J. Dairy Sci. 99:1–7
http://dx.doi.org/10.3168/jds.2015-10702

Effects of different heat treatments on lysozyme quantity and antimicrobial activity of jenny milk

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ABSTRACT

Thermal treatments are used to improve milk microbial safety, shelf life, and biological activity of some its components. However, thermal treatments can reduce the nutritional quality of milk, affecting the molecular structure of milk proteins, such as lysozyme, which is a very important milk component due to its antimicrobial effect against gram-positive bacteria. Jenny milk is characterized by high lysozyme content. For this reason, in the last few years, it has been used as an antimicrobial additive in dairy products in alternative to hen egg white lysozyme which can cause allergic reactions. This study aims to investigate the effect of pasteurization and condensation on the concentration and antimicrobial activity of lysozyme in jenny milk. Furthermore, lysozyme quantity and activity were tested in raw and pasteurized milk after condensation at 40 and 20% of the initial volume. Reversed-phase HPLC was performed under fluorescence detection to monitor lysozyme in milk samples. The antimicrobial activity of the tested milk was evaluated against *Bacillus megaterium*, *Bacillus mojavensis*, *Clavibacter michiganensis*, *Clostridium tyrobutyricum*, *Xanthomonas campestris*, and *Escherichia coli*. Condensation and pasteurization did not affect the concentration or antimicrobial activity of lysozyme in jenny milk, except for *B. mojaventis*, which showed resistance to lysozyme in milk samples subjected to heat treatments. Moreover, lysozyme in jenny milk showed antimicrobial activity similar to synthetic antibiotics versus some gram-positive strains and also versus the gram-negative strain *X. campestris*.
Key words: condensation, pasteurization, lysozyme, antimicrobial activity, jenny milk

INTRODUCTION

Heat treatment of milk is an essential step in milk processing adopted by the dairy industry. Thermal processing of milk aims to prolong the shelf life and improve the quality of this complex biological fluid by reducing the microbial load (Raikos, 2010). In other cases, if milk is used as a food ingredient in milk-based products, heat treatment is employed to improve the organoleptic properties of such dairy formulations by manipulating the functionality of milk proteins (del Angel and Dalgleish, 2006). One of the most common milk thermal treatments is pasteurization. The main aims of pasteurization are to reduce the microbial population, both pathogenic and spoilage, inactivate enzymes and minimize chemical reactions and physical changes, and to extend the milk shelf life (Gao et al., 2002).

The alteration of the food results in a change of its characteristics, such as decrease of pH, precipitation of calcium phosphate, denaturation of whey proteins and interaction with casein, lactose isomerization, Maillard browning, and modifications to the casein micelle (Walstra and Jenness, 1984). The microorganisms that can contaminate a food are bacteria and fungi (yeasts and molds; Ledenbach and Marshall, 2009). The factors that influence their development in food are pH, the presence or absence of oxygen, radiation, presence of chemicals, temperature, and water content (Dugenest et al., 1999). Water is essential for microorganisms; in fact, microorganisms show poor or absent growth in foods in which the water content is low (Cabral, 2010). To increase the shelf life of milk, treatments, such as lyophilization or pulverization, are applied to remove the water (Ribeiro and Ribeiro, 2010).

Polidori and Vincenzetti (2013) carried out a study on the effects of thermal treatments on jenny milk nutritional characteristics; those authors reported the enzymatic activity of lysozyme decreased up to about 70% in the powdered milk with respect to fresh and frozen milk due to the high temperatures of the atomization in the spray-dry process (up to 200°C). Another technique that can be taken into account is the freeze-drying (or lyophilization), which is much more expensive than

Received December 1, 2015.
Accepted March 27, 2016.
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spray-drying but, in some cases, can lead to products of greater value (Aloisio et al., 2011). Lyophilization does not significantly affect the quality parameters of jenny milk; however, even if the enzymatic lysozyme activity does not change after lyophilization, the lysozyme concentration is slightly reduced after this thermic treatment (Vincenzetti et al., 2011).

Lysozyme is a natural antimicrobial agent that may contribute to gram-positive growth inhibition (Chiavari et al., 2005; Cosentino et al., 2012; Fratini et al., 2015). Moreover, lysozyme is able to kill or to inhibit a large spectrum of pathogens (Zhang et al., 2008; La Torre et al., 2010). Lysozyme is a natural antimicrobial agent because it catalyzes the hydrolysis of glycosidic bonds of mucopolysaccharides in bacterial cell walls. Lysozyme may work synergistically with lactoferrin and immunoglobulins in antimicrobial activity (Lönnerdal, 1985). This enzyme, along with other factors including immunoglobulins, lactoferrin, and lactoperoxidase, may limit the migration of neutrophils into a damaged tissue by behaving as an anti-inflammatory agent (León-Sicairos et al., 2006).

