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Effects of pasteurization and storage conditions on donkey milk nutritional and hygienic characteristics

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Until now there are only few data on the effects of thermal treatments on the nutritional and hygienic characteristics of donkey milk. This Research Communication aims to provide information on the effects of pasteurization (at +65 °C for 30 min) and prolonged storage at refrigeration and freezing temperatures (21 d at + 3 °C ± 2 °C and up to 90 d at −20 °C ± 5 °C) on some nutritional and hygienic characteristics of Amiata donkey milk. The milk was monitored by chemical and microbiological analysis. Pasteurization ensured compliance with EC Regulation No 1441/2007, as Enterobacteriaceae were never found in the milk, or during storage at refrigeration and freezing temperatures. Colony count at 30 °C in pasteurized milk never went beyond 1 log CFU/ml. The heat treatment and the storage did not result in any variations in the main constituents of the milk. Only a decrease in lactose and few variations in some fatty acids at 90 d of freezing were observed. In conclusion, pasteurization was able to achieve and maintain a high hygienic-sanitary quality over time; storage at refrigeration or freezing temperatures did not alter the nutritional quality of fat and the gross composition of the product. These findings are useful to improve knowledge on the milk shelf life in order to guarantee safety and nutritional quality for infants who need small quantities of daily milk.

Keywords: Amiata donkey milk, pasteurization, storage, chemical composition, microbiological quality.

Due to the increasing spread of food allergies worldwide, donkey milk has become of scientific interest for use as an alternative food for children with cow's milk protein allergy.

Raw donkey milk generally has a lower total bacterial count than ruminant milks (Pilla et al. 2010; Ragona et al. 2016). The good hygienic and health characteristics of donkey milk may be due to the content of antimicrobial enzymes such as lysozyme (Vincenzetti et al. 2008), and also to the anatomy of the udder that does not regularly come into contact with the soil. However, despite the low bacterial count, some authors have detected the presence of pathogenic bacterial species (Pilla et al. 2010).

Since the consumption of raw milk may be a serious health risk to consumers due to the possible contamination with foodborne pathogens of animal or environmental origin, which may develop during the milking process or the milk storage, good hygienic practices and thermal treatment are important to prevent microbiological risk.

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Pasteurization is one of the most common thermal treatments performed on milk. Although the effects of thermal treatments and storage on the quality and shelf life of cow milk are well known, few studies have been performed on the effects of thermal treatments and storage on hygienic quality of donkey milk (Polidori & Vincenzetti, 2010; Addo & Ferragut, 2015; Giacometti et al. 2016). The importance of monitoring nutritional characteristics are related to the fact that children who are allergic to cow milk proteins are at particularly high risk for developing growth retardation and nutritional deficiency (Mehta et al. 2013). Therefore, they require a careful management for nutrition, from a quantitative and qualitative point of view, in order to avoid conditions of undernourishment and malnutrition. A further issue is that donkey milk is a niche product so it is not always or easily available on the market and domestic freezing of donkey milk is a common practice.

We designed a study aimed at providing information on the effects of thermal treatment and prolonged storage at refrigeration and freezing temperatures on some nutritional and hygienic characteristics of Amiata donkey milk.

Materials & methods

Once a week, three bulk raw milk samples were collected in duplicate from the morning milking of 20 jennies reared in the province of Grosseto (Central Italy). The jennies were routinely machine milked by a raised milking parlour as described by Bibbiani et al. (2017).

From each sampling, two raw milk aliquots were made: one was refrigerated at +3 °C, whereas the other one had previously undergone Holder type pasteurization (+65 °C for 30 min). The pasteurized milk aliquot was divided into 9 sub-aliquots, one of which was analysed on the day of pasteurization. The other pasteurized subaliquots were stored for up to 21 d at +3 °C (±2 °C) and up to 90 d at −20 °C (±5 °C) and analysed during storage (see online Supplementary Fig. S1).

The following analyses were carried out at the Department of Veterinary Science of University of Pisa and at the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana (Florence) on the milk:

(a) Chemical analysis: pH was measured by pH meter, dry matter and ash were determined by gravimetric method of residues after drying and incineration respectively, fat was evaluated by gravimetric method after extraction of an ethanol-ammonia solution by ethyl ether, protein was calculated as total nitrogen (N) (determined by Kjeldahl) multiplied by 6·38, lactose was determined by infrared analysis; fatty acids were methylated using sodium methoxide solution 0·5 M in methanol (Sigma-Aldrich S.r.l. *Via* Gallarate 154 Milan, Italy, 20151) and analysed by gas chromatography.
(b) Microbiological Analysis: Colony count at 30 °C, *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter* spp., coagulase-positive Staphylococci, Enterobacteriacee.

Detailed methods are reported in the online Supplementary Table S1.

Statistical analysis

Data on the chemical composition of raw donkey milk and pasteurized were compared (JMP, 2002) using ANOVA with heat treatment (presence or absence) as a fixed effect. Quality data for refrigerated and frozen milk, were evaluated using ANOVA for repeated mesurement that included the storage period as a fixed effect.

