Lait 85 (2005) 481–490
© INRA, EDP Sciences, 2005
DOI: 10.1051/lait:2005031

Original article

Use of donkey's milk for a fermented beverage with lactobacilli

Cristiana CHIAVARI[a]*, Fabio COLORETTI[a], Mauro NANNI[b], Elena SORRENTINO[c], Luigi GRAZIA[a]

[a] Dipartimento di Protezione e Valorizzazione Agroalimentare (DIPROVAL), *Alma Mater Studiorum*-Università di Bologna, Via F.lli Rosselli, 107, 42100 Reggio Emilia, Italy
[b] Tinval s.r.l., Spin Off *Alma Mater Studiorum*-Università di Bologna, Italy
[c] Dipartimento di Scienze e Tecnologie Agroalimentari, Ambientali e Microbiologiche (DISTAAM) dell'Università del Molise, Campobasso, Italy

Received 28 January 2005 – Accepted 27 April 2005

Published online 1 October 2005

Abstract – The possibility of producing a fermented beverage from donkey's milk using the probiotic bacterial strains *Lactobacillus rhamnosus* AT 194, CLT 2/2, and *Lactobacillus casei* LC 88, isolated from Parmigiano Reggiano cheese was investigated. The chemical-physical and microbiological properties of the raw milk demonstrated that it has a low microbiological load and an elevated content of lysozyme. The bacterial strains employed for fermentation had a good growth capacity in donkey's milk only after an initial adaptation phase. An extremely high percentage of viable bacteria were found in the final beverage, even after a 30-day shelf life. Likewise, the activity of lysozyme was virtually unchanged with respect to initial values. Sensorial analysis permitted the individuation of differences between the three bacterial strains used for fermentation in terms of descriptors relative to aromatic-olfactory qualities. Based on the above results, technology can be proposed for production of a fermented beverage from donkey's milk that can be utilized by small producers. This would allow the production of a beverage that would be well accepted by consumers interested in a product with favorable therapeutic properties integrated with probiotic bacteria.

donkey's milk / fermented beverage / *Lactobacillus rhamnosus* / *Lactobacillus casei*

摘要 – 乳杆菌发酵驴奶生产发酵乳饮料。本文主要探讨了从 Parmigiano Reggiano 干酪（帕尔马 • 勒佐安诺干酪）中分离的益生菌菌株鼠李糖乳杆菌 (*Lactobacillus rhamnosus*) AT194, CLT2/2 和干酪乳杆菌 (*Lactobacillus casei*) LC88, 以驴奶为原料应用这三株益生菌生产发酵乳饮料的可行性。经理化和微生物性质研究表明，原料乳的菌数较低而溶菌酶含量较高。发酵用的菌株在驴奶培养基中经过适当的驯化后在驴奶中具有很好的生长能力。在最终的产品中，活菌数极高，即使经过了 30d 的保藏，活菌数变化不显著。同时，溶菌酶活性与原始样品相比也基本上没有变化。在感官分析过程中，依据香气和香味的特性来描绘三株菌发酵制得的饮料之间的区别。基于上述结果，认为驴奶发酵生产乳饮料在技术上是可行的，这种技术可用于小规模的乳饮料生产。这种乳饮料尤其结合了益生菌特定的治疗作用，可以很好的被消费者所接受。

驴奶 / 发酵饮料 / 鼠李糖乳杆菌 / 干酪乳杆菌

Résumé – Utilisation du lait d'ânesse pour la production d'une boisson fermentée avec des souches de lactobacilles. Cette étude concerne l'utilisation du lait d'ânesse pour la production d'une boisson fermentée en utilisant les souches probiotiques *Lactobacillus rhamnosus* AT 194,

* Corresponding author (通讯作者): cristiana.chiavari@unibo.it

CLT 2/2 et la souche *Lactobacillus casei* LC 88 toutes isolées du fromage Parmigiano Reggiano. Le lait, caractérisé du point de vue physico-chimique et microbiologique, a comme caractéristiques prédominantes une charge microbienne faible et un contenu en lysozyme élevé. Les souches utilisées ont montré une bonne capacité de développement dans le lait d'ânesse seulement après une phase d'adaptation. Les bactéries inoculées sont restées vivantes plus de trente jours dans les boissons obtenues, garantissant ainsi une longue durée de conservation. Au terme de l'essai, l'activité du lysozyme n'a présenté aucune variation sensible par rapport à l'activité initiale. L'analyse sensorielle à laquelle ont été soumises les boissons fermentées a permis de discriminer les différences induites par les trois souches utilisées en ce qui concerne quelques descripteurs relatifs au tableau aromatique et olfactif. Une technologie de production d'une boisson fermentée applicable à de petits ateliers artisanaux a donc été proposée, permettant d'obtenir un produit en mesure d'être accepté par un consommateur intéressé à la fois par les caractéristiques thérapeutiques du lait d'ânesse et les propriétés bénéfiques pour la santé des bactéries probiotiques.

