

Influence of Myrtle Juice and Syrup on Microbiological, Physicochemical and Sensory Features of Goat's Milk Yogurt Made with Indigenous Starter Culture

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Abstract

This study evaluated the effect of Myrtle Juice (MJ) and Syrup (MS) on microbiological, physicochemical and sensory features in goat milk yogurt fermented by indigenous *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* during 30 days of storage. Generally, in all samples, the high LAB number at the end of incubation and the pH values ranging from 4.1 to 4.6 indicates a good effectiveness of the used starter on fermentation process. *L. delbrueckii* subsp. *bulgaricus* compared to *S. thermophilus* was the most abundant in yogurt with MJ (YMJ) after 30 days of storage compared to YMS. On the contrary, *S. thermophilus* demonstrated the highest viability (7 log cfu/g) in the yogurt with MS throughout the storage period. Spoilage and pathogen microorganisms were absent in fresh products and during the storage period. Overall, physicochemical were very similar in all samples, Myrtle juice addition has positively influenced the increase of the lactic acid L(+), acetaldehyde and Free Fatty Acids (FFAs) content. All samples resulted well for flavor and acidity attributes as well as astringency parameter is highly expressed in the YMJ.

Keywords: Myrtle juice; *Streptococcus thermophilus*; Goat Milk; Yogurt; Fermented dairy foods

Introduction

Fermented dairy foods have constituted a vital part of human diet in many regions of the world since times immemorial. Approximately 400 generic names are applied to the traditional and industrialized fermented milk products manufactured throughout the world. They are mainly related to the type of milk used, the microorganisms involved and the technology applied.

Yogurt can be regarded as functional food [1] and human health benefits have been associated with its consumption [2]. Cow milk yogurts are certainly the most wide spread, well-known and marketed fermented milk products around the world and are recognized as healthy by consumers. Recently, there is an increasing in consumer demand for alternative products, such as goat fermented milk [3] supplemented and/or fortified with different ingredients such as prebiotic compounds, cereals [4] fruits [5] and fibers [6].

Goat milk health benefits such as high digestibility, hypoallergenicity, high calcium and high essential amino acids content compared to cow and sheep milks have been reported [7-10]. Furthermore, goat milk contains similar amount of vitamin B6 and pantothenic acid, more niacin (about 3.5-fold), but less vitamin B12 (about 4-fold) and folic acid (approximately 6-fold) than bovine milk [11]. Folic acid deficiency is one of the main charges against the goat's milk to be used as a product for infant nutrition. To overcome this issue, folate bio-enrichment of goat dairy products was done using native folate producing starter cultures [12]. Recent studies furthermore demonstrated that raw goat milk is a source of potential bio-preservatives cultures for fermented food production [13,14]. In general, goat milk products present an unpleasant "goaty" flavor, but the evaporation procedure used in the yogurt preparation process improves the consistency and reduces this flavor in the end product [15]. Myrtle (*Myrtus communis* L.) is a typical spontaneous plant of the Mediterranean area, it is widely used in Sardinia (Italy) to produce a typical liqueur by hydroalcoholic infusion [16], and the wood smoke of myrtle is used for flavoring meat or cheeses, such as the PDO Fiore Sardo cheese.

Myrtus leaves hydroalcoholic extracts and essential oils have been shown good biological activity such as antioxidant [17] and antimicrobial activities on some pathogenic bacteria particularly *Escherichia coli*, *Listeria monocytogenes*, *S. aureus* and *Candida albicans* [18]. Nowadays there is a high demand by consumers for alternatives to cow's milk due to problems associated with allergenicity, gastrointestinal disorders and desire for novel dairy products with enhanced healthy properties [10]. Numerous studies have shown that several fruits increase the nutritional value to food, because they are potential beneficial for human health [19]. Therefore supplementation of goat's milk yoghurt with myrtle berries can enhance its nutritional quality and provide therapeutic value too.

The objectives of this study were to develop a new goat milk yoghurt added with myrtle berries juice and syrup and assessed for evaluate: i) a possible inhibitory effects of these on the yogurt starter microorganisms and their activity; ii) a possible negative influence on the physicochemical and sensory characteristics of the goat's milk yoghurt during the storage period. To our knowledge, this is the first study of myrtle juice and syrup effects on the microbiological and physicochemical parameters of goat milk yogurt.

Materials and Methods

Culture starter

Streptococcus thermophilus SL1 (*S. thermophilus*) and *Lactobacillus*

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delbrueckii subsp. *bulgaricus* LY1 (*L. bulgaricus*) strains, isolated from goat milk and with suitable technological properties [12] were used as starter cultures. Lactobacilli strains to be used in the experimental batches were grown overnight in MRS medium (Oxoid, Milan, Italy) at 42°C, streptococci in M17 medium (Oxoid) at 42°C.

