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

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Short title: **Quality of donkey milk: pasteurization and storage**

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Summary

Until now there are only few data on the effects of thermal treatments on the nutritional and hygienic chacteristics of donkey milk, this Research Communication aims to provide preliminary information on the effects of pasteurization (at 65°C for 30 minutes) and prolonged storage at refrigeration and freezing temperatures (21 days at +3°C±2°C and up to 90 days 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 a decrease in lactose and few variations in some fatty acids at 90 days 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.*, 2015). The good hygienic and health characteristics of donkey milk, may be due to the content of antimicrobial enzymes such as lysozyme, and also to the anatomy of the udder that does not regularly come in contact with the soil. However, despite the low bacterial count, some authors have detected the presence of undesirable 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 the good hygienic practices amd thermal treatment are important to prevent microbiological risk.

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, there have been few studies on the effects of thermal treatments and storage on hygienic quality of donkey milk (Addo & Ferragut, 2015; Giacometti et al., 2016) and no one include the effects on nutritional characteristics.

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 to provide preliminary 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. 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 minutes). 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 days at +3°C (±2°C) and up to 90 days at -20°C (±5°C) and analysed during storage (see Fig.1 supplementary file).

The chemical and microbiological analysis were carried out on the milk by the methods reported in the supplementary Table 1s.

Statistical Analysis

Data on the chemical composition of raw donkey milk and pasteurized were compared (JMP, 2002) using a statistical model with heat treatment (presence or absence) as a fixed effect. Quality data for refrigerated and frozen milk, were evaluated using a statistical model 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 $P < 0.05$.

Results and Discussion

The heat treatment did not significantly affect the gross composition of the milk (Table 1). The only statistically detectable variations in the chemical composition during storage were related to the lactose, which signifincantly decreased at day 7th in refrigerated milk and at 14^{th} and 30^{th} day in freezed milk.

Table 1 near here

Storage up to 21 days 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 days) were observed significant changes in some fatty acids (decrease in c9,12-18:2 and increase in 6:0, 14:0, 14:11, t9-18-1, 21:0, 20:3n-3 and n3/n6 ratio)

Table 2 near here

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 the storage. The constant quality of fat is relevant for the nutrition of infants, in which dietary lipids also fulfill numerous metabolic and physiological functions critical to their growth and health (Delplanque et al., 2015). The unchanged SFA/UFA ratio indicated a lack of degradation and/or oxidation processes during prolonged cold storage.

The pH mean value of the milk was 7.19, consistent with the values reported in the literature (Addo and 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 154 x 10^3 CFU/mL (4.84 log CFU/mL), much lower than the limit required by the Regulation (EC) 853/2004 for total plate count at 30°C (≤1.500 x 10^3 CFU/mL). Pasteurization resulted in a reduction of colony count at 30°C of 4-log, which remained low thereafter. In addition colony count was lower than that described in other studies on donkey pasteurized milk (Giacometti et al., 2016).

Coagulase-positive Staphylococci were found in the raw milk with a count of 2.23 log CFU/mL (1.7x10^2 CFU/mL) on average, lower compared with the results of Malissiova *et al.*

(2016). However in the heat-treated milk Staphylococci were always lower than the detection limit of the method (<1 CFU/mL).

The *Enterobacteria* count was lower than 1 CFU/mL in the pasteurized milk and during storage, in compliance with Regulation (EC) No 1441/2007.

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 heat treatment was able to achieve and maintain a high hygienic-sanitary quality over time. This study highlights that pasteurization and storage at refrigeration or freezing temperatures, do not alter the milk gross composition and the nutritional quality of the fat. Considering that donkey milk is often not easily procurable 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 extended beyond the normal duration of cow's milk; further investigations to guarantee the quality of donkey milk during an extended shelf life are required.

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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 days and 90 days, respectively.

		Raw	Pasteurized		Pasteurized stored at +3°C(±2°C)					Pasteurized stored at -20°C (±5°C)						
				SEM	D 1[1]	D 7	D 14	D 21	SEM	D 1	D 7	D 14	D21	D 30	D 90	SEM
Fat	g/100ml 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	[2]	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[3]	<1		<1	<1	<1	<1		<1	<1	<1	<1	<1	<1	
Coagulase positive Staphylococci	log CFU/mL	2.23	0													

A,B,C: P≤0.01

[1] D1, 7, 14, 21, 30, 90=number of days of storage

[2] log CFU/mL or CFU/mL if <1

[3] Analysis not performed

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

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	Pasteurized donkey milk					Pasteurized donkey milk						
Fatty acid methyl ester	Stored at +3°C(±2°C)					Stored at -20°C (±5°C)						
	D 1[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)[2]	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)[3]	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)[4]	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[5]	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[6]	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[7]	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[8]/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

175 In the table only the significant differences and the fatty acids classes and ratio are shown (the full table is availabe as supplementary file)

176 A,B: P≤0.01; a,b: P≤0.05

177 [1] D1, 7, 14, 21, 30, 90=number of days of storage

178 [2]SCFA (Short Chain Fatty Acids): (≤ C10); [3]MCFA (Medium Chain Fatty Acids): (≥C11≤C17); [4]LCFA (Long Chain Fatty Acids): (≥C18); [5]SFA

179 (Satured Fatty Acids);[6]MUFA (Monounsatured Fatty Acids); [7]PUFA (Polyunsatured Fatty Acids); [8]UFA (Unsaturated Fatty Acids)

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