lait d'ânesse / boisson fermentée / *Lactobacillus rhamnosus* / *Lactobacillus casei*

1. INTRODUCTION

The properties of equine milk differ from that of other mammals in many ways that include important differences in nutritional value. Moreover, its composition does not permit the production of cheese due to the presence of whey proteins that represent 35–50% of the nitrogen fractions [14, 20]. It also contains lysozyme, which is practically absent in the milk of cows, ewes and goats [5, 18]; this enzyme possesses bactericidal properties as it hydrolyzes the polysaccharides of bacterial cell walls and inhibits development.

Among various equine milks, the best known is the mare's milk that is traditionally produced and consumed by the nomadic peoples of Central Asia (Mongolia, Siberia and Kazakhstan) after lactic acid and alcoholic fermentation called Koumiss [16]. Even to date, Koumiss is the national drink of the people in that area and is also quite popular in countries bordering the Russian federation. It has been suggested that Koumiss has probiotic properties and has even been prescribed as a cure for patients with various diseases in Russian hospitals [17]. It is practically unknown in the rest of Europe and the Western world.

Donkey's milk has a composition similar to that of mare's milk [20], but is characterized by a higher lysozyme [7] content, which makes it somewhat selective with regards to the bacteria it can host. Its consumption, though extremely limited, has increased due to pediatric studies carried out over the last decade for the treatment of intolerance to cow's milk in young children [6, 8, 11]. It is not used, either fresh or after fermentation, in the diets of adults even if it has been suggested to have favorable pharmaceutical and nutritional properties [9, 10, 20]. Considering the numerous benefits of donkey's milk, including its health-promoting characteristics and probiotic effects, Coppola et al. [7] suggested the possibility of using donkey's milk for probiotic purposes. Accordingly, these authors investigated the fermentative properties of donkey's milk in addition to investigating the survival of selected *Lactobacillus rhamnosus* strains.

The aim of the present study was to establish the methodology for the production of a fermented beverage made from donkey's milk using only lactic acid bacteria with probiotic activity. This would allow for the retention of the beneficial properties of the milk in addition to maintaining the presence of microorganisms. We also evaluated the shelf life of the product with particular regard to the survival of the inoculated lactic acid bacteria and the resulting sensorial characteristics as a function of the bacteria used for fermentation.

2. MATERIALS AND METHODS

2.1. Milk

The milk was taken from a herd of around 150 donkeys in the province of Reggio Emilia, Italy. In particular, four jennets that

had given birth one month previously were selected and mechanically milked for 150 d. On each day, the first milking was carried out 4 h after the colt was removed and the second milking was 3 h later. The colt was then returned to its mother 2 h after the second milking. During 5 months of milking, a sterile milk sample (250 mL) was subjected to microbiological and physical-chemical analyses every month.

2.2. Starter cultures

The strains *Lactobacillus rhamnosus* AT 194 and CLT 2/2, isolated from Parmigiano Reggiano cheese, were from the Department of Agro-industrial, Environmental and Microbiological Sciences' (DISTAAM) collection (University of Molise), classified as probiotic [22]. The strain *Lactobacillus casei* LC 88, isolated from Parmigiano Reggiano cheese, was from the Department of Agri-food Protection and Improvement (DIPROVAL) at the University of Bologna. All strains were preserved in MRS agar (Oxoid, Unipath, Basingstoke, UK) at 4 °C.

To prepare starter cultures, cells were grown in 10 mL of MRS broth for 24 h, harvested by centrifugation (5000 rpm for 10 min), washed twice in a solution of 0.9 $g \cdot L^{-1}$ NaCl and re-suspended in 10 mL of sterile skimmed milk (Oxoid) at 37 °C for 4 h. Heat-treated (110 °C for 10 min) pasteurized whole milk (90 mL) was then inoculated with approximately 10^8 $cfu \cdot mL^{-1}$of each strain and incubated at 37 °C for 12 h prior to the next culture passage (pre-culture).

