J. Dairy Sci. 99:1–9
http://dx.doi.org/10.3168/jds.2015-10741

Shelf life of donkey milk subjected to different treatment and storage conditions

Federica Giacometti,*[1] Lia Bardasi,† Giuseppe Merialdi,† Michele Morbarigazzi,‡ Simone Federici,‡ Silvia Piva,* and Andrea Serraino*
*University of Bologna, Department of Veterinary Medical Sciences, Via Tolara di Sopra 50-40064 Ozzano Emilia, Bologna, Italy
†Experimental Institute for Zooprophylaxis in Lombardy and Emilia Romagna, Via P. Fiorini 5, 40127 Bologna, Italy
‡HPP Italia Srl, Via Carbognani 6, 43029 Traversetolo, Parma, Italy

ABSTRACT

The aim of this study was to investigate the effects of different treatment conditions on microbiological indicators of donkey milk hygiene and their evolution during shelf life at 4 and 12°C from 3 to 30 d, simulating a farm-scale pasteurization and packing system. Four treatment conditions were tested: no treatment (raw milk), pasteurization (65°C × 30 min), high-pressure processing (HPP), and pasteurization plus HPP. The microbiological quality of the raw donkey milk investigated was not optimal; our results highlight the importance of raw milk management with the need for animal hygiene management and good dairy farming practices on donkey farms to improve handling procedures. The raw milk treated with HPP alone showed visible alterations with flocks, making the milk unfit for sale. The microbiological risk posed by consumption of raw donkey milk was significantly reduced by heat treatment but farm-scale packing systems cannot guarantee an extended shelf life. In contrast, the pasteurization plus HPP treatment was the most effective method to maintain microbiological milk quality. Microflora growth had little effect on pH in donkey milk: pH values were significantly different only between raw milk and pasteurized and pasteurized plus HPP milk stored at 12°C for 3 d. Alkaline phosphatase activity and furosine could be used as indicators of proper pasteurization and thermal processing in donkey milk. Moreover, the presence and growth of *Bacillus cereus* in the case of thermal abuse hamper the wide-scale marketing of donkey milk due to the potential consequences for sensitive consumers and therefore further tests with time/temperature/high-pressure protocols associated with *B. cereus* are needed. Finally, our study shows that an HPP treatment of pasteurized milk after packing extends the shelf life of donkey milk and assures its microbial criteria up to 30 d if properly stored at 4°C until opening; therefore, combined heat treatment and storage strategies are recommended to enhance the shelf life of donkey milk.
Key words: donkey milk, pasteurization, high-pressure processing (HPP), shelf life

INTRODUCTION

Even though nonruminant milk accounts for less than 0.1% of global milk production (Claeys et al., 2014), donkey milk is receiving increasing interest in Europe as an alternative to breast milk and infant formula for babies allergic to cow milk (Iacono et al., 1992; Monti et al., 2007, 2012; Mansueto et al., 2013), in case of multiple food intolerance (Carroccio et al., 2000), or when breastfeeding is not possible (Sarno et al., 2012). In addition, donkey milk is appreciated by people eager to try new foods and purchase locally grown produce (Scatassa et al., 2011).

To meet this demand, donkey farming is undergoing a revival in Italy, with new donkey dairies opening in several regions. With few exceptions, farms are small (<10 to 150 donkeys and from 5 to 30 milking jennies), family-run, and usually located in mountainous or hilly areas. Jennies are milked once a day using milking machines adapted from goat or cow milking equipment (Cavallarin et al., 2015) and usually produce about 1.5 L of milk a day. Daily milk production on the farm does not usually exceed 50 to 100 L and, due to the long distances between donkey farms, logistical organization of milk collection and distribution is lacking.

Currently, donkey milk for human consumption is sold as raw milk directly at farms or from vending machines, or it is pasteurized (or, rarely, UHT-treated or freeze-dried), packed in cartons or polyethylene terephthalate (**PET**) or glass bottles, and sold in shops, pharmacies, or online. By Italian law, raw milk has a shelf life of 3 d, whereas the shelf life of pasteurized and UHT milk is usually fixed by manufacturers at 4 to 6 d for pasteurized milk under storage conditions between 0 and 4°C and at 6 mo for UHT, with the recommenda-

Received December 10, 2015.
Accepted February 9, 2016.
[1]Corresponding author: federica.giacometti3@unibo.it

tion to refrigerate it at a maximum temperature of 5°C after opening and to consume it within 3 d. Nevertheless, these conditions raise some problems: (1) the 3-d shelf life of raw donkey milk limits the wide-scale marketing of this commodity and the development of donkey milk companies or farms; (2) pasteurization extends the shelf life but not sufficiently to be a viable alternative for wide and efficient distribution given the logistic limitations of donkey farms; (3) UHT and freeze-drying treatments guarantee commercially sterile products but entail irreversible changes in endogenous milk compounds such as whey protein and lipid components (Sorrentino et al., 2006), and could alter flavor. In addition, UHT treatment systems are very expensive for a single farm and require large amounts of milk, which donkey farms are not expected to produce. At the same time, the safety of donkey milk is a potential concern for food-sensitive consumers or individuals with allergies or other problems.

