Comparison of Sensory, Microbiological, and Biochemical Parameters of Microwave Versus Indirect UHT Fluid Skim Milk During Storage

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ABSTRACT

Shelf-stable milk could benefit from sensory quality improvement. Current methods of heating cause flavor and nutrient degradation through exposure to overheated thermal exchange surfaces. Rapid heating with microwaves followed by sudden cooling could reduce or eliminate this problem. The objectives for this study were focused on designing and implementing continuous microwave thermal processing of skim fluid milks (white and chocolate) to compare sensory, microbiological, and biochemical parameters with conventionally prepared, indirect UHT milks. All test products were aseptically packaged and stored at ambient temperature for 12 mo. Every 3 mo, samples were taken for microbiological testing, reactive sulfhydryl determinations, active enzyme analysis, instrumental viscosity readings, color measurements, and descriptive sensory evaluation. Microbiological plate counts were negative on all milks at each time point. Enzymatic assays showed that plasmin was inactivated by both heat treatments. 5,5'-Dithio-bis(2-nitrobenzoic acid) analysis, a measure of reactive sulfhydryl (-SH-) groups, showed that the initial thiol content was not significantly different between the microwave-processed and UHT-treated milks. However, both heating methods resulted in an increased thiol level compared with conventionally pasteurized milk samples due to the higher temperatures attained. Sulfhydryl oxidase, a milk enzyme that catalyzes disulfide bond formation using a variety of protein substrates, retained activity following microwave processing, and decreased during storage. Viscosity values were essentially equivalent in microwave- and UHT-heated white skim milks. Sensory analyses established that UHT-treated milks were visibly darker, and exhibited higher caramelized and stale/fatty flavors with increased astringency compared with the microwave samples. Sweet aromatic flavor and sweet taste decreased during storage in both UHT and

microwave milk products, whereas stale/fatty flavors increased over time. Sensory effects were more apparent in white milks than in chocolate varieties. These studies suggest that microwave technology may provide a useful alternative processing method for delivery of aseptic milk products that retain a long shelf life.

(Key words: ultra-high temperature, microwave, sulfhydryl oxidase, sensory)

Abbreviation key: DTNB = 5, 5'-dithio-bis(2-nitrobenzoic acid), SHOX = sulfhydryl oxidase.

INTRODUCTION

Thermal treatment of milk products has a significant impact on the dairy industry. These processing regimens (a) minimize bacterial growth, providing safer foods, (b) alter shelf life, and (c) affect parameters related to product functionality. Often, heat treatment causes milkfat globule membrane proteins and whey proteins to unfold such that buried sulfhydryl (-SH-) groups, normally masked in the native protein, are exposed to the outer surfaces (Hoffmann and van Mill, 1997). In turn, these processes produce extreme cooked flavors, often attributed to changes in the sulfhydryl and disulfide content of the protein fraction (Swaigood et al., 1987).

Conventional pasteurization methods have long been in place and with the advent of UHT technology, the sterilization of fluid milk was achieved using higher temperature treatments for shorter periods. However, shelf-stable milk has met with limited acceptability by the consumer, especially in the United States, due in part to a high cooked flavor. Several attempts to improve the quality of UHT-treated milk products proved successful to varying degrees. Previously, Swaigood and coworkers used immobilized sulfhydryl oxidase to reduce the thiol content of UHT-heated skim milk and described an improved flavor after enzymatic oxidation to form protein disulfide bonds (Swaigood et al., 1987). Other studies have showed that altering UHT processing parameters, such as indirect vs. direct steam injection systems, cooling rates, and long-term storage conditions have a significant impact on sensory attri-

Received May 13, 2005.

Accepted August 2, 2005.

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butes (Browning et al., 2001). Most recently, epicatechin, a flavonoid compound, was added to UHT milk prior to heating, and the results revealed partial inhibition of thermally generated cooked aroma (Colahan-Sederstrom and Peterson, 2005).