2.3. Production of fermented milk

After milking, the raw milk was poured into sterile 100-mL Sovirel flasks, pasteurized at 63 °C for 30 min, rapidly cooled at 37 °C, inoculated with different amounts of pre-culture (3 mL for the strain AT194, 4 mL for CLT 2/2 and 4 mL for *L. casei*) to obtain about 10^6–10^7 $cells \cdot mL^{-1}$, and incubated at 37 °C. The rate of fermentation was monitored by measuring the pH every 12 h after inoculation. As soon as a pH of 4.5–4.6 was reached, the flasks were chilled and placed in a refrigerator at 4 ± 0.2 °C. At the moment of inoculation ($t = 0$), upon reaching a pH of 4.5–4.6 ($t = 2$) and after 7, 15 and 30 d, microbiological analyses were carried out in order to evaluate the shelf life. Sensory characterization was carried out on the samples after 30 d of storage at 4 ± 0.2 °C.

2.4. Microbiological analyses

Serial dilutions of each sample in 0.9 $g \cdot L^{-1}$ NaCl were prepared and then plated in duplicate on agar plates [21]. Total aerobic bacteria were counted on PC Agar (Oxoid) incubated at 30 °C for 48 h; lactic acid bacteria were counted in MRS agar (Oxoid) in anaerobic jars for 5 d at 30 °C; thermoduric bacteria were counted after pasteurization (80 °C for 10 min) on PC Agar (Oxoid) incubated at 30 °C for 72 h. *Enterobacteriaceae* were counted after plating on Violet Red Bile Glucose Agar (Oxoid) after incubation for 24 h at 37 °C, while fecal coliform bacteria were plated on Violet Red Bile Lactose Agar (Oxoid) for 24 h at 42 °C.

2.5. Lysozyme content

The concentration of lysozyme was measured in milk before and after pasteurization, and in the fermented beverage during the storage phase at 7, 15 and 30 d after inoculation.

Lysozyme was measured as described by Lodi et al. [13], through the evaluation of lytic activity on *Micrococcus lysodeikticus* of the samples compared with standard purified enzyme solutions extracted from eggs. The method used is characterized by the linear relationship (semi-logarithmic curve) between the diameter of the zone of inhibition around the wells at which concentrations of lysozyme between 1 and 4 ppm were layered.

2.6. Chemical and physical analyses

Chemical and physical analyses were carried out using the standard methods: titratable acidity [19], pH was measured using a Hanna Instruments (Padova, Italy)

Table I. Main characteristics of raw donkey's milk (means and standard deviation for 5 milkings of 4 donkeys) in comparison with cow's milk [1].

		Donkey	*Cow*
Density at 20 °C		1.029 ± 0.02	1.032
pH		7.01 ± 0.07	6.68
Dry matter	$g \cdot kg^{-1}$	89.06 ± 0.31	125.0
Ash	$g \cdot kg^{-1}$	3.24 ± 0.24	8.0
Lactose	$g \cdot kg^{-1}$	67.31 ± 0.63	47.0
Fat	$g \cdot kg^{-1}$	2.82 ± 0.45	35.0
True protein	$g \cdot kg^{-1}$	15.88 ± 0.24	31.0

pHmeter, density at 20 °C by Quevenne's densitometer, dry matter was determined by the gravimetric method at 102 °C at constant weight, ash was determined in a furnace overnight at 550 °C, fat was measured using the Rose-Gottlieb method [2], and lactose was determined by the phenol sulfuric method [15]. Total nitrogen and nitrogen fractions were determined using the Kieltec Tecator System following the procedure described by Aschaffenburg and Drewry [3].

2.7. Sensory characterization

Sensory characterization of the fermented milks produced with the probiotic strains was performed by a group of 15 persons (7 male and 8 female). An evaluation card previously studied by trained tasters (staff from DIPROVAL highly familiar with fermented dairy products) was used for the assessment on the basis of a methodology suggested by Bérodier et al. [4] for cheeses. As suggested by the authors, the intensity of each descriptor was evaluated on an arbitrary scale with values from 1 to 7. For the evaluation of the significance of the resulting averages the ANOVA test was applied. The significance levels were set at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Physical-chemical traits of the raw milk

Table I shows the composition of donkey's milk used for production of the fermented beverage. Compared with cow's milk [1], which is the raw material most commonly used in the production of fermented milks, donkey's milk has a notably higher concentration of lactose and lower levels of fat and protein.

