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Research Article

Probiotic, Antimicrobial, Antioxidant and Sensory properties of Fermented donkey milk with Lactobacillus fermentum ME-3 and Lactobacillus acidophilus (ATCC 4356)

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Abstract

The aim of this study was to investigate the ability of the probiotic *L.fermentum* ME-3 and *L.acidophilus* (ATCC4356) to ferment pasteurized donkey's milk, keeping it in cold storage for 14 days and testing end products for antimicrobial, antioxidant activities and sensory properties. Fermented donkey milks had pH values of 4.5 - 4.6 just after fermentation and they maintained high bacterial counts (8.5 log cfu ml^{-1}) during cold storage for 14 days. The antimicrobial activity of *L.fermentum* ME-3 milk was highest against *E.coli* NCTC12241, while *L.acidophjlus* (ATCC4356) milk showed a greater zone of inhibition against *Salmonella typhimurium* NCTC120123. Lysozyme activity of raw, pasteurized and fermented milks remained high and values did not significantly differ. The antioxidant activity (FRAP) of fermented *L. acidophilus* (ATCC4356) was highest in Day 1 (65.4±4.7 μmol^{-1}) when compared to *L.fermentum* ME-3 (46.0±5.1 μmol^{-1}). *L.fermentum* ME-3 milk was assessed ($p<0.05$) as more refreshing, "light" and less viscous than *L.acidophilus* (ATCC4356) milk.

Keywords: donkey milk; fermentation; lactic acid bacteria; antimicrobial activity; antioxidant activity

Introduction

The Donkey (Equus asinus) belongs to the taxonomic order Perissodactyla, family Equidae and genus Equus and were first reported in North Africa in 6000-7000BC and later in 2000BC in Europe (Spain and Italy). In Cyprus, two donkey breeds are present namely; the dark brown and the grey donkey. The dark brown donkey accounting for the 80% of the donkey population in Cyprus is believed to originate from breeds in Western to South-Western France; it is quite distinctive because of its size (1.4-1.8m height, 300kg weight). The smaller grey donkey is almost certain to have originated from Africa [24].

Donkey milk has been used in the past as an alternative to human (breast) milk for infants or children mainly because of its similarities in nutrient composition, hypoallergenicity, immune homeostasis conditions, and antimicrobial activity [29, 23, 27] also state that in Africa (Ethiopia) donkey milk was used for treating whooping cough and other child ailments.

Recent research on donkey's milk has highlighted the need for preserving this valuable milk. It is marketed in some countries (i.e. Italy, Belgium, Netherlands) both as raw milk directly sold from the farm (to be consumed within 48h of milking) and heat treated (i.e. pasteurized, ultra-high temperature). Freeze-dried powder for human consumption (including infants), is also marketed.

Koumiss (Kumis) is a fermented dairy product traditionally made from mare's milk, usually found in the Central Asian steppes. The same product is known as airag in Mongolia. Koumiss and other fermented horse milk products are reported for their therapeutic and nutritional properties [33].

[30] have reported that the sales of fermented milk beverages have risen to $16 million in 2010 accounting for the 80% of the total sales of the functional dairy food market, and also highlight their health benefits (i.e. antitumor activity, prevention of gastrointestinal infections, preventing of atherosclerosis).

The objective of this study was to produce fermented milk by adding selected probiotic lactic acid bacteria in pasteurized donkey's milk and subsequently assessing the microbiological, physicochemical, sensory characteristics and functional properties of the product.

Materials and Methods

Milk Collection

The milk was collected from the farm Golden Donkeys in Larnaca, Cyprus. In particular, sampling was conducted from March to October 2013 and a total of eleven samples were collected by manually milking 8-10 Jennies. The milk samples were collected in sterilized 250 ml containers, placed in cool-boxes and immediately transported to the laboratory at 4-6°C. The samples were subjected to microbiological analysis, and pasteurization (for starter culture production) same day.