Alternative technologies, such as microwave processing, have also evolved. Microwaves, an element of the electromagnetic spectrum, range in frequency between 300 MHz and 3 GHz, and their routine application in heating foods is already prevalent, especially in developed countries. Since the early 1960s, microwave energy has been used for cooking, baking, and thawing. Hamid et al. (1969) were the first group to use the technology for milk pasteurization. Unlike other energy delivery systems, microwave heating involves a rapid and direct heating process that reduces the time required to achieve a desired temperature. Hence, the total cumulative thermal treatment is much reduced, better preserving the thermolabile constituents of foods, such as aromas, vitamins, and pigments (Mudgett, 1986) while maintaining the sterility of the final product by minimizing bacterial growth (Merin and Rosenthal, 1984).

Based on our review of the literature, there are relatively few reports that describe the effects of microwave processing on sensory, microbiological, rheological, and biochemical parameters of heated milk during long-term storage. In fact, we found only one article that discussed chemical and sensorial changes that occurred during cold storage of microwave-treated milk over a 15-d period (Valero et al., 2001). Therefore, our study was designed to evaluate the effects of microwave vs. indirect UHT heating regimens on these attributes using fluid skim milk products, both white and chocolate varieties, stored at room temperature (21°C) over a much longer period (12 mo). Specifically, the objectives were focused on determining the differences between these processes in 1) bacterial counts, 2) plasmin activity, 3) DTNB reactive protein sulfhydryl groups, 4) sulfhydryl oxidase activity, 5) viscosity, 6) color parameters, and 7) descriptive sensory attributes. These results suggested that microwave processing regimens might deliver an equivalent quality fluid milk product that retains a long-term, stable, shelf life.

MATERIALS AND METHODS

Preparation of Milk

Raw milk (1000 kg, $3.2 \pm 0.2\%$ protein, $3.8 \pm 0.2\%$ fat) was collected from the North Carolina State University dairy facility on 2 occasions. Raw skim milk ($0.35 \pm 0.01\%$ fat) was obtained by centrifugal separation and used in subsequent studies. On each occasion, skim milk was evenly divided (500 kg) and assigned to the

respective flavor content (white or chocolate). Chocolate milk was prepared by adding UHT SIPT 8, a UHT chocolate milk formulation comprising cocoa processed with alkali, starch, salt, carrageenan, vanillin, and other artificial flavorings (Benjamin P. Forbes Company, Cleveland, OH). This ingredient was added at the ratio of 1.2 g of cocoa powder mix/100 g of milk in conjunction with cane sugar (7.6 g of sugar/100 g of milk). White and chocolate milks, 250 kg each, were then separated for UHT vs. microwave sterilization. High temperature, short time milk, or pasteurized milk, was prepared at a high temperature for a short time (at least 71.7°C for 15 s or an equivalent combination).

UHT Processing Methodology

Skim milk was subsequently heated using a traditional UHT processing unit that delivers thermal energy via an indirect steam injection system (No-Bac Unitherm model VIII Cherry Burrell, Corp., Louisville, KY). In this paper, the abbreviation UHT is specifically defined as indirect UHT milk, based on this particular method of preparation (indirect steam injection). White milk was heat-treated at 137.8°C for 10 s fastest particle (heating rate: 3.26°C/s, 20-s average hold time, $F_0 = 8$), whereas chocolate milk was thermally processed at 140.6°C for 10 s fastest particle (heating rate: 3.33°C/s, 20-s average hold time, $F_0 = 15$). F_0 is a process temperature equivalent unit, whereby an $F_0 = 1$ indicates that a product received a heat treatment equivalent to 121°C for 1 min. The flow rate was approximately 2.0 L/min. It is important to note that with steam heating, there is a gradation of thermal energy because heat is delivered from the outside area of the processing chamber to the interior (indirect heating). As a result, the outermost surfaces of the milk product were likely superheated.

Microwave Processing Methodology

For microwave processing, a 60-kW continuous flow microwave-heating unit (IMS 60 kW microwave system, Industrial Microwave Systems, Morrisville, NC), operating at 915 MHz, was used to process skim milks. Microwaves were focused to a cylindrical applicator, and the power relayed from the generator was monitored using a control panel supplied by the manufacturer. Microwaves were then delivered to the product by a waveguide of rectangular cross-section, which was split into 2 sections and geared towards 2 specially designed applicators, with a directional coupler in each. A polytetrafluoroethylene tube (0.038 m i.d.) was placed at the center of each applicator and the exposure region was 0.2 m long in each applicator (Coronel et al., 2003).

