound could be involved in fluorescence or browning, but those parameters were not studied. Of course, a combination of the previous explanations is also possible.

The main conclusion obtained in this part of the work is that nonenzymatic browning reactions are different under heat and under MTS treatments. It is not possible to state if this difference is due to an acceleration by ultrasound of reactions that take place in heat treatments or, on the contrary, ultrasound is able to promote different reactions yielding different compounds.

Conclusion. Results obtained in this work show that MTS does not affect significantly nutrient contents studied but changes the behavior of nonenzymatic browning, which could be a problem in the implementation of MTS as an industrial method for food preservation. However, as MTS allows much shorter treatments because of its higher microorganism and enzyme inactivation efficiency, the higher rate of NEB reactions detected under some conditions could be compensated by the shorter time needed to inactivate enzymes and/or microorganisms.

ABBREVIATIONS USED

MTS, manothermosonication; NEB, nonenzymantic browning; HMF, hydroxymethylfurfuraldehyde; F-HMF, free HMF; B-HMF, bound HMF; FIC, fluorescent intermediary compounds; BP, brown pigments; DCPIP, dichlorophenol-indophenol.

LITERATURE CITED

(1) López, P.; Sala, F. J.; de la Fuente, J. L.; Condón, S.; Raso, J.; Burgos, J. Inactivation of peroxidase, lipoxygenase and polyphenol oxidase by manothermosonication. *J. Agric. Food Chem*. **1994**, *42*, 252−256.

(2) Sala, F. J.; Burgos, J.; Condon, S.; Lopez, P.; Raso, J. Effect of heat and ultrasounds on microorganisms and enzymes. In *New Methods of Food Preservation*; Gould, G. W., Ed.; Blackie: Glasgow, U.K., 1996; pp 176−204.

(3) Burgos, J. Manothermosonication. In *Encyclopedia of Food Microbiology*; Robinson, R. K., Batt, C. A., Patel, P. D., Eds.; Academic Press: New York, 1999; pp 1462−1469.

(4) Vercet, A.; López, P.; Burgos, J. Inactivation of heat-resistant lipase and protease from *Pseudomonas fluorescens* by manothermosonication. *J. Dairy Sci*. **1997**, *80*, 29−36.

(5) Sørhaug, T.; Stepaniak, L. Psychrotrophs and their enzymes in milk and dairy products: Quality aspects. *Trends Food Sci. Technol.* **1997**, *8*, 35−41.

(6) Vercet, A.; López, P.; Burgos, J. Inactivation of heat-resistant pectin methylesterase from orange by manothermosonication. *J. Agric. Food Chem*. **1999**, *47*, 432−437.

(7) López, P.; Vercet, A.; Sanchez, A. C.; Burgos, J. Inactivation of tomato pectic enzymes by manothermosonication. *Z. Lebensm. Unters. Forsch*. **1998**, *207*, 249−252.

(8) Raso, J.; Pagan, R.; Condon, S.; Sala, F. J. Influence of temperature and pressure on the lethality of ultrasound. *Appl. Environ. Microbiol*. **1998**, *64*, 465−471.

(9) Raso, J.; Palop, A.; Pagan, R.; Condon, S. Inactivation of *Bacillus subtilis* spores by combining ultrasonic waves under pressure and mild heat treatment. *J. Appl. Microbiol*. **1998**, *85*, 849−854.

(10) Villamiel, M.; Van Hamersveld, E. H.; De Jong, P. Review: effect of ultrasound processing on the quality of dairy products. *Milchwissenschaft* **1999**, *54*, 69−73.

(11) Villamiel, M.; De Jong, P. Influence of high-intensity ultrasound and heat treatment in continuous flow on fat, proteins and native enzymes of milk. *J. Agric. Food Chem*. **2000**, *48*, 472−478.

(12) Clydesdale, F. M.; Ho, C. T.; Lee, C. Y.; Mondy, N. I.; Shewfelt, R. L. The effects of postharvest treatment and chemical interactions on the bioavailability of ascorbic acid, thiamin, vitamin A, carotenoids and minerals. *Crit. Rev. Food Sci*. **1991**, *30*, 599−638.

(13) Gregory, J. F. Vitamins. In *Food Chemistry*; Fennema, O. R., Ed.; Dekker: New York, 1996; pp 531−616.

(14) Ottaway, P. B. Stability of vitamins in food. In *The Technology of Vitamins in Food*; Ottaway, P. B., Ed.; Blackie Academic and Profesional: Glasgow, U.K., 1993; pp 90−113.