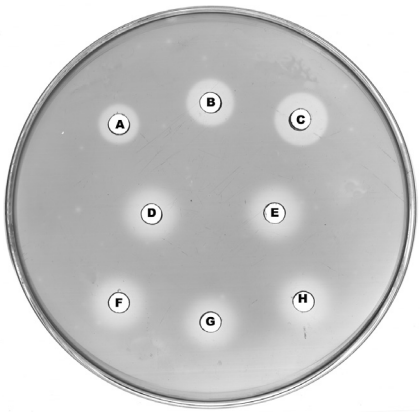

Figure 1. Plate diffusion for milk and donkey's milk fermented beverage. Lysozyme determination: wells A, B, C lysozyme standard solutions (1, 2, 4 ppm); wells D, E diluted milk, respectively before and after pasteurization at 63 °C for 30 min; wells F, G, H diluted fermented beverage 7, 15 and 30 d after inoculation.

From observation of the distribution of nitrogen fractions donkey's milk also has a lower protein content, and in particular casein (Tab. II). However, seroproteins represent 37% of the proteic content in donkey's milk compared with 17% in cow's milk. The richness in whey protein content of donkey's milk, as in all one-stomached animals, makes it more favorable for human nutrition [8, 11]. As expected, lysozyme was present at very high concentrations ($> 3\ g \cdot L^{-1}$). Pasteurization carried out at 63 °C for 30 min did not have any effect on the anti-microbial activity (Fig. 1, wells D and E). This finding is in agreement with those previously reported in donkey's milk [7, 12] and in horse's milk [20], confirming the elevated thermostability of the enzyme.

3.2. Microbiological and pasteurization traits

Bulk milk contained a low microbial content, which as affirmed by Coppola et al.

Table II. Nitrogen fraction distribution in donkey's milk (means and standard deviation for 5 milkings of 4 donkeys) in comparison with cow's milk [1].

		Donkey	*Cow*
Total N	$g \cdot L^{-1}$	2.56 ± 0.03	5.01
Non – casein N	$g \cdot L^{-1}$	1.40 ± 0.03	1.09
Caseinic N	$g \cdot L^{-1}$	1.15 ± 0.07	3.91
True whey protein	$g \cdot L^{-1}$	0.96 ± 0.03	0.84
Lysozyme	$mg \cdot L^{-1}$	3750.0 ± 250.0	0.09*
NPN	$g \cdot L^{-1}$	0.44 ± 0.03	0.25
Distribution total N			
Caseinic N	%	45.26 ± 2.05	78.0
True whey protein	%	37.24 ± 1.55	17.0
NPN	%	17.50 ± 1.32	5.0

* Mean of data reported by the author [1].

[7], can be attributed to the anti-bacterial activity of lysozyme. The raw milk used for production of the fermented beverage was nonetheless subjected to pasteurization (63 °C for 30 min) as an additional safety measure. The results achieved after heat treatment are shown in Table III.

3.3. Fermentations

The strains used showed remarkable fermentative vigor in cow's milk (data not shown), but also demonstrated difficulties at the adaptation stage when inoculated either in small quantities or directly inoculated into pasteurized donkey's milk. This could be overcome by using a 3% inoculum prepared in donkey's milk, previously treated at 110 °C for 10 min, for production of a fermented beverage containing viable cells in quantities greater than 10^8 mL^{-1}. The fermentations took place in a rapid and regular manner, as was also evident by the changes in titratable acidity, and were stopped by refrigeration after reaching pH values between 4.5 and 4.6 after about 48 h (Fig. 2).

3.4. Shelf life

After 2 days following inoculation, the microbial load was about 10^8 $cfu \cdot mL^{-1}$. One month after inoculation the lactic microbe content was still found to be above 10^8 $cfu \cdot mL^{-1}$, demonstrating that the strains used remained viable over time and even thrive, representing almost all of the bacterial content (Tab. IV). The presence of 10^8 $cfu \cdot mL^{-1}$ of probiotic lactic bacteria is sufficient to ensure the daily intake suggested by Vanderhoof and Young [23], even with limited consumption of the beverage. Enterobacteria and coliforms were completely absent. The activity of lysozyme measured after 7, 15 and 30 d did not show any significant changes with respect to raw milk and thermally-treated milk (Fig. 1, wells F, G and H).

Table III. Microbial counts (log $cfu \cdot mL^{-1}$) of donkey's milk before and after pasteurization (means and standard deviation for 5 milkings of 4 donkeys).