A positive displacement pump (model A7000, Marlen Research Corp., Overland Park, KS) was used to move the milk through the system at a flow rate of 3.8 L/min. Temperatures were measured at the inlet of the system, the inlet and exit of each applicator, and at the holding tube exit with thermocouples arranged as previously described (Coronel et al., 2003). Thermal readings were recorded at 4-s intervals using a data logging system (HP 3497A, Hewlett Packard, Palo Alto, CA). Heat processing conditions were essentially equivalent to those previously described in the UHT methodology section (i.e., white milk, heating rate 3.42°C/s, $F_0 = 8$, and chocolate milk, heating rate 3.51°C/s, $F_0 = 15$). In contrast to UHT processing methods, microwave energy, or cold wall heat, was transferred directly into the food product via the microwave itself.

Aseptic Packaging

Milk was aseptically filled (250-mL aseptic filler, model SA-50, International Paper Co., Memphis, TN) and packaged into 250-mL brick-style aseptic cartons formed from standard aseptic roll-stock board composed of laminated layers of polyethylene, aluminum foil, and paperboard (International Paper Co.). The aluminum foil roll-stock provides protection against light and gas penetration. Twenty boxes, collected from the beginning and end of each run, were excluded from the study to obtain uniformly heat-exposed, representative products from each trial. Cartons were stored at 21°C in the dark over a 12-mo period, and sampling was conducted every 3 mo. Time-zero analyses were performed within 96 h after processing. For each testing period (sampling), 20 boxes of each milk type (white and chocolate) were pulled, and 5 boxes aseptically combined for microbiological plate counts, enzymatic assays, instrumental analyses, and descriptive sensory evaluations. Pooled milk samples were stored at 5°C, and microbiological assays were conducted on all experimental milks before sensory analyses.

Bacteriological Tests

Initially, total plate and coliform counts were conducted on raw milks. The microbiological quality of shelf-stable milks was evaluated for total numbers of bacteria including coliforms, spore-forming, and psychrotrophic microorganisms according to standard methods (American Public Health Association, 2004). Briefly, total plate counts were evaluated using standard methods agar (Difco, Sparks, MD) followed by incubation at 32°C for 48 h. Coliforms were enumerated on violet red bile agar (Difco) followed by incubation at 32°C for 48 h. To quantify the numbers of spore-forming

bacteria, 200 mL of milk was heated to 80°C in a water bath and held for 12 min. Afterwards, milk samples were immediately cooled in an ice water bath, and plated in duplicate onto standard methods agar containing 0.1% (wt/wt) soluble starch (Sigma, St. Louis, MO) followed by incubation at 32°C for 48 h. Psychrotrophic bacteria were counted using crystal violet tetrazolium agar. Appropriate dilutions were made using sterile 0.1% peptone water, and plates were incubated at 21°C for 48 h before enumeration of visible colonies.

Plasmin

Plasmin activity was monitored at 25°C using a chromozym PL kit purchased from Roche Molecular Biochemicals (Mannheim, Germany). Hydrolysis of the substrate, tosyl-glycyl-prolyl-lysine-4-nitroanilide-acetate, was followed at A_{405} nm. One unit was defined as that activity liberating 1 micromole of 4-nitroaniline per minute at 25°C under the defined experimental conditions. Two samples from each trial run were measured in duplicate and the numbers averaged.

Reactive Sulfhydryl Groups

The reagent, 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB), was purchased from Sigma Chemical Company. The level of reactive protein sulfhydryl group was measured according to a modified assay technique in which DTNB reacted with free SH groups producing a yellow color with a maximum absorbance at 412 nm (Ellman, 1959). For these experiments, milks were diluted 1:50 with 0.1 M phosphate buffer, pH 8.0, and 1.0 mL of a 1 mM DTNB solution, prepared with 50 mM sodium phosphate, pH 7, was added. The solution was maintained at room temperature (21°C) for 30 min before reading the absorbance at 412 nm. A blank sample was prepared in an analogous manner with the addition of 1 mL of 50 mM phosphate buffer, pH 7, rather than the DTNB reagent, serving to correct for milk turbidity. A DTNB control (minus milk sample) was also prepared to adjust for the slight increase in the absorbancy of the DTNB solution itself, that occurred during the incubation period. This value was subtracted from the experimental sample reading before calculation of the reactive sulfhydryl concentration. Two samples from each trial run were measured in duplicate and the numbers averaged.