Preparation of juice and syrup mirtus

Myrtus berries (collected in Sardinia, Italy) were cleaned under running water and pasteurized at 78°C for 30 minutes and then crushed with a mixer (Turbo Homogeneizer HMHF, Pbi). Crushed berries were squeezed with a press to obtain the juice. After than the juice was filtered by cheese cloth; the syrup was obtained by adding saccharose to the filtered juice up to have a final concentration of 20%. The syrup was obtained by adding to the juice 20% of saccharose and hand mixing.

Experimental yogurt manufacturing

For this work 150 liters of goat milk were pasteurized at 90°C for 30 s and cooled to 42°C.

In order to verify the action of the myrtle berries (juice and syrup) in the best possible manner, three separate batches of 50 liters of milk were performed: batch A (Y): *S. thermophilus* + *L. bulgaricus*; batch B (YMJ): *S. thermophilus* + *L. bulgaricus* + myrtle juice (3%); batch C (YMS) - *S. thermophilus* + *L. bulgaricus* + mirtus syrup (3%). The goat milk was dispensed directly in 125 g plastic containers and then inoculated (~ 6 log ufc/mL of milk) with the *S. thermophilus* and *L. bulgaricus* starter culture in 1:1 ratio and incubated at 42°C for 4 h. At the end of incubation period, the containers were immediately cooled and kept in a cold room at 5°C for 30 days. Raw milk and also four yoghurt samples were collected at the end of the incubation (t=0) and after 5, 15 and 30 days of storage and microbiological and physicochemical analysis were carried out. Sensory evaluation was performed at 30 days of storage.

Microbiological analysis

Raw goat milk and yogurt (10 ml) samples were homogenized in 90 ml of sterile Ringer's solution (Oxoid, Mila, IT) for 2 min in a Stomacher Lab Blender 80 (PBI, Milan, IT). Aliquots (1 ml) were 10-fold diluted in Ringer's solution (Oxoid, Milan, IT) plated on the specific media used to quantify different species and microbial groups. Lactobacilli and lactic streptococci were quantified after anaerobic incubation (Gas-Pack, Oxoid, Milan, IT) at 42°C for 48 h on acidified (pH 5.4) MRS and M17 agar (Oxoid, Milan, IT) respectively; staphylococci and yeasts, were quantified using the method described by Mangia et al. [20]; faecal coliforms were counted as described by Mangia et al. [21]; aerobic spore-forming bacteria were counted on Nutrient agar (Oxoid, Milan, IT) [22]; anaerobic spore-forming bacteria were enumerated after samples heat treatment (80°C for 10 min), inoculation on DRCM broth (Oxoid, Milan, IT) and incubation at 37°C for 48 h in anaerobic conditions (MPN method).

Determination of physicochemical parameters

Yoghurt pH value was determined with a pH-meter (Crison Instruments SA, Barcelona, Spain). Acidity determination was carried out in 10 ml of milk/yogurt titrated with 0.1 N NaOH, phenolphthalein was used as indicator and acidity was expressed as percentage of lactic acid; Dry Mater (DM), ash, fat and chlorides were monitored according to IDF Standard [23-26] respectively; Total Nitrogen (TN) were determined by Kjeldahl according to Butikofer et al. [27]; FFAs were extracted from yogurt and analysed by gas chromatography using the procedures described by De Jong and Badings [28], with some minor modifications detailed by Madrau et al. [29]. Briefly, FFA

was extracted with three steps of lipid extraction from yogurt mixed with 3 g of anhydrous sodium sulphate and 0.3 ml of sulphuric acid. 3 mL of diethyl ether/heptane (1:1 v/v) was added and vortex for 3 min. This operation was repeated three times. The extracted FFA are then isolated by using alumina, and subsequently desorbed with 5 mL of ether containing 6% formic acid. FFAs were separated using a fused silica capillary column Nukol (15 m, 0.53 mm I.D., 0.50 mm Df; Sigma-Aldrich Co.), using an HP 5890 series II gas chromatograph (Hewlett-Packard Co.), equipped with an auto-sampler, flame ionisation detector and a data acquisition system (HP Chemstation Rev. A.06.03 software; Hewlett-Packard Co.). The sum of FFA was calculated and used in the present study. Lactose, glucose, galactose, D(-) and L(+) lactic acid and acetaldehyde were quantified using enzymatic assays (Boehringer Mannheim, R-Biopharm, Germany).

Sensory analysis

Sensory analysis was carried out in agreement with those reported on The Sensory Evaluation of Dairy Products [30] and the IDF standard methods [31-33]. Flavor, consistency, acidity, taste, sweet, astringent and animal-like parameters (aromatics associated with barns and stock) [34] were evaluated by a trained panel of 10 members using a five-point score system in accordance with their preference hedonistic (5 excellent, 1 unacceptable). The sensory profiles were conducted on coded samples at 30 days of storage. Each judge of the 10 sensory panelists has performed the test three times with an interval of 24 hours between sessions.

