J. Dairy Sci. 95:4256–4262
http://dx.doi.org/10.3168/jds.2012-5387

Effect of addition of CO_2 to raw milk on quality of UHT-treated milk

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ABSTRACT

The objective of this study was to evaluate the effect of addition of CO_2 to raw milk on UHT milk quality during storage. Control milk (without CO_2 addition) and treated milk (with CO_2 addition up to pH 6.2) were stored in bulk tanks at 4°C for 6 d. After storage, both samples were UHT processed using indirect heating (140°C for 5 s). Samples were aseptically packed in low-density polyethylene pouches and stored in the dark at room temperature. Raw milk was evaluated upon receipt for physicochemical composition, proteolysis, lipolysis, standard plate count, psychrotrophic bacteria, and *Pseudomonas* spp. counts, and after 6 d of storage for proteolysis, lipolysis, and microbial counts. After processing, UHT milk samples were evaluated for physicochemical composition, proteolysis, and lipolysis. Samples were evaluated for proteolysis and lipolysis twice a month until 120 d. Peptides from pH 4.6-soluble N filtrates were performed by reversed-phase HPLC after 1 and 120 d of storage. A split-plot design was used and the complete experiment was carried out in triplicate. The results were evaluated by ANOVA and Tukey's test. After 6 d of storage, CO_2-treated raw milk kept its physicochemical and microbiological quality, whereas the untreated milk showed significant quality losses. A significant increase in proteolysis occurred during 120 d of storage in both treatments, but the increase occurred 1.4 times faster in untreated UHT milk than in CO_2-treated UHT milk. In both UHT milks, the proteolysis was a consequence of the action of plasmin and microbial proteases. However, the untreated UHT milk showed higher microbial protease activity than the treated UHT milk. The addition of CO_2 to the raw milk maintained the quality during storage, resulting in UHT milk with less proteolysis and possibly longer shelf life, which is usually limited by age gelation of UHT milk.

Key words: carbon dioxide, psychrotrophic bacteria, UHT milk, proteolysis

INTRODUCTION

Refrigeration of raw milk is the most efficient method of storage and is globally accepted for increasing shelf life and eliminating spoilage by mesophilic bacteria (Cousin, 1982). However, cooling of raw milk does not prevent growth of psychrotrophic bacteria, which are present as normal contaminants in raw milk and produce heat-resistant extracellular enzymes that affect the quality of milk and dairy products. Enzyme activity, in general, is significant when the psychrotrophic bacteria count exceeds 10^6 cfu/mL. When that occurs, cheese yield is reduced, age gelation of UHT milk occurs, and off-flavors result in many dairy products (Muir, 1996; Sørhaug and Stepaniak, 1997; Santos and Fonseca, 2001).

Quality and shelf life of UHT milk are directly related to the quality of the raw milk. Age gelation is the main problem that limits the shelf life of UHT milk: an increase in viscosity occurs, immediately followed by formation of a gel and a loss in fluidity (Datta and Deeth, 2001; Manzi and Pizzoferrato, 2006). One of the causes of age gelation is proteolysis due to a native milk protease (e.g., plasmin) or a heat-resistant protease produced by psychrotrophic bacteria (Fox and McSweeney, 1998; Datta and Deeth, 2001). Sensory defects in UHT milk, such as the bitter taste (produced by the release of hydrophobic peptides) and rancid flavor (caused by increased levels of short-chain FFA), can also be caused by the action of proteolytic and lipolytic enzymes produced by psychrotrophic bacteria (Datta and Deeth, 2001; Datta et al., 2002; Manzi and Pizzoferrato, 2006).

Controlling the growth of psychrotrophic bacteria is important to the dairy industry because it extends the storage of raw milk without loss of quality. One method proposed to control psychrotrophic bacteria growth involves treatment with CO_2, which inhibits the

Received January 26, 2012.
Accepted April 5, 2012.
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growth of these microorganisms at low temperatures, reduces enzyme production, and extends raw milk shelf life (King and Mabbitt, 1982; Ma et al., 2003; Martin et al., 2003). Carbon dioxide affects growth and microbial metabolism in at least 3 different ways: (1) the replacement of O_2 by CO_2; (2) a pH reduction due to CO_2 dissolution and formation of carbonic acid; and (3) a direct effect on the metabolism of microorganisms, including changes in membrane fluidity, reductions in intracellular pH, and direct inhibition of metabolic pathways (Loss and Hotchkiss, 2000; Hotchkiss et al., 2006). Carbon dioxide influences the lag phase and generation time of different microorganisms, especially psychrotrophic bacteria, extending the raw milk shelf life. The inhibitory effect of CO_2 is greater at lower storage temperatures (King and Mabbitt, 1982; Martin et al., 2003; Vianna and Gigante, 2010).