Sulfhydryl Oxidase

Sulfhydryl oxidase (SHOX) activity was determined as the rate of oxidation of 0.8 mM glutathione, prepared in 50 mM sodium phosphate buffer, pH 7, at 37°C,

essentially according to the method of Janolino and Swaisgood (1975). Typically, the reaction mixture contained 0.1 mL of test milk added to 1.0 mL of glutathione substrate. At timed intervals, 0.1-mL aliquots were removed and mixed with 5.0 mL of 100 μ M DTNB prepared with 0.1 M sodium phosphate buffer, pH 8. The concentration of remaining substrate (reduced glutathione) was calculated from the absorbance at 412 nm ($\epsilon_{412\text{nm}} = 13,600 \text{ m}^{-1}\text{cm}^{-1}$). Sulphydryl oxidase activity was expressed as micromoles of thiol oxidized per minute (units) under the experimental conditions as defined. Two samples from each trial run were measured in duplicate and the numbers averaged.

Protein Assays

A bicinchoninic acid protein assay kit was purchased from Pierce Chemical Company (Rockford, IL) with BSA (2 mg/mL) as the standard. Protein measurements of skim milks were made to calculate the specific activity of sulphydryl oxidase.

Instrumental Color Analysis

Reflectance measurements of all milks were recorded using a Gardner Spectrogard Color system (Pacific Scientific Instrument Division, Silver Spring, MD) with daylight illumination. The tristimulus parameters were measured in the $L^*a^*b^*$ mode, in which L^* represents the lightness value, and a^* and b^* represent chromaticity coordinates. The colorimeter was calibrated against white and black tile standards, and all samples were tempered to 25°C before performing duplicate readings of each skim milk product.

Viscosity Analysis

Apparent viscosity readings for all milk samples were achieved using a stress-controlled rheometer (Stress-Tech, Rheologica Instruments AB, Lund, Sweden) equipped with a CC 25 concentric cylinder geometry. Two samples from each trial run were measured in duplicate, the numbers averaged, and the apparent viscosity reported as the mean shear rate of the measurement taken at 50 s^{-1} .

Descriptive Sensory Analysis

Milk products, at each sampling, were evaluated in triplicate by each member of a trained descriptive panel ($n = 9$, 7 females, 2 males, ages 22 to 45). Sensory testing was conducted in compliance with North Carolina State University's Institutional Review Board for Human Subjects. Each panelist had at least 60 h of previous

training on sensory analysis of dairy products using the Spectrum descriptive analysis technique (Meilgaard et al., 1999). Twelve 30-min training sessions were conducted to focus on sensory properties of shelf-stable milks. Flavor, mouthfeel, and color terms identified and selected by the panelists are listed in Table 1. Throughout all training sessions, panelists evaluated and discussed milks to clarify descriptor concepts and to consistently scale product attributes. The statistical analysis of data collected from these training sessions confirmed that panel results were consistent and that terms were not redundant (Drake et al., 2003). Descriptive analysis was conducted by each panelist in triplicate on each milk using a randomized block design. Samples (20 mL) were served in 59-mL plastic cups fitted with plastic lids (Sweetheart Cup Co., Owings Mills, MD) and labeled with 3-digit codes. Milks were served at $10 \pm 2^\circ\text{C}$, and panelists evaluated 4 milks per session. Chocolate milks were evaluated in separate sessions. Visual attributes were determined at different times to prevent the influence of flavor and mouthfeel attributes on appearance. For tasting purposes, panelists were first presented with a warm-up sample of commercial shelf-stable milk, previously discussed during training sessions. Panelists then proceeded to evaluate each sample. Ambient-temperature spring water was available for palate cleansing.