Data on pH and total mesophilic count variations were evaluated separately using the PROC ANOVA of SAS/STAT® (SAS, 2004), considering the storage condition as a fixed effect. Significant differences between data were considered at $P < 0{\cdot}05$.

Results and discussion

The heat treatment did not significantly affect the gross composition of the milk (Table 1). The only statistically

Table 1. Gross compostion, pH and micorbiological analysis on raw and pasteurized donkey milk stored +3 °C (±2 °C) and −20 °C (±5 °C) for 21 and 90 d, respectively.

		Raw	Pasteurized		Pasteurized stored at +3 °C(±2 °C)					Pasteurized stored at −20 °C (±5 °C)					
				SEM	D 1†	D 7	D 14	D 21	SEM	D 1	D 7	D 14	D21	D 30	D 90	SEM
Fat	g/100 ml of milk	0·34	0·36	0·050	0·36	0·33	0·35	0·39	0·068	0·36	0·35	0·41	0·38	0·40	0·30	0·052
Proteins		1·69	1·67	0·054	1·67	1·70	1·77	1·87	0·156	1·67	1·66	1·72	1·80	1·76	1·65	0·148
Dry matter		9·52	9·52	0·504	9·52	9·76	9·88	9·39	0·425	9·52	9·62	9·29	9·14	9·13	9·14	0·496
Ash		0·37	0·38	0·014	0·38	0·38	0·37	0·40	0·029	0·38	0·37	0·39	0·38	0·38	0·34	0·008
Lactose		5·90	6·04	0·225	6·04A	5·68B	5·47B	5·41B	0·307	6·04A	5·57AB	5·37B	5·20B	4·68C	4·67C	0·278
pH	pH units	7·19	7·14	0·026	7·14	7·13	7·19	7·16	0·020	7·14	7·11	7·21	7·21	7·20	7·34	0·022
Colony count at 30 °C	‡	4·84	0·39		0·39	0·73	0·16	0·32	0·127	0·39	<1	0·58	0·16	0·32	0·43	0·111
Enterobacteriaceae	CFU/ml	NP§	<1		<1	<1	<1	<1		<1	<1	<1	<1	<1	<1	
Coagulase-positive Staphylococci	log CFU/ml	2·23	0													

A,B,C: $P \leq 0{\cdot}01$
†D1, 7, 14, 21, 30, 90 = number of days of storage
‡log CFU/ml or CFU/ml if <1
§Analysis not performed

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Table 2. Fatty acid composition (g/100 g of total fatty acids) of pasteurized donkey milk stored for 21 and 90 d, respectively

Fatty acid methyl ester	Pasteurized donkey milk					Pasteurized donkey milk						
	Stored at + 3 °C (±2 °C)					Stored at −20 °C (±5 °C)						
	D 1[†]	D 7	D 14	D 21	SEM	D 1	D 7	D 14	D 21	D 30	D 90	SEM
6 : 0	0·20	0·18	0·20	0·13	0·043	0·20B	0·16B	0·16B	0·19B	0·17B	0·31A	0·043
14 : 0	7·32	7·35	7·40	7·28	0·505	7·32B	7·45B	7·30B	7·30B	7·29B	7·67A	0·505
14 : 1	0·36	0·33	0·35	0·33	0·027	0·36B	0·33B	0·33B	0·34B	0·36B	0·39A	0·027
t11–18 : 1	0·01	0·02	0·01	0·01	0·010	0·01b	0·01b	0·02b	0·01b	0·02b	0·03a	0·010
c9,12–18 : 2	13·01	12·88	13·02	13·15	1·426	13·01a	12·83a	12·80a	13·12a	13·20a	11·83b	1·426
21 : 0	0·29	0·33	0·20	0·22	0·093	0·29b	0·22b	0·20b	0·20b	0·20b	0·39a	0·093
20 : 3n-3	0·24	0·27	0·17	0·18	0·073	0·24b	0·16b	0·18b	0·17b	0·16b	0·34a	0·073
SCFA (≤C10)[‡]	14·24	14·37	13·91	14·04	0·920	14·24	14·30	14·45	14·53	14·28	14·75	0·920
MCFA(≥C11≤C17)[§]	43·10	43·84	44·03	43·98	1·551	43·10	44·52	44·15	43·82	43·92	44·68	1·551
LCFA(≥C18)[‖]	42·65	41·79	42·06	41·97	2·388	41·62	41·18	41·40	41·64	41·80	40·57	2·388
SFA**	55·55	56·74	56·38	56·52	2·575	56·65	57·24	56·80	56·84	56·44	58·04	2·575
MUFA[††]	22·21	21·78	21·59	22·27	1·141	22·17	21·88	22·20	21·86	22·37	21·99	1·141
PUFA[‡‡]	21·08	21·48	22·04	21·22	0·487	21·18	20·88	21·00	21·30	21·19	19·96	0·487
UFA[§§]/SFA	0·79	0·78	0·79	0·80	0·030	0·10	0·10	0·09	0·11	0·11	0·17	0·040
n-3/n-6	0·59	0·64	0·64	0·57	0·060	0·20B	0·16B	0·16B	0·19B	0·17B	0·31A	0·043