Raw donkey milk quality

Serial dilutions of each sample in physiological solution (0.85% NaCl, w/v) were prepared and then plated in duplicate on agar

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plates. Total aerobic bacteria were counted on PC Agar (Merck, Darmstadt, Germany) incubated at 30 °C for 72h (ISO 4833-2:2013); lactic acid bacteria were counted on MRS agar (Oxoid, Basingstone, Hamshire, UK) in anaerobic jars for 72h at 37 °C, on acidified MRS (pH 5.7) (Oxoid) in anaerobic jars for 72h at 30 °C and on M17 agar (Oxoid) for 48h at 45 °C and then for 4d at 20°C [15] ; *Enterobacteriaceae* were counted after plating on Violet Red Bile Glucose Agar (BD, Franklin Lakes, NJ USA) after incubation for 24 h at 37 °C [18], and Staphylococci were plated on Baird Parker Agar (Himedia) for 48h at 37°C [16]. Yeast and Moulds were counted on Dichloran Rose Bengal Chloramphenicol (DRBC) (BD) incubated at 25°C for 5 days [19]; Listeriamonocytogenes was detected by using the ISO 11290-1:1996 and *Salmonella spp* was detected by using the [17]. Anaerobic conditions were achieved by using CampyGensachets (Oxoid).

Chemical composition of raw donkey milk

Chemical analyses of raw donkey milk were performed by using standard methods i.e. total nitrogen content [22], fat (ISO 2246:2008), ash (AOAC 945.46), acidity (AOAC 947.05) and pH (HANNA, Woonsocket, RI, USA).

Milk fermentation

The strains *Lactobacillus fermentum* ME-3 and *Lactobacillus acidophilus* (ATCC 4356), were used for the production of the fermented milk(s). The selection of the strains was based on their properties (probiotic, antimicrobial and/or antioxidant). *L. fermentum* ME-3 (owning all 3 properties and previously used in dairy products [31] was kindly offered by the University of Tartu (Estonia), while the probiotic culture of *L. acidophilus* (ATCC 4356) was purchased from the CCUG (Gothenburg, Sweden) as freeze-dried ampoules. All strains were preserved in MRS agar (Oxoid) at 4°C.

To prepare the starter cultures; 25ml pasteurized donkey milk (63°C/30 min) was inoculated by approximately 10^8cfu ml^{-1} of *L. fermentum* ME-3 και *L. acidophilus* (ATCC 4356). The milks were incubated at 37°C until the pH value reached 4.6. To prepare fermented milks; pasteurized milk samples were immediately placed in an ice bath, rapidly cooled to 37°C and subsequently inoculated with (5%,v/v) of the mother culture of each of the starter culture (10^8cfu ml^{-1}) and incubated at 37°C.

The rate offer mentation was monitored by measuring pH every 2 h after inoculation. Acidity (% lactic acid) was also determined during fermentation. At a pH value of 4.6–4.7, the milk was immediately placed in a refrigerator at 4 ± 0.2 °C. This procedure was replicated thrice.

Bacterial Counts

Enumeration of bacteria was performed for starter cultures used and during cold storage of the fermented products at 4 ± 0.2 °C (day 1, 7 and 14) in order to evaluate the shelf life and the viability of lactic acid bacteria during storage. Total aerobic bacteria, lactic acid bacteria (Lactobacilli), *Enterobacteriaceae*, *Staphylococci*, and yeasts and moulds were enumerated as described above.

Proteolytic activity

The proteolytic activity of the *L. fermentum* ME-3 and *L. acidophilus* (ATCC 4356) was determined using a spot cultivation test in anaerobic conditions [26]. Spot cultivation tests were conducted under anaerobic conditions: 10 μL of the selected lactic acid bacteria were spotted on skim milk agar medium (5%, 10%, 15% and 20% w/v) and incubated at 37°C for 48 h. The diameter of the clear zone surrounding each culture was recorded.

