Scientific Reviews & Chemical Communications

Research | Vol 7 Iss 3

A Preliminary Study on the Development of Donkey Milk Based Fermented Product

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Received: November 10, 2017; **Accepted:** January 15, 2018; **Published:** January 19, 2018

Abstract

Donkey milk's nutritional properties make it similar to human milk more than any other mammal milk, it is rich in antimicrobials and it has also been classified as hypoallergenic. Nowadays, donkey milk is again under investigation for the exploration of its functions. Due to its high price and its low fat, donkey milk is not considered ideal for commercial use especially for fermented products but due to the increased awareness for allergies it could be an excellent alternative. The aim of this study was to explore ways of producing a yoghurt-style product from donkey milk using only traditional material and methods. Donkey milk was used alone and in combination with other milks and also different cultivation methods were tested. Finally, the most successful products were tested for their sensory characteristics and their acceptance by the consumers. The results are encouraging and they indicate that a yoghurt-style product from donkey milk, without additives, is possible with the addition of other milk, in limited amount, and its sensory characteristics may be well received.

Keywords: Donkey milk; Fermented products; Hypo-allergenic food; Sensory acceptance; Functional food

Introduction

Donkey milk has been acknowledged for its unit properties since the ancient times. Hippocrates as well as Pliny the Elder believed that donkey milk can act therapeutically in numerous cases, such as for liver problems, infections, fever and asthma [1]. It has nutritional properties that make it similar to human milk more than any other mammal milk [2], it is rich in natural antimicrobials [3] and it has been classified as hypoallergenic [4]. In addition, positive effects of the nano-galactose are recorded in the osteogenesis process, in the treatment of arteriosclerosis as a vasodilator, in the rehabilitation of patients with coronary artery disease or premature aging and in low cholesterol diets [5]. Finally, many studies have suggested that the addition of linolenic acid to diet can contribute to the treatment of atopic dermatitis [6], and that name can also be used as a food source of linolenic acid for children suffering from this condition [7].

Citation: Tsakali, Bosdra, Giannopoulos, et al. A Preliminary Study on the Development of Donkey Milk Based Fermented Product. Sci Revs Chem Commun. 2017; 7(3):113.

Donkey milk's high content of the serum protein makes it desirable for human nutrition [8], as confirmed by Chiavari [9]. Its protein composition differs significantly from that of cow's milk: the total content is lower (13-28 mg/mL) and quite similar to that of human and mare milk; this prevents an excessive concentration of proteins in kidneys [10]. The main difference is found in the proportion of serum proteins, which makes up 35-40% of the nitrogen fraction and in cow's milk represents only 20% [11]. Donkey milk suggests a lower fat content compared to human and this may explain its reduced energy content [12]. Its Lactose content (7%) is similar to that of human milk [2], as confirmed by Gubic et al., and is much higher compared to cow's milk [13].

In cases of allergies a food substitute should be sub- or anti-allergic, not cause cross-reactions, be nutritionally adequate and palatable [1]. The widespread use of cow's milk in human nutrition has shown that a significant percentage of people are allergic to some of its protein components. In particular, cow's milk, which was used as a substitute for breastfeeding when breast milk was not available or desirable, represents the main source of allergies in the infant population (0.3-7.5%) [1]. Cow's milk allergy is clinically a non-natural immune response to cow's milk proteins, which may be due to the interaction between one or more milk proteins as well as one or more immune mechanisms, resulting in immediate reactions due to in Lg-E [14] although etiology mechanisms have not been elucidated [15]. Mare and donkey milk, which are used in popular practices for allergic children, are valid alternative sources of protein when properly assessed from the health point of view [2]. Clinical studies have shown that donkey milk can be considered a good substitute for cow's milk for the feeding of children with severe protein allergy syndrome (Ig-E) when it is impossible to provide human milk [16]. For these patients, the donkey milk is not only useful but also safer compared to the milk obtained from other mammals [17], due to its increased similarity to human milk, especially considering the content of protein fractions [2].