Statistical Analyses

Data were analyzed using SAS software (version 8.2; SAS Institute, Inc., Cary, NC). Significant effects and interactions of heat treatment and storage time on all parameters listed were evaluated by ANOVA with means separation (least squares means).

RESULTS AND DISCUSSION

Microbiological Results

To date, there are a limited number of publications that describe the pasteurization of milk products using microwave energy. In an early work, Villamiel et al. (1996b) heated raw bovine and goat milks using continuous microwave processing temperatures ranging from 73.1 to 96.7°C, and showed low bacterial counts ($\leq 2 \log \text{cfu/mL}$) after treatment under several temperature and time combinations. Ultra-high temperature technology has also been developed for food safety applications. Browning et al. (2001) demonstrated the impact of UHT heating parameters on sterility and presented a spreadsheet for predicting safety parameters, such as lethality and microbial inactivation, using UHT milk products subjected to different heating and cooling profiles and varying holding times in a continuous heat exchanger.

Table 1. Descriptive sensory language for UHT- and microwave-treated milks.¹

Descriptor	Reference	Preparation
Cooked/caramelized	Heated milk	Heat-pasteurized skim milk to 85°C for 45 min
Sweet aromatic/cake mix	Pillsbury white cake mix Vanillin	Dilute 5 mg of vanillin in skim milk
Chocolate flavor (chocolate milks only)	Aromatics associated with alkali processed cocoa	Hershey's Hot Chocolate recipe (on box) using skim milk
Fatty/stale	(E,E)-2,4-Decadienal	2 ppb in skim milk
Sweet taste	Sucrose	5% sucrose solution
Bitter	Caffeine	0.5% caffeine solution
Astringent	Tea	Soak 6 tea bags in water for 10 min
Color intensity	Pasteurized skim milk Condensed skim milk Sweetened condensed skim milk	Color intensity from light to dark (brown)

¹Adapted from Drake et al. (2003) and Thompson et al. (2004).

The raw milk used for these studies showed 1600 ± 350 cfu/mL when plated on standard methods agar, and 25 ± 20 cfu/mL when grown on violet red bile medium. Both UHT and microwave processing regimens virtually eliminated all bacterial growth in the milk as evidenced by the lack of colony formation using various microbiological media. Furthermore, sterility was maintained throughout a 1-yr storage period, indicative of effective aseptic packaging methodologies.

Plasmin Enzyme Assays

Plasmin, an enzyme naturally occurring in milk, cleaves casein and often causes coagulation and sometimes gelation in milk products over time. Plasmin activity is often associated with the development of off-flavors, especially bitterness, during storage (Baker, 1983). Previously, Alichanidis et al. (1986) noted that plasmin retained as much as 30 to 40% of the total activity even after exposure to UHT processing conditions; however, in our own work, plasmin was totally inactivated after both UHT and microwave thermal treatments. Presumably, the absence of plasmin activity may improve the long-term shelf stability of the final fluid milk product.

Reactive Protein Sulfhydryl Groups and SHOX Activity

Off-flavors in heated milk often result from creation of organic sulfur compounds that arise during the decomposition of reactive protein sulfhydryl groups associated with the amino acids, methionine and cysteine. Often, their concentration has been correlated with the sensory detection of undesirable "cooked" flavors in milk samples exposed to high temperatures (Swaigood et al., 1987). Several examples of such compounds produced upon heating include hydrogen sulfide (rotten

egg odor), dimethyl sulfide (rotten vegetables), dimethyl disulfide (putrid), and dimethyl trisulfide (onion, garlic), all of which have extremely low thresholds for detection (DMI, 2003).

Protein sulfhydryl groups also contribute directly to undesirable effects such as these. Whey protein sulfhydryl groups, typically buried within the core of the protein structure, are exposed to the surface because of heating. Furthermore, protein sulfhydryl groups may be formed because of hydrolysis or β -elimination of disulfide bonds during thermal treatment (Patrick and Swaisgood, 1976). β -Lactoglobulin, a major protein component of the whey fraction (accounting for over 50% of the total protein) is often involved in these processes (Mulvihell and Kinsella, 1987). The dimeric structure of β -lactoglobulin comprises 2 free [-SH-] groups and 4 disulfide [-S-S-] bonds with a molecular weight of 36,000 (McKenzie et al., 1972). Thus, during heat treatments, the protein is rapidly denatured and aggregated through disulfide interchange.