The addition of CO_2 to raw bulk milk during refrigerated storage can improve and extend the shelf life of UHT milk, which represents about 80% of fluid milk consumed in Brazil. The objective of this study was to evaluate the effect of CO_2 addition to raw milk on quality of raw milk and UHT milk during storage.

MATERIALS AND METHODS

Milk Collection and Treatments

After milking, the raw milk was cooled in a plate heat exchanger at 4 ± 1°C. Raw milk (200 L) was divided into 2 portions that were stored in bulk tanks (model KW 250-2, Etscheid Techno SA, Pirajuí, SP, Brazil) with stirring and automatic temperature control (4 ± 1°C). One of the portions, the untreated milk, was used as control milk. Food-grade CO_2 was bubbled into the second portion for around 20 min until a pH 6.20 ± 0.05 was reached. Untreated and CO_2-treated milks were kept in closed bulk tanks at 4 ± 1°C for 6 d before UHT processing. The CO_2-treated milk was monitored daily for pH and CO_2 concentration (~1,000 ppm). Variations in pH were corrected daily by short injections (~5 min) of CO_2. After 6 d of storage, refrigerated milk was preheated at 80°C under stirring in a batch pasteurization tank to eliminate CO_2 elimination and restore pH. Milk was then cooled to 4°C and 0.1% sodium citrate was added. This practice is adopted by the Brazilian dairy industry to prevent destabilization of the caseins during heat treatment and is supported by legislation. The UHT milk was processed in a plate heat exchanger (Sumá Indústria e Comércio Ltda., Campinas, SP, Brazil) at 140°C for 5 s. Refrigerated milk was pumped through the heating section of the heat exchanger until it reached 68 to 72°C, homogenized at pressure of 210 and 40 kgf/cm^2 in the first and second stages, respectively (Alitec Comércio e Indústria Ltda., Pindamonhangaba, SP, Brazil), cooled to 20°C, aseptically packaged in pouches of low-density polyethylene (white pigmented with titanium dioxide) and stored in the dark at 25°C. Sterilization of the packaging was done in an immersion bath with 35% hydrogen peroxide at 35 ± 2°C for 13 s, followed by UV radiation exposure of 260 μW/cm^2 at 254 nm for at least 1.0 s delivered by a lamp installed 10 mm away from the film.

Sampling and Analysis

Raw Milk. The SCC of raw milk was determined by flow cytometry with a Bentley Somacount 500 instrument (Bentley Instruments Inc., Chaska, MN); antibiotic residue by Delvotest kit (DSM Food Specialties Dairy Ingredients, Delft, the Netherlands); pH (Digimed model DM-20, Digicron Analítica Ltda, São Paulo, Brazil) by introducing the electrode directly into the sample (25°C); total solids (AOAC, 1995; method 925.23; 33.2.09/A); fat by Gerber method (British Standards Institution, 1989); ash (AOAC, 1995; method 935.42; 33.2.10); and lactose by difference between total solids and other milk solids. Total nitrogen (**TN**; AOAC, 1995; method 991.20; 33.2.11), pH 4.6 soluble nitrogen (**pH 4.6-SN**; AOAC, 1995; method 998.05; 33.2.64) and 12% TCA-soluble nitrogen (**12% TCA-SN**; AOAC, 1995; method 991.21; 33.2.12) were determined by the Kjeldahl method. Protein was estimated by using a conversion factor of 6.38. True protein (**TP**) and casein content were calculated as follows: TP = (TN – 12% TCA-SN) × 6.38, and CN content = (TN – pH 4.6-SN) × 6.38, respectively. The casein content as a percentage of true protein (CN/TP) was considered as a proteolysis index for raw milk.

The FFA content (mEq of FFA/kg of milk) was determined by copper soap method (Shipe et al., 1980; modified by Ma et al., 2003) and was used as an index of lipolysis. Raw milk was evaluated for SPC and psychrotrophic bacteria count by using plate count agar followed by incubation at 35°C for 48 h and at 7°C for 10 d, respectively (American Public Health Association, 2001). *Pseudomonas* spp. count was carried out using Pseudomonas isolation agar, followed by incubation at 30°C for 24 h (Hayes and Nielsen, 2000). All microbiological culture media used in this study were from Difco Laboratories (Detroit, MI). During cold storage, pH and CO_2 concentration (MOCON Pac Check 650, Minneapolis, MN) were measured at 24-h intervals according to Ma et al. (2003). Total N, N fractions (pH 4.6-SN and 12% TCA-SN), FFA, pH, and microbial