(15) Ball, G. F. M. Riboflavin and other flavins. In *Bioavailability and Analysis of Vitamins in Foods*; Ball, G. F. M., Ed.; Chapman and Hall: London, U.K., 1998; pp 293−317.

(16) Mouly, P. P.; Gaydou, E. M.; Lapierre, L.; Corsetti, J. Differentiation of several geographical origins of single strength Valencia orange juices using quantitative comparasion of carotenoids profiles. *J. Agric. Food Chem*. **1999**, *47*, 4038−4045.

(17) Yaylayan, V. A. Classification of the Maillard reaction: a conceptual approach. *Trends Food Sci. Technol.* **1997**, *8*, 13−18.

(18) Labuza, T. P.; Baisier, W. M. The kinetics of nonenzymatic browning. In *Physical Chemistry of Foods*; Schwartzberg, H. G., Hartel, R. W., Eds.; Dekker: New York, 1992; pp 595−649.

(19) Van Boeckel, M. A. J. S. Effect of heating on Maillard reactions in milk. *Food Chem*. **1998**, *62*, 403−414.

(20) O'Brien, J. Reaction Chemistry of lactose: nonenzymatic degradation pathways and their significance in dairy products. In *Advanced Dairy Chemistry, Vol 3. Lactose, Water, Salts and Vitamins*; Fox, P. F., Ed.; Chapman and Hall: London, U.K., 1997; pp 155−231.
(21) Kanner, J.; Fishbein, J.; Shalom, P.; Harel, S.; Ben-Gera, I. Storage stability of orange juice concentrate packaged aseptically. *J. Food Sci.* **1982**, *47*, 429−431.
(22) Kimball, D. A. Processing contamination. In *Citrus Processing: Quality Control and Technology*; Kimball, D. A., Ed.; AVI Book, Van Nostrand Reinhold: New Yorx, 1991; pp 258−278.
(23) Del Castillo, D.; Corzo, N.; Polo, M. C.; Pueyo, E.; Olano, A. Changes in amino acid composition of dehydrated orange juice during accelerated nonenzymatic browning. *J. Agric. Food Chem.* **1998**, *46*, 277−280.
(24) Del Castillo, D.; Corzo, N.; Olano, A. Early stages of Maillard reaction in dehydrated orange juice. *J. Agric. Food Chem.* **1999**, *47*, 4388−4390.
(25) Morales, F. J.; Romero, C.; Jiménez-Pérez, S. Chromatographic determination of bound hydroxymethylfurfural as an index of milk protein glycosylation. *J. Agric. Food Chem.* **1997**, *45*, 1570−1573.
(26) Morales, F. J.; Romero, C.; Jiménez-Pérez, S. Fluorescence associated with Maillard reaction in milk and milk-resembling systems. *Food Chem.* **1996**, *57*, 423−428.
(27) Rashid, I.; Potts, D. Riboflavin determination in milk. *J. Food Sci.* **1980**, *45*, 744−745.
(28) AOAC. Thiamine in foods. Fluorimetric method. In *Official Methods of Analysis*; Helrich, K., Ed.; AOAC: Arlington, VA, 1990; Vol. 2, pp 1049−1051.
(29) AOAC. Vitamin C in vitamin preparations and juices. In *Official Methods of Analysis*; Helrich, K., Ed.; AOAC: Arlington, VA, 1990; Vol. 2, pp 1058−1059.
(30) Casas, A.; Mallent, D.; Montoro, R. Rapid evaluation of the total carotenoids content of orange juice. *Rev. Esp. Cienc. Tecnol. Aliment.* **1976**, 503−507.
(31) Crawley, H. Natural occurrence of vitamins in food. In *The Technology of Vitamins in Food*; Ottaway, P. B., Ed.; Blackie Academic and Professional: Glasgow, U.K., 1993; pp 19−41.
(32) Öste, R.; Jägerstad, M.; Anderson, I. Vitamins in milk and milk products In *Advanced Dairy Chemistry, Vol. 3. Lactose, Water, Salts and Vitamins*; Fox, P. F., Ed.; Chapman and Hall: London, U.K., 1997; pp 347−402.
(33) Higby, W. K. A simplified method for determination of some aspects of the carotenoid distribution in natural and carotene-fortified orange juice. *J. Food Sci.* **1962**, 42−49.
(34) Basedow, A. M.; Ebert, K. H. Ultrasonic degradation of polymers in solution. *Adv. Polym. Sci.* **1977**, *22*, 83−148.