	Raw milk	Pasteurized milk 63 °C for 30 min
Total aerobic bacteria	4.24 ± 0.12	NR
Lactic acid bacteria	<1.00	NR
Thermoduric bacteria	<1.00	<1.00
Enterobacteriaceae	2.01 ± 0.07	NR
Fecal coliform bacteria	NR	NR

NR = none recovered.

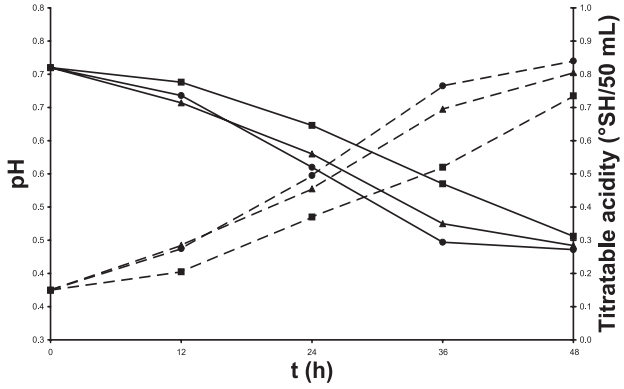

Figure 2. pH (continous line) and titratable acidity (dotted line) variation during fermentation of the three strains used.(●: *L. rhamnosus* AT 194; ■: *L. rhamnosus* CLT 2/2, ▲: *L. casei* LC 88).

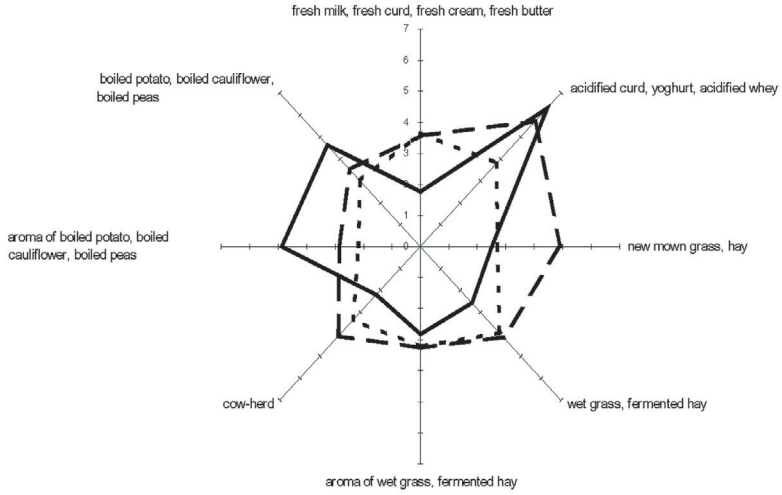

Figure 3. Presentation of the 8 descriptors (average of the evaluation by 15 consumers), which allows the differentiation of beverages fermented with the three strains (—— *L. rhamnosus* AT 194, ---- *L. rhamnosus* CLT 2/2, ····· *L. casei* LC 88).

3.5. Sensorial testing

The various taste analyses by the trained panel allowed the identification of the descriptors normally used for the testing of cheeses, which were most useful to describe the fermented beverage from a sensorial point of view. Evaluation of the fermented beverages was carried out according to a precise order: (1) visual characteristics; (2) odor; (3) aroma; (4) four basic tastes; (5) trigeminal sensations; (6) aftertaste and persistence; and (7) pleasantness.

On the basis of the table drawn up by the trained panel, the fermented beverages were evaluated by a group of 15 consumers. Table V contains each descriptor and the average of the taste evaluations. No significant differences were found among the beverages produced with the three strains of lactic bacteria in terms of the visual test, the four basic tastes, the trigeminal sensations, or the aftertaste and persistence. However, the individual strains did bring to light notable differences in some descriptors regarding the range of smells, as shown in Figure 3.

Table IV. Monitoring of microbic flora (log cfu·mL^{-1}) in fermented milks at different days (0, 2, 7,15 and 30 d).

	L . rhamnosus AT 194				
	0 d	2 d	7 d	15 d	30 d
Total aerobic bacteria	6.85	8.63	8.97	8.43	8.11
Lactic acid bacteria	6.77	8.54	8.94	8.40	8.06
Thermoduric bacteria	<1.00	<1.00	<1.00	<1.00	<1.00
Enterobacteriaceae	NR	NR	NR	NR	NR
Fecal coliform bacteria	NR	NR	NR	NR	NR

NR = none recovered.