Antimicrobial assay

The agar well diffusion assay was performed as described by [12]. The antimicrobial activity of the following samples a) fermented donkey milk with *L. fermentum* ME-3, b) fermented donkey milk with L. acidophilus (ATCC 4356), c) raw donkey milk, d) overnight culture of *L. fermentum* ME-3 and e) overnight culture of *L. acidophilus* (ATCC 4356) against three bacterial strains (*Salmonella typhimurium* NCTC 12023, *Staphylococcus aureus* NCTC 12981, *Escherichia coli* NCTC 12241) were tested.

The antibacterial activity (zone of inhibition; zoi) represented the difference between the diameter of the clear zone surrounding wells containing the antimicrobial agents and that of the control sample. A diameter >1mm around the well was considered as a positive result. It was assumed that the greater the diameter of the zoi, the greater the antimicrobial activity of the sample.

Lysozyme activity

Lysozyme activity was determined by the EnzChek Lysozyme Assay Kit (Life Technologies, Carlsbad, CA, USA) in raw and fermented donkey milk. The assay is based on the assessment of the lytic activity of lysozyme in the cell walls of Micrococcus lysodeikticus which have been labeled with the fluorescent dye fluorescein. The test was performed according to the manufacturer's instructions.

Antioxidant activity

The antioxidant activity of prepared [34] fermented milk samples was investigated by the ferric reducing antioxidant power (FRAP) assay as described by [4]. The antioxidant activity was expressed as Trolox Equivalent Antioxidant capacity (TEAC, μmol^{-1}). All determinations were carried out in triplicate from 3 independent experiments.

Organoleptic characteristics

The sensory assessment of the final products was conducted with a panel of 31 assessors (13 men and 18 women) from age of 20 to 65 years old according to the Quantitate Descriptive Analysis (QDA) as described by [25]. The panelists were all members of the Department of Agricultural Sciences, Biotechnology and Food Science and they were formally trained prior to tasting and completion of the questionnaire. The panelists were served approximately 30 mL of each sample (8°C) in disposable clear 50 ml PET bottles, coded with three-digit random numbers. Potable water at room temperature was used to rinse the palate prior to and between samplings. The panelists assessed the fermented milk products presented in monadic form and quantified the perceived intensities of each attribute using a 10 cm unstructured scale ranging from 0 (low) to 10 (high). The attributes evaluated were the following: appearance (colour), aroma (odour), taste (acidity), mouthfeel (refreshing, "light", viscosity) and after taste. As the probiotic fermented donkey milks could be proposed as new products therefore the panel was also asked to assess the overall acceptance (on a 9 point hedonic scale), purchasing possibility, and purchasing possibility after learning about product's functionality.

Statistical Analysis

Data obtained were statistically analysed (SPSS Statistics 20, IBM, Armonk, New York, USA) by t-test (t-Test: Paired Two Sample for Means) to determine whether ($p<0.05$) variation occurred among the means of each treatment.

Results and Discussion

Donkey milk's microbiological characteristics

Raw donkey milk used in the experiments was characterized by a very low bacterial load i.e. total aerobic bacteria 3-4 log cfu ml^{-1}, an absence of pathogens (i.e. *Listeria monocytogenes*, *Salmonella spp.*, *Staphylococcus aureus*, *Enterobacteriaceae*) and spoilage bacteria (yeasts and moulds). Lactic acid bacteria represented the major microbial population (Fig. 1). Similar findings are reported by [10].

Donkey milk's chemical characteristics

Donkey milk is characterized by low fat and protein contents, a high lactose content high pH value and low acidity (Table1).The results are comparable to the work presented by [29], while [7] states that mare milk from Arabia has higher pH and lower acidity than bovine milk due to the lower concentration of casein and phosphate. The chemical composition of donkey milk is considered as favorable for the production of fermented milks (i.e. low fat and low protein) [30].

