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Quality characteristics and plasmin activity of thermosonicated skim milk and cream

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ABSTRACT

The shelf life of pasteurized milk is limited by heat-stable proteases, which cause gelation and bitter taste upon extended storage of milk. Ultra-high temperature processing inactivates proteases but detrimentally affects milk's sensory quality. An adjunct to pasteurization is desired to extend milk's shelf life while maintaining quality characteristics. In this study, the effects of combined heat and ultrasound (thermosonication) on total plasmin activity and various quality characteristics of skim milk and cream were studied. Thermosonication, at an average power of 115 W (152 $\mu m_{p\text{-}p}$, where p-p = peak-to-peak amplitude) for 3 min, decreased the total plasmin activity (i.e., plasmin- and plasminogen-derived activity) by nearly 94% in fresh skim milk and cream. Enzyme activity in thermosonicated skim milk samples measured at the end of 30 d was 5- to 10-fold higher than on d 0, but remained stable in thermosonicated cream. Descriptive analysis of odor attributes was conducted for up to 4 wk with 8 trained panelists. No significant differences were observed between the intensities of offensive "eggy" and "rubbery" odor attributes of thermosonicated skim milk and pasteurized commercial skim milk and cream. In addition, lightness (L*) values and viscosity were not adversely affected by thermosonication. Thermosonication decreased the fat globule size in skim milk and cream, and the homogenizing effect increased with treatment time. Thermosonication at average powers of 104 W (133 $\mu m_{p\text{-}p}$) and 115 W (152 $\mu m_{p\text{-}p}$) for 1 and 3 min destroyed coliforms and over 99% of the total aerobic bacteria. The total aerobic bacteria counts of thermosonicated skim milk and cream samples were less than 20,000 cfu/mL on d 30. Because thermosonication did not induce off-aromas or viscosity changes but did inactivate microorganisms and protease enzymes, thermosonication may be an appropriate adjunct to pasteurization to extend milk shelf life.

Key words: bacteria, sensory, shelf life, ultrasound

INTRODUCTION

Milk is a highly perishable product but it competes in a market rich with beverages that have a long shelf life, such as bottled water, fruit juice, soda, and sports beverages. Milk processors and dairy scientists seek methods to improve the shelf life of fluid milk to improve competitiveness. The shelf life of milk depends on various factors, such as the quality of incoming raw milk, processing time and temperature, survival of spoilage microorganisms, storage temperature, exposure to light, and postprocessing contamination (Bylund, 1995; Simon and Hansen, 2001). Conventional pasteurization [i.e., HTST continuous pasteurization and low-temperature, long-time (LTLT) batch pasteurization] destroys all pathogens and many spoilage microorganisms, rendering milk a shelf life of approximately 14 to 21 d when bottled and stored under refrigerated conditions (Allen and Joseph, 1985; Boor, 2001). Ultrahigh temperature processing, where the milk is heated to 138°C and held there for at least 2 s (FDA, 2009), can extend milk's shelf life to at least 60 d at room temperature (Boor and Nakimbugwe, 1998). However, extreme heating of milk can lead to Maillard or caramelization reactions (Clare et al., 2005) and an increase in sulfur compounds and off-flavors (Christensen and Reineccius, 1992). Chapman et al. (2001) showed that children aged 6 to 11 found 2% UHT milk to be undesirable. Thus it is important that any proposed treatment does not compromise the sensory quality and nutritional properties of milk.

When milk is pasteurized, spores of thermoduric microorganisms, as well as some native and microbial enzymes, survive the heat treatment and can cause spoilage under extended storage. Proteases are one of the major types of enzymes that limit the shelf life of fluid milk (Fox and Kelly, 2006). Proteases can be

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indigenous or produced by microorganisms that survive milk pasteurization or postpasteurization contaminants, such as *Pseudomonas* spp. or *Bacillus* spp. (Sorhaug and Stepaniak, 1997). Proteases can have either beneficial or detrimental effects on the flavor and texture of dairy products (Ismail and Nielsen, 2010). However, in fluid milk, proteases that hydrolyze milk proteins into bitter peptides or lead to flocculation or gelation are particularly troublesome.