Commercial donkey milk is a valuable product and can be used in multiple applications for the production of dairy products as well as for cosmetics and soaps [18]. The dairy applications include pasteurized Tetra Pak packaged milk, milk powders and cheese. Taking into account the many benefits of donkey milk -including its beneficial health characteristics and probiotic effects- Coppola et al. suggested its use for probiotic purposes [9].

Yoghurt is a semisolid, acidified fermented dairy product which is made from heat-treated and standardized milk with the contribution of the symbiotic microorganisms *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* [19]. The nutritional value of yogurt depends exclusively on its ingredients. The raw ingredients used, the ingredients added as well as the production process followed will have an impact on vitamins, proteins, fat and minerals. In general, it is accepted that consumption of traditional yogurt containing only *S.thermophilus* and *L.delbrueckii subsp. bulgaricus* allows people with lactose intolerance to derive a nutritional benefit from the available milk [20], and can help to stabilize the normal microflora of the small intestine [21].

The aim of this study was to explore ways of producing a yoghurt-style product from donkey milk using only traditional material and methods, as fermented dairy products are ideal alternatives to expand the self-life of milk.

Material and Methods

Donkey (Equus asinus) milk samples from the Arcadian breed, frozen at -18^{o}C, were purchased from the company ONOS at Meligala Messinias Greece between May and November. Ovine milk from Karagouniko and Lacaune breeds in a mix of 60:40 was purchased unpasteurized from a small farm at Parnassos, Greece during the same period. The commercial pasteurized bovine milk which was used was from Delta Company. Yogurt starter cultures of *S. thermophilus* and *L. delbrueckii subsp. Bulgaricus* were obtained from Laboratory of Milk and Dairy Technology and Quality of T.E.I. of Athens.

All three types of milk (donkey, ovine and bovine) were tested for their composition and pH. Fat content was determined by Gerber method (ISO 2446:2008 I IDF 226:2008), protein content by Kjeldahd method (ISO 8968-1:2014 I IDF 20-1:2014), lactose content and total solids were determined by MilcoScan 134A/B of Foss Electric while pH was measured with Mettler Toledo pH meter. All milk samples were tested for inhibitory agents (antibiotics residues) using Delvotest P (Gist-Brocades, NY) [22]. The same methodology was followed for the testing of the most accepted final products.

Yogurt style products were prepared following set type yogurts' procedure. Nine different combinations of milk and amount of starter culture were used as shown in **TABLE 1**. First the milk was heated at 90^{o}C for 10 minutes; it was then cooled at 37^{o}C before the starter culture was added in the appropriate amount each time. The milk and starter culture mix was then packaged in 200 mL containers and they were placed for incubation at 45^{o}C until pH dropped to 4.6-4.8 (14 hours approximately). The final product was then placed under refrigeration (4^{o}C) for at least one more day and maximum three before it was further tested [23].

Milk	**% Culture**
100% Donkey milk	3.0
100% Donkey milk	4.5
100% Donkey milk	6.0
90% Donkey-10% Ovine milk	3.0
80% Donkey-20% Ovine milk	3.0
70% Donkey-30% Ovine milk	3.0
90% Donkey-10% Bovine milk	3.0
80% Donkey-20% Bovine milk	3.0
70% Donkey-30% Bovine milk	3.0

TABLE 1. **Combinations of milk mixes and culture amount tested**

Ranking test was used for the sensory evaluation of the best three produced yogurt style products, namely 100% donkey milk- 6% starter culture, 70:30 donkey/ovine milk-3% starter culture and 70:30 donkey/ bovine milk-3% starter culture. Simple rank-sum statistics were based on Kramer's corrected tables [24]. The panel consisted of 19 non-trained

testers between 18 and 65 years old. The parameters examined were texture, colour, taste, acidity. Finally, testers were asked which of the three examined products would be more likely to buy and whether they were willing to spend more money on this if they knew that donkey milk was used.

Results and Discussion

Chemical analysis

All milk samples were found of regular composition and pH. No antibiotics residues were traced in any sample. The results of the chemical analysis of milk are presented in **TABLE 2**.