Table 1. The gross chemical composition of raw donkey milk

Parameter	Value (g $100ml^{-1}$)
Total protein content	0.77 ± 0.05
Fat	0.50 ± 0.07
Ash	0.34 ± 0.01
Acidity (% lactic acid)	0.09± 0.04
pH	7.30± 0.16

Results are the means ± (SD) of eleven milk samples

Milk fermentation and cell viability during cold storage of fermented milks

L. fermentum ME-3 fermented pasteurized donkey milk to pH 4.7 (0.31% w/v lactic acid) in 12h, while *L. acidophilus* (ATCC 4356) presented a faster fermentation (9h) at pH 4.7 (0.34% w/v lactic acid). The results showed that a day after inoculation, the population of lactic acid bacteria in fermented milks were 9.02 log cfu ml^{-1} for L. fermentum ME-3 and 8.78 log cfu ml^{-1} for *L. acidophilus* (ATCC 4356). At the end of storage (14 days) the population of lactic acid

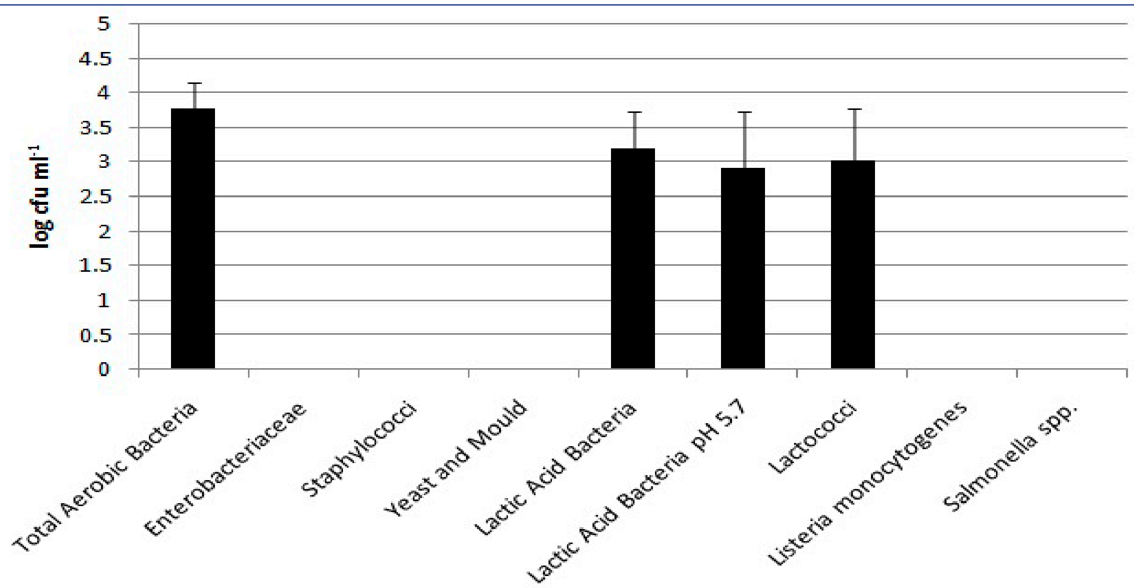

Figure 1. Total microbial counts of raw donkey milk. Error bars show standard deviation (SD) of triplicate measurements of eleven raw milk samples.

bacteria remained high; 8.53 log cfu ml^{-1} for *L. fermentum* ME-3 and 8.59 log cfu ml^{-1} for *L. acidophilus* (ATCC 4356) indicating that the strains were viable over time, representing almost exclusively the total aerobic bacterial population. *Enterobacteriacae*, *Stapylococci* and yeast and moulds were completely absent. In a similar study, [11] used donkey's milk to produce *L.rhamnosus* probiotic fermented milk. They also reported high counts of the lactobacillus used for 15 days at 4°C and pH values of 3.7-3.8. Additionally, [34] added two different probiotic strains in donkey's milk and after lyophilization the cultures remained viable for 2 months reaching counts of 2x10^{8}cfu g^{-1}.This illustrates that donkey milk is a very good medium for starter cultures (low initial aerobic counts), high lactose content, while lysozyme does not inhibit the growth of lactic acid bacteria.

According to the [9] the composition of fermented milk must satisfy the following criteria: (a) less than 10% (w/v) fat (b) minimum titratable acidity (% lactic acid) 0.3% (w/v) and (c) total bacterial count in starter culture >10^{6} cfug(or ml)$^{-1}$. Results obtained for fermented donkey milk in this study satisfy all of the above.