Statistical analysis

Microbiological and physicochemical analyses were carried out in triplicate on yogurt samples from each batch (n=3). Mean values of microbiological and physicochemical data at a specific storage time for each yoghurt were compared using the Student's T test and differences were deemed statistically significant at P<0.05. Sensory analysis data were analyzed with Statgraphics Centurion version XV.

Result and Discussion

Microbiological analysis

Overall the microbiological quality of milk used in this experiment was acceptable (Table 1). The initial count of lactobacilli and lactic streptococci in the raw milk were higher than counts found in raw goat milk by others authors [35,36], even though the heat treatment applied eliminates all microbial groups of the raw milk (data not shown).

LAB numbers increased during the incubation time reaching higher count values compared to the results of Ranadheera et al. [3], but similar at the results of Xanthopoulos et al. [37]. On average the counts of *L. bulgaricus* in the different experimental products decreased about 2 log units during the storage period with different behaviour between the different batches. In Y and YMJ the counts of *L. bulgaricus* increased up to 15 days and then decreased slightly at 30 days of storage. While in the YMS the counts decreased after 5 days of storage. These trends disagreed with the results of Eissa et al. [38] which showed that *L. bulgaricus* count in caprine yoghurt increased in the first ten days of storage and then decreased. The *S. thermophilus* counts decreased during storage; this is agreed with the results of Ranadheera et al. [3] but in contrast with several previous studies that detected in goat's milk yogurt [39] and cow's milk yogurt [40] a slight increase of *S. thermophilus* counts in the first week of storage. In particular, *S. thermophilus* count decrease was variable depending of the batch. In Y and YMJ the initial counts decreased at 30 days of storage more

	Raw milk	Y	YMJ	YMS	Y	YMJ	YMS	Y	YMJ	YMS	Y	YMJ	YMS
Time	t0				5			15			30		
Microbial groups													
Lactobacilli\ <i>L. bulgaricus</i>	5.0 ± 0.01	9.8 ± 0.20 ^b	10.8 ± 1.05 ^a	9.2 ± 0.01 ^c	9.8 ± 0.11 ^a	9.8 ± 0.01 ^a	8.8 ± 2.10 ^b	9.9 ± 0.01 ^a	9.9 ± 0.01 ^a	8.3 ± 4.00 ^b	7.98 ± 0.26 ^b	8.8 ± 0.01 ^a	6.9 ± 0.01 ^c
Streptococci\ <i>S. thermophilus</i>	5.3 ± 0.15	9.3 ± 0.52 ^b	9.7 ± 0.90 ^a	9.2 ± 0.05 ^c	7.0 ± 1.73 ^b	7.2 ± 0.24 ^b	9.1 ± 3.09 ^a	6.0 ± 0.01 ^b	6.0 ± 0.09 ^b	9.0 ± 2.70 ^a	5.4 ± 0.05 ^b	4.3 ± 0.26 ^c	7.5 ± 2.99 ^a
Fecal coliforms	2.6 ± 0.10	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Staphylococci NC	3.3 ± 2.20	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Yeasts and Moulds	2.4 ± 0.01	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
ASFB*	3.0 ± 0.13	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
AnSFB*	7	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3

Presented values are the average ± SD. ASFB: Aerobic spore-forming bacteria; AnSFB: Anaerobic spore-forming bacteria. *MPN Method. For each sampling time, values in the same row with different superscript letters differed significantly ($P < 0.05$).

Table 1: Evolution of microbial groups (log cfu g⁻¹) of raw milk, yogurt (Y), yogurt added myrtle juice (YMJ) and yogurt added myrtle syrup (YMS) at the end of incubation (t0) and at 5, 15 and 30 days of storage at 5°C.