	Donkey Milk		**Ovine Milk**		**Bovine Milk**	
	Obtained value	**Reference value [25]**	**Obtained value**	**Reference value [26]**	**Obtained value**	**Reference value [26]**
Proteins (%)	1.53	1.4-2.0	4.94	4.5-7.0	3.21	3.0-3.9
Fat (%)	0.56	0.3-1.8	6.00	5.0-9.0	3.50	3.3-5.4
Lactose (%)	6.97	5.8-7.4	5.61	4.1-5.9	4.61	4.4-5.6
Total solids (%)	9.49	8.8-11.7	16.55	8.0-10.0	11.42	7.0-8.0
pH	7.3	7.0-7.2	6.6	6.5-6.8	6.7	6.5-6.8

TABLE 2. **Composition of milk samples.**

Yogurt preparation

The coagulation mechanism of yogurt includes the use of lactose from the starter culture -as an energy source- for the production of lactic acid. Subsequently the pH drop will cause the removal of Ca and P from the casein micelles into the soluble phase and in combination with the reduction of micelles' electric charge, the protein destabilization will start and it will be completed at the isoelectric point of caseins (pH 4.6). Since the milk was thermal treated in high temperature (90^{o}C-10min) β-galactoglobulin is integrated in the micelles which assists in the hydrophilic properties of the yogurt gel. Finally, fat is also enclave in final gel [27]. From the mechanism described it is obvious that milk with high protein and fat content will be easier coagulated. Also, the amount of starter culture assists in the production of lactic acid, thus pH drop. Finally, the amount of total solids plays an important role in the texture of yogurt [28].

Based on the mechanism described it is obvious that milk with high protein and fat content, and increased total solids with will be easier coagulated. Also, the amount of starter culture assists in the production of Lactic acid, thus pH drop. The results of this study confirm these suggestions. In the first set of experiments, when only donkey milk with 3-4.5% starter culture was used, no coagulation was achieved. When 6% starter culture was used a partly coagulation was achieved. This product does not qualify as yoghurt, but it can be described as fermented beverage, as it has been suggested in the past by Coppola et al. [29]. The next set of experiments included the addition of ovine milk in concentrations of 10, 20 and 30%.

Coagulation was obtained only when 30% ovine milk was used with 3% starter culture. Exactly the same results were obtained when bovine milk was used in the same amounts with 3% starter culture. The composition of the three produced yogurts is presented in **TABLE 3**.

	Final product obtained from		
	Donkey Milk	**Donkey-Ovine Milk 70:30**	**Donkey-Bovine Milk 70:30**
Proteins (%)	1.62	2.19	1.93
Fat (%)	0.59	1.76	1.38
Lactose (%)	4.93	3.52	3.95
Total solids (%)	8.92	9.25	9.07

TABLE 3: **Composition of yogurt style experimental products.**

Sensory analysis

The results of the sensory analysis showed that the yogurt prepared by Donkey–Ovine milk (70:30) had significantly the best texture and the most well accepted acidity (significance level 0.05). On the other hand, the yogurt prepared by Donkey – bovine milk (70:30) was described as the one with the better colour and taste but it was not significantly better that the other two samples (significance level 0.05). There was also no statistically significant difference between the yogurt prepared by Donkey – Ovine milk (70:30) and the yogurt prepared by Donkey – Bovine milk (70:30) regarding the question "Which of the three- examined products- it would be more likely to buy". Finally, 67% of the panel testers responded that were willing to spend more money on a yogurt product made by donkey milk.

Conclusions

The results of this study are encouraging and they indicate that a yoghurt-style product from donkey milk, without additives, is possible with the addition of other milk in limited amount, and it may be well received in the market for its sensory characteristics. Although, the price of donkey milk is significantly much higher than other mammal milks, the consumers are willing to spend more money in order to benefit from its unique properties.

Acknowledgements

This work was supported by project PFV/10/002 (Center of Excellence OPTEC-Optimization in Engineering) of the KU Leuven Research Fund; project G.0930.13 of the Fund for Scientific Research-Flanders, and the Belgian Program on

Interuniversity Poles of Attraction, initiated by the Belgian Federal Science Policy Office (IAP Phase VII/19 DYSCO). The writers would like to thank ONOS farms (Mr Eliopoulos) for the donkey milk provided.

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