(35) Doullah, M. S. A proposed mechanism for the degradation of addition polymers in cavitating ultrasonics fields. *J. Appl. Polym. Sci.* **1978**, *22*, 1735−1743.
(36) Suslick, K. S. Sonochemistry. *Science* **1990**, *247*, 1439−1445.
(37) Lund, D. Effects of heat processing on nutrients. In *Nutritional Evaluation of Food Processing*; Karmas, E., Harris, R. S., Eds.; AVI Book, Van Nostrand Reinhold: New York, 1988; pp 319−354.
(38) Mulley, E. A.; Stumbo, C. R.; Hunting, W. M. Kinetics of thiamine degradation by heat. Effect of pH and form of the vitamin on its rate of destruction. *J. Food Sci.* **1975**, *40*, 989−992.
(39) Mulley, E. A.; Stumbo, C. R.; Hunting, W. M. Kinetics of thiamine degradation by heat. A new method for studying reaction rates in model systems and food products at high temperature. *J. Food Sci.* **1975**, *40*, 985−988.
(40) Lathrop, P. J.; Leung, H. K. Rates of ascorbic acid degradation during thermal processing of canned peas. *J. Food Sci.* **1980**, *45*, 152−153.
(41) Morales, F. J.; Romero, C.; Jiménez-Pérez, S. New methodologies for kinetic study of 5-(hydroxymethyl)-furfural formation and reactive lysine blockage in heat-treated milk and model systems. *J. Food Prot.* **1995**, *58*, 310−315.
(42) Esteve, M. J.; Frígola, A.; Martorell, A.; Rodrigo, C. Kinetics of green asparagus ascorbic acid heated in a high-temperature thermoresistometer. *Z. Lebensm. Unters. Forsch.* **1999**, *208*, 144−147.
(43) Vercet, A.; López, P.; Burgos, J. Free radical production by manothermosonication. *Ultrasonics* **1998**, *36*, 615−618.
(44) Riesz, P.; Kondo, T. Free radical formation induced by ultrasound and its biological implications. *Free Radical Biol. Med.* **1992**, *13*, 247−270.
(45) Yaylayan, V. A.; Huyghues-Despointes, A. Chemistry of Amadori rearrangement products: analysis, synthesis, kinetics, reactions and spectroscopic properties. *Crit. Rev. Food Sci.* **1994**, *34*, 321−369.
(46) Friedman, M. Food browning and its prevention: an overview. *J. Agric. Food Chem.* **1996**, *44*, 631−653.
(47) Van Boeckel, M. A. J. S. Kinetic modelling of sugar reactions in heated milk-like systems. *Neth. Milk Dairy J.* **1996**, *50*, 245−266.
(48) Beveridge, T.; Harrison, J. E. Nonenzymatic browning in pear juice concentrate at elevated temperatures. *J. Food Sci.* **1984**, *14*, 1335−1336.
(49) Toribio, J. L.; Lozano, J. E. Heat induced browning of clarified apple juice at high temperatures. *J. Food Sci.* **1986**, *51*, 172−175.
(50) Ajandouz, E. H.; Puigserver, A. Nonenzymatic browning reaction of essential amino acids: effect of pH on caramelization and Maillard reaction kinetics. *J. Agric. Food Chem.* **1999**, *47*, 1786−1793.
(51) Hidalgo, A.; Pompei, C. Hydroxymethylfurfural and furosine reaction kinetics in tomato products. *J. Agric. Food Chem.* **2000**, *48*, 78−82.
(52) Overby, L. R.; Frost, D. V. The effects of heat on the nutritive value of protein hydrolysates with dextrose. *J. Nutr.* **1952**, *46*, 539.
(53) Cerruti, P.; Resnik, S. L.; Seldes, A.; Fontan, C. F. Kinetics of deteriorative reactions in model food systems of high water activity: glucose loss, 5-hydroxymethylfurfural accumulation and fluorescence development due to nonenzymatic browning. *J. Food Sci.* **1985**, 627−630.
(54) Adhikari, H. R.; Tapel, A. L. Fluorescent products in a glucose-glycine browning reaction. *J. Food Sci.* **1973**, *38*, 486−488.
(55) Portenlänger, G.; Heusinger, H. Polymer formation from aqueous solutions of α-D-glucose by ultrasound and γ-rays. *Ultrason. Sonochem.* **1994**, *1*, 125−129.

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