Using Ellman's (1959) procedure, we measured the concentration of reactive protein sulfhydryl groups, generated because of each heat treatment, by performing DTNB assays. The results showed that there were no significant differences between the microwave-treated skim milk products (both white and chocolate varieties) compared with the indirect UHT-processed milks (Figure 1). However, both heating methods resulted in an increased thiol content as compared with conventionally pasteurized milk samples due to the higher thermal temperatures attained during processing. Within 3 mo of storage at room temperature, the (-SH-) content for all heated test milks decreased to essentially equivalent low level values (~ 0.20 to 0.30 $\mu\text{mol/g}$ of protein), and remained at minimal concentrations throughout the remainder of the study (data not shown). Interestingly, Patrick and Swaisgood (1976)

Figure 1. Effect of heat treatment on reactive sulfhydryl content in skim milk at time zero. The protein concentration in skim milk was measured at ~32 g/L. Duplicate analyses from 2 separate trial runs were analyzed and the data reported as the mean $\pm$ SEM. ^{a,b}Letters above bars within each product type represent significant differences ($P < 0.05$).

noted similar data patterns using UHT-treated skim milk stored at 25°C.

Sulfhydryl oxidase, an enzyme identified in raw milk, catalyzes the oxidation of milk protein sulfhydryl groups (Janolino and Swaisgood, 1975; Clare et al., 1981). In early studies, an immobilized form of SHOX was used in the continuous treatment of UHT milk to remove cooked flavors. The results showed a direct correlation between treatment time (or degree of SH oxidation) and the level of cooked flavor as determined by an experienced milk-judging panel (Swaisgood et al., 1987). In fact, SHOX-treated milk was deemed indistinguishable from commercially pasteurized milk based on flavor attributes although trained panelists could differentiate UHT test samples from enzyme-treated milk samples by headspace odor alone.

Previously, it was noted that SHOX retained enzymatic activity after pasteurization; however, UHT treatment resulted in a significant loss of catalytic capacity (H. E. Swaisgood, Raleigh, NC; personal communication). Therefore, we measured SHOX activity in both heated milk products to investigate the retention of enzymatic catalytic capacity after each type of thermal exposure (microwave vs. indirect UHT). The results established that residual SHOX activity was detected in microwave-processed skim milks (white and chocolate) compared with UHT-sterilized samples that were essentially devoid of active enzyme (Figure 2). Seemingly, microwave conditions favored retention of SHOX activity because of shorter ramping times required to reach the target temperature and uniform thermal distribution throughout the milk. Both of these parameters

Figure 2. Effect of heat treatment on sulfhydryl oxidase activity in skim milk at time zero. Duplicate analyses from 2 separate trial runs were analyzed and the data are reported as the mean $\pm$ SEM. ^{a,b,c}Letters above bars within each product represent significant differences ($P < 0.05$).

may significantly affect denaturation of the enzyme. Previously, others noted a low degree of whey protein denaturation, specifically β -lactoglobulin, after application of continuous microwave treatment using both cow and goat milk (Villamiel et al., 1997).

The retention of SHOX activity in the microwave milks may represent at least one of the parameters that contribute to the sensory differences observed between the 2 types of heated milk products, at least at the beginning points of the storage trial. As previously stated, thermal treatment increases the concentration of “free” protein SH groups that are available for participation in subsequent oxidative reactions and ultimately contribute to the production of reactive oxygen species, such as singlet oxygen and hydroxyl radicals. Such processes often influence the development of many undesirable sensory characteristics. Therefore, rapid enzymatic catalysis of reactive protein sulfhydryl groups by SHOX might lessen the production of off-flavors in the microwave milk products. However, it is important to emphasize that such effects would be most significant at the early stages of sensory testing because the concentration of reactive sulfhydryl groups decreased over time in both the UHT- and microwave-processed milk as evidenced by DTNB analysis.