	L . rhamnosus CLT 2/2				
	0 d	2 d	7 d	15 d	30 d
Total aerobic bacteria	6.27	7.61	8.64	8.11	8.07
Lactic acid bacteria	6.17	7.30	8.56	8.11	8.02
Thermoduric bacteria	<1.00	<1.00	<1.00	<1.00	<1.00
Enterobacteriaceae	NR	NR	NR	NR	NR
Fecal coliform bacteria	NR	NR	NR	NR	NR

NR = none recovered.

	L. casei LC88				
	0 d	2 d	7 d	15 d	30 d
Total aerobic bacteria	6.70	8.42	8.25	8.15	8.07
Lactic acid bacteria	6.10	8.28	8.14	8.00	8.02
Thermoduric bacteria	<1.00	<1.00	<1.00	<1.00	<1.00
Enterobacteriaceae	NR	NR	NR	NR	NR
Fecal coliform bacteria	NR	NR	NR	NR	NR

NR = none recovered.

Overall, the *L. casei* strain proved to be most capable of producing a beverage with a more balanced aromatic olfactory profile in the individual descriptors. The milk fermented with strain AT194 demonstrated a clear hint of boiled vegetables and acidic milk, whereas strain CLT 2/2 gave a higher average relating to the smell of fresh milk, grasses and animal odors.

Among the elementary tastes more commonly perceived, albeit at slightly different levels for each strain, were sweetness and acid. The fermented milk that was preferred from an organoleptic point of view by the 15 tasters was produced with strain AT194, which was also the best for production of the fermented beverage from donkey's milk.

3.6. Suggested technology

The tests carried out permit us to suggest production technology for a fermented beverage with probiotic strains that can be easily applied to a local setting in conjunction

Table V. List of descriptors and sensorial analysis of the fermented beverages with the three strains used. Values are means and standard deviation for $n = 15$ consumers.

	AT 194	CLT 2/2	LC 88
Visual characteristics			
Color (white)	5.45 ± 0.85	5.33 ± 0.98	5.33 ± 0.98
Homogeneity	4.90 ± 2.09	4.80 ± 1.85	4.95 ± 1.01
Grittiness	0.73 ± 2.02	0.73 ± 2.10	0.80 ± 1.42
Odor			
Fresh lactic	1.77 [a] ± 1.98	3.56 [b] ± 1.93	3.65 [b] ± 1.93
Acidified lactic	6.30 [a] ± 0.66	5.70 [b] ± 0.82	3.78 [c] ± 0.44
Grass	2.50 [a] ± 1.92	4.91 [b] ± 2.25	2.70 [a] ± 1.17
Fermented grass	2.56 [a] ± 0.52	4.12 [b] ± 1.48	3.92 [b] ± 1.33
Boiled vegetables	4.61 [a] ± 2.10	3.50 [b] ± 0.58	3.00 [b] ± 0.90
Toasted seeds	0.36 ± 0.78	0.58 ± 0.38	0.40 ± 0.81
Very toasted	0.71 ± 0.46	0.80 ± 0.60	0.90 ± 0.56
Cow-herd	2.19 [a] ± 1.02	4.09 [b] ± 1.91	3.35 [c] ± 1.78
Butyric, rancid	2.00 ± 0.35	2.20 ± 0.67	2.47 ± 1.05
Putrid, sulphuric, silage	0.44 ± 1.31	0.50 ± 1.27	0.56 ± 1.33
Aroma			
Fresh lactic	0.79 ± 0.46	0.78 ± 0.69	0.89 ± 0.66
Acidified lactic	5.30 ± 1.32	5.06 ± 0.75	4.96 ± 0.58
Grass	2.71 ± 0.46	2.75 ± 0.76	2.92 ± 0.94
Fermented grass	2.81 ± 0.52	3.25 ± 0.53	3.22 ± 0.51
Boiled vegetables	4.88 [a] ± 1.27	2.86 [b] ± 1.59	2.18 [c] ± 0.31
Toasted seeds	0.60 ± 1.04	0.50 ± 1.07	0.50 ± 0.98
Very toasted	0.75 ± 1.12	0.78 ± 1.39	0.89 ± 1.69
Cow-herd	1.89 ± 0.56	2.04 ± 0.46	2.24 ± 1.05
Butyric, rancid	2.57 ± 0.74	2.42 ± 0.64	2.42 ± 0.64
Putrid, sulphuric, silage	0.67 ± 1.07	0.70 ± 1.34	0.55 ± 1.21
Basic tastes			
Sweet	5.50 ± 1.35	5.36 ± 1.77	4.97 ± 1.45
Salty	0.10 ± 0.18	0.12 ± 0.30	0.09 ± 0.31
Acid	5.15 ± 0.83	4.85 ± 0.71	4.88 ± 1.10
Bitter	0.20 ± 0.60	0.20 ± 1.26	0.36 ± 1.15
Trigeminal sensations			
Astringent	0.46 ± 0.41	0.40 ± 0.25	0.30 ± 0.12
Aftertaste and persistence	2.86 ± 0.91	2.46 ± 1.11	2.22 ± 1.04
Pleasantness	5.11 [a] ± 1.62	2.96 [b] ± 1.49	2.17 [c] ± 1.15 [c]