In the table only the significant differences and the fatty acid classes and ratio are shown (the full table is available as Supplementary File)
A,B: $P \leq 0{\cdot}01$; a,b: $P \leq 0{\cdot}05$
†D1, 7, 14, 21, 30, 90 = number of days of storage
‡SCFA (short-chain fatty acids): (≤C10)
§MCFA (medium-chain fatty acids): (≥C11≤C17)
‖LCFA (long-chain fatty acids): (≥C18)
**SFA (saturated fatty acids)
††MUFA (monounsaturated fatty acids)
‡‡PUFA (polyunsaturated fatty acids)
§§UFA (unsaturated fatty acids)

detectable variations in the chemical composition during storage were related to lactose, which significantly decreased at day 7 in refrigerated milk and at day 14 and 30 in frozen milk. The lack of changes in the gross composition during storage ensures a constant quality of the product and this is of interest as donkey milk is frequently used by allergic children in which nutritional deficiencies such as lower intakes of protein and fat have been reported (Henriksen et al. 2000).

The major fatty acid composition of the stored milks is shown in Table 2 and the full fatty acid profile is shown in online Supplementary Table S2. Storage for up to 21 d at +3 °C(±2 °C) did not affect the total fatty acid profile of the refrigerated milk, while only with extended storage at −20 °C (90 d) did we observe significant changes in some fatty acids (decrease in c9,12–18 : 2 and increase in 6 : 0, 14 : 0, 14 : 11, t9–1 : 1, 21 : 0, 20 : 3n-3 and n3/n6 ratio). Furthermore, the saturated/unsaturated fatty acids ratio (SFA/UFA), the total polyunsaturated fatty acid (PUFA) and some essential fatty acids, such as 18 : 3n-3 (ALA), 20 : 5 (EPA) and 22 : 6 (DHA) were not affected by storage. The unchanged SFA/UFA ratio indicated a lack of degradation and/or oxidation processes during prolonged cold storage.

ALA, EPA and DHA are essential fatty acids (EFA), namely fatty acids that the human body is not able to synthesize, and that must be obtained from the diet. In infants, dietary lipids fulfil numerous metabolic and physiological function (Delplanque et al. 2015), however, food allergy in children may lead to insufficient supply of EFA through allergic symptoms and food restriction. In addition, in allergic children dietary fat should provide a balanced combination of saturated, monounsaturated and polyunsaturated fatty acids (Paassilta et al. 2014). Therefore, due to the higher risk for developing growth retardation and nutritional deficiency a constant quality of fat is relevant to support normal growth and mental development (Delplanque et al. 2015).

The mean pH value of the milk was 7·19 (standard deviation: 0·03; range values: 7·17–7·22), consistent with the values reported in the literature (Addo & Ferragut, 2015; Giacometti et al. 2016) and did not show significant differences over the period of study either in the refrigerated or the frozen aliquots.

The average colony count at 30 °C of the raw milk was 4·84 log CFU/ml (standard deviation: 0·68; range values: 4·30–5·60), corresponding to 154×10^3 CFU/ml, much lower than the limit required by the Regulation (EC) 853/2004 (European Commission, 2004) for total plate count at 30 °C ($\leq 1{\cdot}500 \times 10^3$ CFU/ml). Pasteurization resulted in a reduction of colony count at 30 °C of 4-log at day 1, which remained <1 log CFU/ml during storage. In addition, colony count at 30 °C was lower than that described in

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other studies on donkey pasteurized milk (Giacometti et al. 2016).

Coagulase-positive Staphylococci were found in the raw milk with an average count of 2·23 log CFU/ml (standard deviation: 0·03; range values: 2·20–2·26), corresponding to $1{\cdot}7 \times 10^2$ CFU/ml, lower compared with the results of Malissiova et al. (2016), and in the pasteurized milk they were always lower than the detection limit of the method (<1 CFU/ml).

The Enterobacteriaceae count was lower than 1 CFU/ml in the pasteurized milk and during storage, in compliance with Regulation (EC) No 1441/2007 (European Commission 2007). In addition, in both raw and pasteurized milk samples, the bacteria responsible of food-borne outbreaks (*Salmonella* spp., *Listeria monocytogenes*, *Campylobacter* spp.) were never isolated.

In conclusion, the pasteurization adopted in this study was able to achieve and maintain a high hygienic quality over time. This study highlights that pasteurization and storage at refrigeration or freezing temperatures does not alter the milk gross composition and the nutritional quality of the fat. Considering that donkey milk is often not easily available on the market and it is a food intended for vulnerable groups of consumers, these findings are useful to improve knowledge on the milk shelf life in order to guarantee safety and nutritional quality for infants who need small quantities of daily milk. Our results suggest that donkey milk shelf life would extend beyond the normal duration of cow's milk; further investigations to guarantee the quality of donkey milk during an extended shelf life are required.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S0022029918000687

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