Viscosity and Color Measurements

Casein micelles play a major role with respect to the viscosity parameters of skim milk (Walstra and Jenness, 1984); therefore, any factor that affects the aggregational state of the micelle such as ionic strength, pH, or heat will influence resistance to flow. Furthermore,

Figure 3. Effect of heat treatment on viscosity parameters in skim milk. The milk samples were UHT-treated white milk (UW); microwaved white milk (MW); UHT-treated chocolate milk (UC); and microwaved chocolate milk (MC). Duplicate analyses from 2 separate trial runs were analyzed and the data are reported as the mean $\pm$ SEM. ^{a-c}Letters above bars within each product type represent significant differences ($P < 0.05$).

when milk is heated above 70°C, there is a notable effect on the viscosity attributed to the denaturation of whey proteins. As discussed before, denatured whey proteins often undergo sulphydryl-disulfide interchange reactions, an effect that additionally promotes increased viscosity.

As depicted in Figure 3, both UHT- and microwave-treated chocolate skim milks exhibited the highest viscosity throughout the entire experimental trial. This result would likely be anticipated because chocolate milk formulations were prepared by adding UHT SIPT 8, a UHT chocolate ingredient mixture prepared with cocoa processed with alkali, starch, salt, carrageenan, vanillin, and other artificial flavorings. In addition, the Fo heating parameters were higher for chocolate milks, a factor that could elevate these readings. Statistical analysis verified that the viscosity of the microwave-treated chocolate skim milk was comparable to that of the UHT-heated samples at each sampling time during the storage study.

Statistical differences were noted between UHT- and microwave-treated white milks at various storage times, but these differences were small from a practical perspective (Figure 3). The viscosity of all skim milks

increased during storage at room temperature although this observation was most apparent at 6 mo. A slight increase in these values occurred at 9 mo, after which time these measurements remained similar for the duration of the study (12 mo). Viscosity changes may be at least partially attributed to age gelation effects, an aggregation phenomenon that typically affects shelf-stable, sterilized dairy products, although the actual mechanism has not yet been fully defined.

With regard to color, there were no major variations between chocolate milks processed by either heat treatment ($P > 0.05$); however, significant differences were observed between white UHT- and microwave-treated samples ($P < 0.05$; Figure 4). Ultra-high temperature—treated white milks exhibited less green (a^*) and more yellow (b^*) hues compared with microwave-treated milks (Figure 4). Over time, the lightness (L^*) of both white milks decreased ($P < 0.05$), whereas redness decreased in both chocolate milks ($P < 0.05$).

Sensory Analyses

In heated skim milk, the potential sources of undesirable odors and flavors are often attributed to lipolysis

Figure 4. Instrumental color analysis of UHT- vs. microwave-treated white and chocolate skim milk samples. The milk samples were UHT-treated white milk (UW); microwaved white milk (MW); UHT-treated chocolate milk (UC); and microwaved chocolate milk (MC). Test samples from 2 separate trial runs were analyzed in duplicate and the data are reported as the mean $\pm$ SEM. A significant interaction between heat treatment and storage time was observed ($P < 0.05$). ^{a-d}Letters above bars within each product type represent significant differences ($P < 0.05$).

Figure 5. Sensory attributes of UHT- vs. microwave-processed white skim milks. There was no significant interaction between the type of heat treatment vs. storage time ($P > 0.05$); therefore, mean intensities for significant attributes were averaged across all time intervals. Attribute intensity (y-axis) was scored using a 5-point universal Spectrum intensity scale. Other traits, such as sweet taste (see Table 1), did not differ with the type of heat treatment ($P > 0.05$). ^{A,B}Attribute means with different letters are dissimilar ($P < 0.05$).

and increased Maillard reaction products (Renner, 1988; Valero et al., 2001). For example, lipid oxidation may give rise to stale “oxidized” flavors caused by the formation of aldehydes and ketones (Contarini et al., 1997). Typically, aged UHT skim milk exhibits higher proteolytic activity, leading to an increased concentration of free amino groups available for reactivity via the Maillard reaction (Valero et al., 2001).

Villamiel et al. (1996b) were among the first to show that microwave processing by continuous flow could deliver a sterile fluid milk product with satisfactory sensory quality. In later work, Valero et al. (2001) established that trained sensory panelists were unable to distinguish between microwave treated vs. conventionally pasteurized milk samples based on taste and odor differences. In fact, the 2 products remained indistinguishable based on their sensory qualities, even after storage at refrigeration temperatures over a 15-d period.