Proteolytic (Caseinolytic) Activity

Determination of the proteolytic capacity of *L. fermentum* ME-3 and *L. acidophilus* (ATCC 4356) was performed on agar plates with skim milk at different concentrations (5, 10, 15 and 20% w/v). The protein (casein) hydrolysis was observed by the production of clean clear zones around isolated colonies of the two microorganisms. The greatest proteolytic activity observe don plates supplemented with 5% skim milk agar, with a clear zone of 10.5 ± 0.8mm and 13.8 ±1.8 mm for *L. fermentum* ME-3 and *L. acidophilus* (ATCC 4356), respectively. The 10% skim milk agar, there was a reduction on the proteolytic activity with *L. fermentum* ME-3 and *L. acidophilus* (ATCC 4356) giving a clear zone of 3.0 ± 0.0 mm and 5.3 ± 0.8mm respectively. Both lactic acid bacteria didn't show any visible proteolytic activity on plates with skim milk concentrations of 15 and 20% (w/v). [6] tested the proteolytic activity of *L. helveticus* M92 και other probiotic lactic acid bacteria, including *L. acidophilus* (ATCC 4356) which showed apotent proteolytic (caseinolytic) activity on 2% (w/v) skim milk agar. The proteolytic activity of *L.fermentum* ME-3 described as weak [32].

Antimicrobial activity

The agar well diffusion method was used to assess the antimicrobial activity of the selected LAB and their corresponding fermented milks (at day 14 of cold storage). Their antimicrobial properties were tested against three major food-borne pathogens namely *E. coli* NCTC 12241, *S. aureus* NCTC 12981, and *Salmonella typhimurium* NCTC 12023.

Raw donkey milk showed the highest antimicrobial activity (19mm) against *Salmonella typhimurium* NCTC 12023, followed by *L. acidophilus* (ATCC 4356) (9.5mm) and *L. fermentum* ME-3 (7mm). Corresponding fermented milk products showed inhibition zones against *Salmonella typhimurium* NCTC 12023 of (13 mm) for *L. fermentum* ME-3 milk and (10.5 mm) for *L. acidophilus* (ATCC 4356).

Antimicrobial activity against *E. coli* NCTC 12241 (Fig.4) was highest in both the model organism *L. fermentum* ME-3 (19mm) and the corresponding fermented milk product (19 mm). *L. acidophilus* (ATCC 4356) showed antimicrobial activity (13.5 mm) against the pathogen *E. coli* NCTC 12241while this activity was increased to (17 mm) when the corresponding fermented milk product was tested. Raw donkey milk produced an inhibition zone (10.5mm) against *E. coli* NCTC 12241.

The lowest antimicrobial activity i.e. zoi 2mm, was recorded against *S. aureus* NCTC 12981 by raw donkey milk, while lactic acid bacteria and their corresponding fermented milks did not show any activity.

The above results reveal that donkey milk; both raw and fermented, and probiotic lactobacilli cultures have antimicrobial activities against specific food pathogens. [36] reported that donkey milk had demonstrated antimicrobial activity against Salmonella choleraesuis and Shigelladysenteriae. Similarly, in our results raw milk exhibited the highest antimicrobial activity against *Salmonella typhimurium* NCTC 12023. This finding could also be linked with the fact that *Salmonella spp* was not isolated from raw donkey milk samples as seen in Fig. 1.Tidonaet al. (2011) showed that donkey milk digested (with gastrointestinal enzymes) had antimicrobial effect against *E.coli*. Likewise, the *E.coli* NCTC 12241 strain we tested was inhibited both by the raw and the fermented milk (Fig. 2). Additionally the fermented milks showed an increase (in mm) of the zones of inhibition, mainly because of the fact that the presence of biomolecules released during the proteolysis (either by lactic acid bacteria or human enzymes) may further contribute to the antimicrobialactivity of donkey milk (Nazzaroet al., 2010; Tidonaet al.,2011). The antimicrobial activity of *L.fermentum* ME3 against *Salmonella typhimurium*, *E. coli* and *S. aureus* was reported by [31]. The above are partly in accordance to our results as we did not observe an antimicrobial activity by the lactobacilli or their fermented milks against the *S. aureus* strain we tested. On the contrary, raw donkey milk showed a minor (2mm) zoi against *S.aureus*.