The lysozyme content is higher in jenny milk [from 1.0 to 3.7 mg/mL, according to Zhang et al. (2008) and Galassi et al. (2012)], than in other species [cow: 0.13 μg/mL (Shahani et al., 1973); ewe: 0.20 μg/mL (Fratini et al., 2006); goat: 0.25 μg/mL (Scharfen et al., 2007)]. In the recent years, some authors have proposed the use of lysozyme from jenny milk as a substitute of hen egg white (**HEW**) lysozyme, currently used as natural antibacterial additive in commercial dairy products (Cosentino et al., 2013, 2015a,b). Although the major egg allergens are ovalbumin and ovomucoid, several studies have shown that lysozyme is indeed an allergen (Frémont et al., 1997; Pérez-Calderón et al., 2007). On the contrary, lysozyme from jenny milk is not derived from a known allergenic source (Galassi et al., 2012), in fact Vincenzetti et al. (2014) presented evidence that demonstrate the hypoallergenicity of jenny milk in humans. To carry out its antimicrobial role it is important that the lysozyme quantity and activity are not lost during milk thermal treatment.

We proposed that condensation is a valid alternative to lyophilization and pulverization for decreasing the water content of the milk. Condensed milk is the product obtained by evaporating part of the water of whole milk or fully or partly skim milk (Van Den Berg, 1962). Cosentino et al. (2015c) previously showed that pasteurization and condensation do not significantly affect the total antioxidant capacity of jenny milk. The object of the current study was to investigate the effects of condensation and pasteurization on lysozyme quantity and antimicrobial activity in jenny milk.

MATERIALS AND METHODS

The research was carried out on bulk milk of 15 Martina Franca multiparous jennies, in midlactation (180 d after foaling), between 7 and 10 yr of age. Jennies were fed on ad libitum oat hay with an integration of 1 kg/head per day of concentrate, characterized by the following mixture: 37% flaked corn, 30% oats, 9% locust bean crushed, 8% wheat bran, 8% dehydrated alfalfa, 6% dried beet pulp, and 2% mineral and vitamin supplement. After milking, milk aliquots were immediately refrigerated at 4°C and transported to the laboratory, where the following chemical parameters were determined by Milkoscan FT 6000 (Foss Electric, Hillerød, Denmark): protein, fat, lactose, and DM. The effect of different levels of condensation on the lysozyme quantity and antimicrobial activity of jenny milk was evaluated on raw (**R**) and on pasteurized (**P**) milk. Pasteurization was performed by heating raw jenny milk to 63°C for 30 min. Milk was condensed by rotary vacuum evaporation using system RV8 (IKA-Werke GmbH and Co., Staufen im Breisgau, Germany) equipped with a vacuum pump model PC 3001 Vario (Vacuumbrand GmbH, Wertheim, Germany), under the following conditions: water bath temperature 35°C; pressure 4 kPa; processing time 2 and 3 h for condensation at 40 (**RC40** and **PC40**) and 20% (**RC20** and **PC20**) of the initial volume, respectively. Before determining lysozyme concentration and activity, to reconstitute the initial water volumes, 6 mL of MilliQ water (Millipore, Billerica, MA) were added to 4 mL of RC40 or PC40, and 8 mL of MilliQ water were added to 2 mL of RC20 or PC20. Each heat treatment was repeated 3 times using 3 different batches of jenny milk.

Lysozyme Extraction

For lysozyme extraction, a modified method of Pellegrino and Tirelli (2000) was employed. Ten milliliters of raw, pasteurized, and condensed jenny milk were mixed with 30 mL of 1 *M* NaCl (pH 6). The samples were kept under magnetic stirring previously at 40°C for 10 min, and then at room temperature for 1 h. Afterward, the extract was acidified to a pH 2.2 adding 1 *M* HCl to precipitate the caseins. Finally, the extracts were filtered over a paper filter and then with a 0.20-μm filter (Minisart NML syringe filters in cellulose acetate; Sartorius, Gottingen, Germany).

Chemicals for HPLC Analysis

A freeze-dried lysozyme standard, Fluka/34046-Vetranal, HPLC-grade acetonitrile, and trifluoroacetic