For the purposes of this study, the most notable sensory-perceived differences were observed between white milks. Statistical analysis of the data showed that the major dissimilarities between the 2 heated milk products could be attributed to the type of thermal treatment used, i.e., UHT vs. microwave processing ($P < 0.05$), independent of storage time ($P > 0.05$). At all time points, microwave milk products were characterized by lower caramelized flavor, decreased astringency, less fatty/stale flavors, and less brownish hues (Figure 5). Such sensory traits are considered negative qualities of shelf-stable milks generally associated with high heat treatment or extended storage time. As these

attributes were consistently lower in microwave-heated milks, the results suggested that microwave technology may deliver milk products that exhibit superior sensory characteristics at given time intervals. Future work should be designed to address consumer acceptance and perception of such products. Other sensory attributes did not differ between the 2 heat treatments ($P > 0.05$; data not shown).

Ultra-high temperature methodologies may be more prone to Maillard reactivity because thermal energy is delivered from the outside surfaces of the processing tank to the innermost fluid milk. In this event, some of the milk is overheated, especially at the contact surface; a process that may cause more browning. Such findings were observed by the panelists as shown in Figure 5. It should be noted that there is relatively little information with respect to Maillard reactivity in microwave milk products. In one report, Villamiel et al. (1996a) detected elevated levels of furosine (an indicator of the Maillard reaction) in microwave milks compared with conventionally treated milk, a finding attributed to uneven heating in the microwave oven. In contrast, the unique microwave system used for this study offers an infinitely variable, continuous power source with improved temperature control. Such a system should circumvent potential problems associated with nonuniform temperature distribution and deliver a product with low potential for excessive Maillard browning.

The length of the storage time influenced the sensory profiles of UHT- and microwave-sterilized milk products in a similar manner (Figure 6). In each case, sweet aromatic flavors and sweet taste decreased with time, whereas fatty/stale flavor, astringency, and color intensity were increased ($P < 0.05$). These attributes were influenced by storage time regardless of type of heat treatment (no statistical interaction, $P > 0.05$). Other sensory attributes did not change with storage time ($P > 0.05$; data not shown). Sensory differences were not detected between UHT- vs. microwave-treated chocolate skim milks ($P > 0.05$; data not shown); chocolate flavor alone may have masked any subtle differences. However, after 12 mo of storage at room temperature, visible separation occurred in microwaved chocolate milks, as observed by all sensory panelists, indicating a failure of the stabilizer system over this time. Brief swirling of the container eliminated the phase separation. Similar effects were not observed in the indirect UHT-treated chocolate milk samples, which undergo more shearing throughout processing, a factor that could contribute to greater constancy of the stabilizer during storage.

Figure 6. Changes in sensory attributes of UHT- and microwave-treated white skim milks during storage. Attribute intensity (y-axis) was scored using a 5-point universal Spectrum intensity scale. Sensory attributes were impacted by storage time independent of heat treatment. There was not a treatment by storage time interaction ($P > 0.05$) so attributes were averaged across heat treatments for given time intervals. Other traits (see Table 1) did not differ with storage time ($P > 0.05$). ^{A-D}Attribute means with different letters are dissimilar ($P < 0.05$).

CONCLUSIONS

In the past, UHT methodologies have proven effective for delivering a sterile milk product that can be stored at room temperature for extended periods. In many cases; however, the off-flavors typically associated with the end products were met with limited acceptance by consumers. Hence, alternative methods such as microwave processing may afford new opportunities to develop fluid milk products that exhibit a long shelf life, with sensory characteristics that are equivalent to, if not better than, those achieved with indirect UHT-treated milk. With the continued development of successful microwave manufacturing practices, the technology holds much promise for the future.

ACKNOWLEDGMENTS

Funding for this project was provided by Dairy Management Incorporated (DMI). Manuscript FSR 05-25 of the Department of Food Science, North Carolina State University. The use of trade names in the publication does not imply endorsement by these organizations nor criticisms of ones not mentioned.

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