Plasmin, a fibrinolysin secreted into the milk from blood plasma through the mammary cells, is the major indigenous protease found in milk (Bastian and Brown, 1996; Fox and Kelly, 2006; Kelly et al., 2006). The activity of the plasmin system, consisting of plasmin (**PL**), plasminogen (**PG**), plasminogen activators (**PA**), plasminogen activator inhibitors, and plasmin inhibitors, depends greatly upon the processing and storage conditions of milk (Ismail and Nielsen, 2010). Although inhibitors are soluble in the milk serum, the rest of the plasmin system (i.e., PL, PG, and PA) is associated with casein micelles (Bastian and Brown, 1996; Fox and Kelly, 2006). Upon storage, PG is cleaved at the Arg^{557}-Ile^{558} peptide bond by proteases such as urokinase and tissue-type PA (Bastian and Brown, 1996; Fox and Kelly, 2006). This proteolytic cleavage converts inactive PG into active PL, resulting in higher PL activity (Fox and Kelly, 2006). Furthermore, the plasminogen activator inhibitors are inactivated by commercial thermal pasteurization, whereas PL is stable to pasteurization (Fox et al., 2003). Thus, PL and PG survive pasteurization and are somewhat resistant to HTST and UHT treatments (Newstead et al., 2006; Ismail and Nielsen, 2010). Alichanidis et al. (1986) reported that at least 30% of the porcine plasmin activity remained in skim milk that was subject to UHT treatment (143°C) and that the D-value (time required for 90% reduction in activity) in skim milk at 143°C was approximately 10 s.

Emerging technologies such as high-pressure processing (García-Risco et al., 2003; Borda et al., 2004; Bilbao-Sainz et al., 2009), pulsed electric field (Bendicho et al., 2005), and ultrasound (Vercet et al., 2002) have been explored to investigate their potential to inactivate shelf life-limiting enzymes in milk, particularly proteases. Ultrasound is a form of energy generated by cyclic sound pressure waves of frequencies that are greater than the upper limit of human hearing range, typically above 18 to 20 kHz (Patist and Bates, 2008). High-power ultrasound is typically produced at lower frequencies (ranging from 16 to 100 kHz) and thus is typically used for emulsion generation and cell disruption where cavitation is the dominant effect and free radical generation ("sonochemistry") is limited. When ultrasound waves are passed through a liquid food material, alternating regions of high and low pressures (i.e., compression and expansion, respectively) are created, which induce cavitation and form gas or vapor bubbles (Nguyen and Anema, 2010; Pingret et al., 2012). These gas bubbles expand because of increased gas diffusion during the expansion cycle and rapidly condense (implode) when the bubbles reach an unstable size. The condensed bubbles collide violently, resulting in shock waves (mechanical or shear forces)—regions of high temperature and pressure. The streaming increases heat and mass transfer in the milk (Jayasooriya et al., 2004; Zheng and Sun, 2006), resulting in spatially confined high temperatures and pressures; the average bulk temperature increases only moderately compared with the temperature in a collapsing bubble (Nguyen and Anema, 2010). Cavitation results in a localized pasteurization effect without causing a significant increase in macro-temperature (Tiwari et al., 2009) but because of streaming, the bulk milk is treated. In addition to cavitation, the shearing effect between the sonication horn and milk also produces heat (Pingret et al., 2012). Destruction of microorganisms or inactivation of enzymes can be induced by one or more of these consequences of sonication. However, the cavitation created depends on many factors, such as frequency and intensity of ultrasound waves, ambient temperature and pressure, and product properties such as viscosity, surface tension, vapor pressure, among others.

Although not yet widely used in the dairy industry, ultrasound has a broad range of applications, including but not limited to cleaning (Kivelä, 1996); promoting nucleation and reduction of ice crystal size in ice cream (Zheng and Sun, 2006); reducing the size of fat globules and creating emulsions (Villamiel and de Jong, 2000b); increasing water-holding capacity and viscosity and reducing syneresis in yogurt (Wu et al., 2001); reducing viscosity and controlling the rate of age thickening of concentrated skim milk (Zisu et al., 2013); and decreasing the fermentation time of yogurt (Wu et al., 2001). Wu et al. (2001) showed that high-power ultrasound (450 W) is capable of homogenizing fluid milk better than commercial homogenization. Villamiel and de Jong (2000b) demonstrated that ultrasonication at higher temperatures (between 70° and 75°C) resulted in a more homogeneous particle distribution than heat or sonication alone.

High-power ultrasound has also proven to be useful in inactivating microorganisms (Wrigley and Lorca, 1992; Villamiel and de Jong, 2000a; Cameron et al., 2008, 2009), suggesting good potential for extension of fluid milk shelf life. However, ultrasound has only been effective in enzyme inactivation when combined with other factors, such as heat or pressure (Villamiel and de Jong, 2000b; Chouliara et al., 2010). Protease