Lysozyme Activity

Lysozyme exerts its antibacterial activity against a number of bacteria catalyzing the hydrolysis of β1→4 glycosidic bonds between N-acetyl muramic acid (Mur2Ac) and N-acetyl glucoseamine (GlcNAc) in the bacterial cell walls polysaccharides [13]. The activity of lysozyme was determined in donkey milk prior and after pasteurization, as well as in the fermented milks during cold storage. In order to compare the lysozyme activity in donkey milk, cow, goat and sheep milk's activity was also determined.

The lysozyme activity in raw and pasteurized donkey milk was

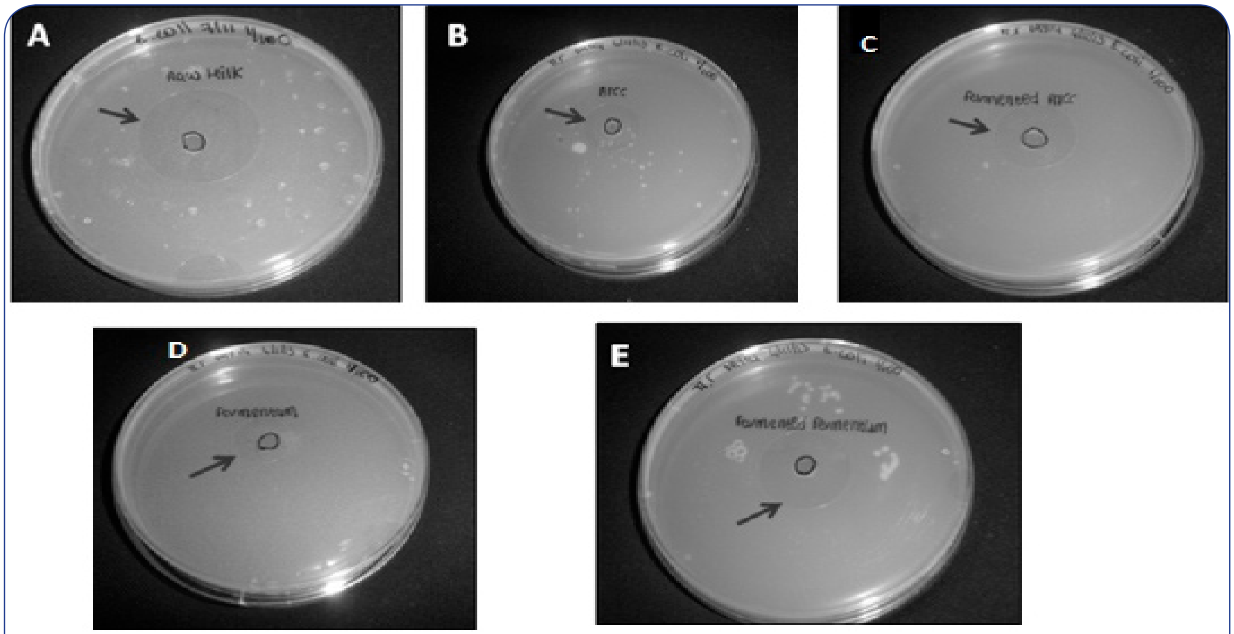

Figure 2: The clear zones of inhibition (zoi) of E. coli NCTC12241 by raw donkey milk (A), L. acidophilus (ATCC 4356) (B), fermented donkey milk with L. acidophilus (C), L. fermentum ME-3 (D) and fermented donkey milk with L. fermentum ME-3.

similar, with a value of 60372 and 61383 U ml^{-1}, respectively. Similar to our results [8] stated that pasteurization of donkey milk at 63°C/30 min had no effect on lysozyme's antimicrobial activity, while Polidori and Vincenzetti (2010) described donkey's milk lysozyme as a thermostable enzyme, since its thermal denaturation starts when thetemperature reaches 70°C.