Values in the same row with different letters are significantly different: $P < 0.05$.

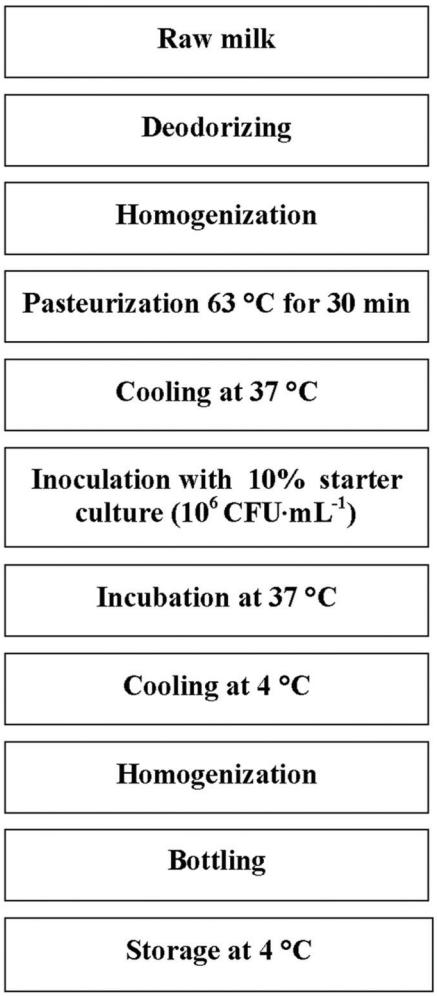

Figure 4. Technology proposed for the production of a beverage made from donkey's milk fermented with probiotic lactic acid bacteria.

with a donkey herd. The most delicate stage of the technique is adaptation of the bacterial strains to donkey's milk, which was overcome by adaptation in cow's milk and sterilized donkey's milk. After adaptation in donkey's milk, the strains were able to undergo rapid fermentation, especially following abundant inoculation.

After eventual deodorization and homogenization, the procedure outlined in Figure 4 entails thermal pasteurization at 63 °C for 30 min. Inoculation at 10% is done with previously obtained fermented donkey's milk. The fermentation takes place at 37 °C and its progress is followed by measuring the pH. Upon reaching pH 4.5–4.6, fermentation is stopped by refrigeration at 4 °C, which may be followed by homogenization, bottling, and storage at 4 °C. The process can be easily performed by small businesses and does not require complex machinery.

4. CONCLUSION

Donkey's milk can be easily used for the production of a beverage fermented with probiotic strains of lactic bacteria that retain a high degree of viability after fermentation and storage. Such a product is obtained by setting up appropriate measures during inoculation that allow the bacterial cultures to replicate despite the high concentration of lysozyme present in the raw material. Moreover, strains of lactic bacteria that confer pleasant sensorial traits to the fermented product are used.

In fact, using the described procedure, a product is obtained that would be well accepted from a sensorial standpoint by consumers looking for the therapeutic qualities of donkey's milk integrated with probiotic bacteria. While the method described within is suitable for small-scale production, in the case of industrial production it would be worthwhile to apply additional procedures such as the standardization of the raw material, homogenization, and the addition of flavorings such as fruit juice.

The data obtained confirms that donkey's milk is a possible basis for a fermented beverage as it contains several advantageous qualities, such as low microbial activity and high amounts of lysozyme, as well as being a vehicle for the consumption of probiotic bacteria.

Further studies may be warranted in order to select other bacterial strains with probiotic properties that are even better adapted to donkey's milk.

Acknowledgements: We thank G. Borghi and his family, Azienda Agrituristica Montebaducco, Salvarano di Quattrocastella (Reggio Emilia, Italy) for providing the milk used in this trial.