For these reasons, it is useful to evaluate alternative approaches to donkey milk sanitation and shelf-life extension. High-pressure processing (**HPP**) is a nonthermal food preservation technology with minimal adverse effects on food quality (Cullen et al., 2012). It relies on the use of high pressure (generally 100–600 MPa) to process liquid or solid foods to inactivate spoilage and pathogenic microorganisms and extend the shelf life (Evelyn and Silva, 2015). The effects of HPP on foods were first studied in the late 19th century, when processing cow milk at 670 MPa for 10 min resulted in 5- to 6-log microbial reductions, extending shelf life up to 4 d after processing (Hite, 1899). However, the complexity of foods and the wide variety of phenomena that occur under pressure make it difficult to predict effects of HPP on foods (Palou et al., 2007). For these reasons, HPP conditions must be evaluated in each specific food.

Little data exists in the literature on the effects of heat treatments on the chemical and microbiological parameters of donkey milk, and no study has hitherto addressed the effects of HPP on its microbial contents. The aim of this study was to investigate the effect of different treatment conditions on the microbiological indicators of donkey milk and their evolution during shelf life at different temperatures from a minimum of 3 d and a maximum of 30 d, simulating a farm-scale pasteurization and packing system.

MATERIALS AND METHODS

Milk Sample Preparation

Four treatment conditions were tested: no treatment (raw milk), pasteurization, HPP, and pasteurization plus HPP. Three batches of raw donkey milk were collected from local farms for 3 consecutive weeks in June 2015. After postmilking refrigeration, each batch (30 L) was transported to the cheese factory of the Department of Veterinary Medical Sciences, Bologna, and then it was divided into 2 portions: (1) 20 L of raw donkey milk was pasteurized (65°C for 30 min) using a commercial farm-scale pasteurization system (Caseus, Plastitalia group, Milan, Italy) and packed into 26 PET spout pouches (250 mL each): 10 were used for the pasteurization test and 16 were transported to a local industry for HPP treatment for the pasteurization plus HPP test; ii) 10 L of raw donkey milk were packed into 16 PET spout pouches (250 mL each): 6 for the raw milk test and 10 for HPP treatment. The HPP treatment was performed by Avure Technologies (Quintus Food Press QFP350L-600; Middletown, OH): milk packs were initially treated at a constant pressure of 600 MPa and at temperatures in the range of 4 to 6°C for 180 s; under working conditions, the temperature increased by approximately 10°C due to pressure buildup (approximately 100 MPa/min). After HPP treatment, the milk was visually inspected for any changes that could affect the marketing of donkey milk. Due to the appearance of clotting in the HPP-treated milk, the pressure was reduced from 600 to 400 MPa for 180 s for pasteurized milk and the HPP treatment was further reduced to 400 MPa for 100 s for raw milk destined for the HPP-only treatment.

For each treatment condition, all samples were divided and stored at 4 and 12°C to simulate optimal storage conditions and domestic storage, respectively (Beaufort et al., 2008): raw milk samples were stored for 3 d (according to Italian legislation); pasteurized and HPP samples for 15 d; and pasteurization plus HPP samples for 30 d.

Samples were analyzed from each PET spout pouch at d 0 (before treatment) and for each storage condition at d 1 and 3 for raw milk; at d 1, 3, 7, 10, 15 for pasteurized and HPP milk; and additionally at d 21, 25, and 30 for the pasteurization plus HPP samples.

Microbiological and Chemical Analyses

The following microbiological analysis were performed in each sample of type of milk as described above: total mesophilic colony count (**TMC**; UNI EN ISO 4833-2:2013/Cor.1:2014; ISO, 2013a); enumeration of *Enterobacteriaceae* (ISO, 2004), *Pseudomonas* spp. (ISO, 2009), presumptive *Bacillus cereus* (ISO, 2005a), and only for raw and HPP samples, enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species; ISO, 1999). The pH value of each sample was measured by an automatic temperature