Comparing the lysozyme activity of raw donkey milk with the other milks (cow, goat and sheep) donkey milk's activity is approximately 200 fold higher (Papademas, unpublished data). [29] reported that lysozyme concentration cow's milk was found in traces. Lysozyme activity of both fermented milks remained high during cold storage (14 days) and no statistically significant differences were determined either between the samples or during cold storage (Table 2). Tidonaet al. (2011) described that lysozyme resists degradation by gastric enzymes and acid hence reaching the gut relatively intact to exert its antimicrobial activity. Polidori and Vincenzetti (2010) also reported that lysozyme in fresh donkey's milk retained completely its activity when the milk was stored for 1 month at 4°C.

Antioxidant activity

The antioxidant activity of fermented milks inoculated with *L. fermentum* ME3 and *L.acidophilus* (ATCC 4356) strains was determined with respect to reducing power of fermented milks. The assay results shown in Table 3 indicate that the milks fermented with the strains of *L. acidophilus* (ATCC4356) and *L. fermentum* ME-3 presented higher antioxidant activity (65.4±4.7, 46.0±5.1μmol^{-1}, respectively) when compared to that of raw donkey milk (31.2±3.1μmol^{-1}).The fact that proteolytic enzymes of lactic acid bacteria hydrolyse milk proteins to smaller peptides of biological activity is well established [1].

It was also noted, that fermented milk inoculated with *L. acidophilus* (ATCC4356) showed higher antioxidant activity at Day 1 which decreases during storage at 4°C, while the antioxidant activity of *L. fermentum* ME-3 fermented milk, increased during storage. [35] described the antioxidant activity of selected lactic acid bacteria including *L.acidophilus* (ATCC 4356), during milk fermentation for 24h reporting that *L.acidophilus* (ATCC 4356) had a high

Table 2. Lysozyme activity (U ml $^{-1}$) of fermented donkey milk with 2 different starter cultures during cold storage

	Storage (days)		
Samples	1	7	14
Fermented donkey milk with *L. acidophilus* (ATCC 4356)	64859.86 ± 6857.45	57111.42± 6025.87	54804.64± 6808.36
Fermented donkey milk with *L.fermentum* ME-3	53575.76± 6277.07	61975.03± 4275.30	58646.57± 2131.83

The results are means ±(SD) of triplicate measurements of three independent experiments. The results show no statistical difference (p<0.05).

proportion of peptides, representing a molecular mass of 4–20 k Da. The development of 4–20 k Da peptides was found to correlate well with high antioxidant capacity.

The fact that *L. acidophilus* (ATCC 4356) is more proteolytic than *L. fermentum* may explain the fact that at Day 1 the *L. acidophilus* (ATCC 4356) milk showed higher antioxidant activity which remained high (but declining-not statistically significant) during cold storage to day 14. On the contrary, *L. fermentum* ME 3 showed an increasing antioxidant activity during cold storage and the values between the Day 1 and Day 14 were statistically significant ($p<0.05$) (46.0±5.1 to 53.2±2.6μmol^{-1}, respectively). The weak proteolytic activity of *L.fermentum* ME 3 [31], may have resulted in a late hydrolysis of milk casein fractions to bioactive peptides (4-20 k Da), thus reaching higher values at day 14 in cold storage.

In general, [35] highlighted that higher antioxidant capacityis obtained when milk is fermented with mixed cultures of LAB rather than milk fermented with single bacterial strains. Moreover, it was stated that the development of antioxidant activity was dependent on the strain used.