REFERENCES

[1] Alais C., Science du lait. Principes des Techniques Laitières, 4th edn., Sepiac, Paris, France, 1984.

[2] AOAC, Official methods of analysis of the association of official analytical chemists, 13th edn., Washington, DC, USA, 1980.

[3] Aschaffenburg R., Drewry J., New procedure for the routine determination of the various non casein proteins of milk, in: 15th Proc. Int. Dairy Congress, London, England, 1959, pp. 1631–1637.

[4] Bérodier F., Zannoni M., Herrero L., Lavanchy P., Casals J., Adamo C., Guide to the smell, aroma and taste evaluation of hard and semi-hard cheese, European Research Program AIR-CT94-2039, GECOTEFT, Poligny, France, 1997.

[5] Blanc B., Les protéines du lait à activité enzymatique et hormonale, Lait 62 (1982) 350–395.

[6] Carroccio A., Cavataio F., Montalto G., D'Amico D., Alabrese L., Iacono G., Intolerance to hydrolysed milk proteins in infants: clinical characteristics and dietary treatment, Clin. Exp. Allergy 30 (2000) 1598–1603.

[7] Coppola R., Salimei E., Succi M., Sorrentino E., Nanni M., Ranieri P., Belli Blanes R., Grazia L., Behaviour of *Lactobacillus rhamnosus* strains in ass's milk, Ann. Microbiol. 52 (2002) 55–60.

[8] Curadi C., Giampietro P.G., Lucenti P., Orlandi M., Use of mare's milk in pediatric allergology, in: 14th Proc. ASPA Congress, Firenze, Italy, 2001, pp. 647–649.

[9] Doreau M., Le lait de jument, Prod. Anim. 4 (1991) 297–302.

[10] Herrouin M., Mollé D., Fauquant J., Ballestra F., Maubois J.L., Léonil J., New genetic variants identified in donkey's milk whey proteins, J. Protein Chem. 19 (2000) 105–115.

[11] Iacono G., Carroccio A., Cavataio F., Montalto G., Soresi M., Balsamo V., Use of ass's milk in multiple food allergy, J. Pediatr. Gastroenterol. Nutr. 14 (1992) 177–181.

[12] Jauregui-Adell J., Heat stability and reactivation of mare milk lysozyme, J. Dairy Sci. 58 (1975) 835–839.

[13] Lodi R., Todesco R., Carini S., Indagine preliminare sull'impiego del lisozima nella tecnologia del formaggio Provolone, Ind. Latte 20 (1984) 37–50.

[14] Malacarne M., Martuzzi F., Summer A., Mariani P., Protein and fat composition of mare's milk: some nutritional remarks with reference to human and cow's milk, Int. Dairy J. 12 (2002) 869–877.

[15] Marier J.R., Boulet M., Direct analysis of lactose in milk and serum, J. Dairy Sci. 42 (1959) 1390–1391.

[16] Montanari G., Zambonelli C., Grazia L., Kamesheva G.K., Shigaeva M.K.H., *Saccharomyces unisporus* as the principal alcoholic fermentation microorganism of traditional Koumiss, J. Dairy Res. 63 (1996) 327–331.

[17] Nassal J., Rembalski C., Hygienische Forderungen bei der Produktion von Stutenmilk und Kumyß, Arch. Lebensm. Hyg. 31 (1980) 209–213.

[18] Pagliarini E., Solaroli G., Peri C., Chemical and physical characteristics of mare's milk, Ital. J. Food. Sci. 5 (1993) 323–332.

[19] Politi I., Chimica del latte e dei latticini, Centro Sperimentale del Latte, Milano, Italy, 1958.

[20] Solaroli G., Pagliarini E., Peri C., Composition and nutritional quality of mare's milk, Ital. J. Food. Sci. 5 (1993) 3–10.

[21] Speck M.L., Compendium of methods for the microbiological examination of food, American Public Health Association, Washington, D.C., USA, 1984.

[22] Succi M., Sorrentino E., Coppola R., Nanni M., Chiavari C., Grazia L., I formaggi a lunga stagionatura prodotti con latte crudo: una fonte di batteri utili, in: Proc. Conference on "The cheese: technology, ripening and characterization", Parma, Italy, 2002, pp. 89–98.

[23] Vanderhoof J.A., Young R., Use of probiotics in childhood gastrointestinal disorderes, J. Pediatr. Gastroenterol. Nutr. 27 (1998) 323–332.