Organoleptic Characteristics

Fermented milk produced with *L. fermentum* ME-3 was rated as more refreshing and "light" and less viscous than the *L.acidophilus* (ATCC 4356) fermented milk. The two samples did not significantly differ in sensory attributes such as colour; odour; acidity and after taste (Table 4). Therefore, the sensory profile of both fermented milks could be described as products of white colour, with a neutral odour and aftertaste, mild acidity, quite refreshing and "light", with none apparent viscosity (Table 4).Regarding the acceptance testing, results showed that 45% of the panelists for both products had a positive (like extremely, like very much, like moderately, like slightly) opinion; 32% and 38% had a neutral (neither like nor dislike) opinion; 23% and 16% had a negative (dislike extremely, dislike very much, dislike moderately, dislike slightly) opinion for *L.fermentum* ME-3 milk product and *L.acidophilus* (ATCC 4356) milk product, respectively. Additionally, when the panelists were asked whether they would purchase the products; 19% and 22% answered "yes", 48% and 45% answered "probably" for *L.fermentum* ME-3 and *L.acidophilus* (ATCC 4356) while 32% were negative for both products. Moreover, once the panelists were asked if they would purchase the product if they knew of product's functionality (i.e. probiotic) there was a major shift in the "yes" answer by 32% and 28 % for *L.fermentum* ME-3 καιτον *L. acidophilus* (ATCC 4356). In general, more than half of the panelists who had a negative opinion on purchasing the products changed their answer to "yes" or "probably".

This finding illustrates that consumers will appreciate a product that might be related to health benefits (added-value) and highlights the need for revealing possible functional properties of fermented milks. The above statement is supported by Annunziata and Vecchio (2013) where they concluded that their study confirmed the importance of health claims in gaining consumer acceptance of functional foods. Additionally they mention that carriers (i.e. dairy products) of functional ingredients have the largest relative importance in consumers' valuation of functional foods with probiotics as the functional component.

Table 3. Trolox Equivalentantioxidant capacity (TEAC) measured by the FRAP method of raw and fermented donkey milk during cold storage

	TEAC (μmol^{-1})		
	Storage (Days)		
Samples	**1**	**7**	**14**
Fermented donkey milk with *L. acidophilus* (ATCC 4356)	65.4^{a} ± 4.7	57.3^{a} ± 3.2	49.1^{a} ± 3.0
Fermented donkey milk with *L.fermentum*ME-3	46.0^{a} ± 5.1	46.6^{ab} ± 1.6	53.2^{b} ± 2.6

The results are means ± (SD) of triplicate measurements of three independent experiments. Different letters indicate significant difference within rows ($p<0.05$)

Table 4. Sensory evaluation of fermented donkey milks produced with different starter cultures

Sensory attributes	**Fermented donkey milk**		**Statistics**
	***L. acidophilus* (ATCC 4356)**	***L.fermentum* ME-3**	***p-value* (<0.05)**
Colour	1.24 ± 1.17	1.49 ± 1.67	NS
Odour	4.68 ± 2.08	4.55± 2.37	NS
Acidity	3.65± 2.58	4.02 ± 2.58	NS
Refreshing	5.20± 2.29	6.11 ± 2.01	*
"Light"	6.37± 2.14	7.32 ± 2.14	*
Viscosity	2.99± 2.36	1.75 ± 1.66	*
After-taste	4.18 ± 2.32	4.79 ± 2.50	NS

The results are the means ± (SD), statistically different means ($p<0.05$) are denoted by the asterisk (*), NS = non-significant

Conclusion

Both strains of the probiotic lactic acid bacteria used in this study i.e. *L. fermentum* ME-3 and *L.acidophilus* (ATCC 4356) were able to grow well in pasteurized donkey milk reaching bacterial counts of 8-9 log cfu ml^{-1}, producing an acceptable to tasters fermented milk. Moreover, high bacterial counts were maintained during cold storage of the fermented milk products for 14 days, thus qualifying it to be labeled "probiotic fermented milk". The high lysozyme activity of donkey's milk did not hinder the growth of the lactobacilli used, while it probably contributed to the antimicrobial activity to pathogens tested i.e. *Salmonella typhimurium* NCTC 12023, *Escherichia coli* NCTC 12241. The antioxidant activity of the fermented milks was also determined illustrating that potential activity of fermented milk products is strictly strain specific.

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