Samples	Pasteurised milk	Y	YMJ	YMS	Y	YMJ	YMS	Y	YMJ	YMS
Time		T ₀			15			30		
pH	6.48	4.10 ^a	4.67 ^b	4.50 ^b	4.07 ^a	3.82 ^b	4.00 ^a	4.03 ^a	3.86 ^b	3.92 ^b
Acidity %	0.19	1.12 ^a	0.75 ^a	0.77 ^a	1.19 ^a	1.15 ^a	1.32 ^a	1.20 ^a	1.26 ^a	1.25 ^a
Lactose g/100g	5.68	2.82 ^a	2.52 ^a	2.99 ^a	2.27 ^a	2.35 ^a	2.50 ^a	2.10 ^a	1.90 ^a	2.20 ^a
Glucose g/100 g	-	0.01	0	0	0	0	0	0	0	0.01
Galactose g/100 g	-	1.44 ^a	0.69 ^b	0.83 ^b	1.18 ^a	0.67 ^b	0.69 ^b	1.20 ^a	0.83 ^b	0.71 ^c
Lactic acid D(-)	-	2.23 ^a	2.57 ^a	3.46 ^b	2.56 ^a	2.32 ^a	3.82 ^b	3.47 ^a	4.68 ^b	4.72 ^b
Lactic acid L(+)	-	7.70 ^a	8.32 ^a	6.90 ^b	8.90 ^a	8.56 ^a	6.69 ^b	9.00 ^a	9.12 ^a	6.77 ^b
Acetaldehyde	-	3.70 ^a	4.80 ^b	2.40 ^c	3.40 ^a	3.00 ^a	0.40 ^c	3.30 ^a	2.80 ^b	0.40 ^c
DM	-	16.13 ^a	15.82 ^a	16.86 ^a	16.12 ^a	15.58 ^a	16.68 ^a	16.02 ^a	15.69 ^a	16.90 ^b
Ash	-	0.91 ^a	0.87 ^a	0.84 ^a	0.92 ^a	0.88 ^a	0.85 ^a	0.95 ^a	0.93 ^a	0.85 ^b
Fat	-	5.58 ^a	4.07 ^b	4.83 ^c	5.30 ^a	5.40 ^a	4.78 ^a	4.78 ^a	4.92 ^a	4.74 ^a
Chlorides %	-	0.47 ^a	0.52 ^a	0.44	0.47 ^a	0.48 ^a	0.45 ^a	0.49 ^a	0.47 ^a	0.41 ^a
TN	-	4.25 ^a	4.07 ^a	3.95 ^a	4.15 ^a	4.10 ^a	3.90 ^a	4.34 ^a	4.19 ^a	3.96 ^a
FFA	-	69.62 ^a	55.70 ^b	47.47 ^c	62.80 ^a	70.80 ^b	73.10 ^b	79.36 ^a	85.06 ^b	77.25 ^a

For each sampling time, values in the same row with different superscript letters differed significantly ($P < 0.05$).

Table 2: Physicochemical analysis of pasteurized milk, yogurt (Y), yogurt added myrtle juice (YMJ) and yogurt added myrtle syrup (YMS) at the end of incubation (t 0), at 15 and 30 days of storage at 5°C.

than 4 log units while in the YMS it decreased about 1.5 log units. In contrast with previous observations by Dave and Shah [41,42] and Eissa et al. [38], *S. thermophilus* count in Y and YMJ remained below that of *L. bulgaricus* at the end of the storage period. This different rate of decrease can be explained by the better tolerance to higher concentration of sucrose in YMS yogurt of *S. thermophilus* compared to *L. bulgaricus* [43] rather than to a negative effect of the myrtle itself. The addition of juice myrtle to yoghurt have a positive effect on the growth of lactobacilli, the count in fact, was significantly higher respect to the other two experimental trials during the entire storage period. A similar stimulant effect of fruits juice was previously detected in goat's yogurt supplemented with aronia juice [44]. However the effect of natural fruit juices on yogurt starter culture growth has been reported to be species and strain specific [45]. Though, high initial temperature (42°C) used in these trials favor the lactobacilli growth; Güler-Akın and Akın [39] observed a significantly higher number of *L. bulgaricus* in yogurt incubated at 42°C rather than at 37°C. *L. bulgaricus* is considered the main responsible of the low pH attained in the yogurt after 1 day of storage, view the low rate of acidification of *S. thermophilus*. Indeed, has been showed that this species take about 10 h of incubation to reach pH 4.5 in goat's milk [46].

Spoilage and pathogens bacteria were absent in fresh products and also throughout the storage period. This result reflects the effectiveness

of the heat treatment on the milk used to make yoghurt, the absence of contamination during the process and the use of low temperature throughout storage. Furthermore due to its low pH, yogurt exhibits unsuitable environment for the growth of pathogens and spoilage microorganisms.

Physicochemical analysis

The results of the physicochemical analysis are shown in Table 2. DM, ash, fat, chlorides and TN parameters were much like in all samples and the acidification process took place with regular increases in acidity. In all samples, the evolution of acidity and pH over time are very similar. However, at the end of incubation phase the pH values ranged from 4.10 to 4.6. The degradation of lactose occurred mainly during the incubation phase and did not undergo major changes during storage as well as glucose. The galactose instead is degraded only in part, because most of the strains of *S. thermophilus* are galactose negative (Gal-) [47].

The increase of lactic acid L(+) was particularly evident for batches Y and YMJ where lactobacilli were more numerous than streptococci LAB and also presented higher acidification activity than *S. thermophilus*. The ratio of L(+) / D(-) lactic acid could be used to assess the quality of yoghurt which is of great dietary interest and puts fermented goat milk in the "good yoghurt" category [48].

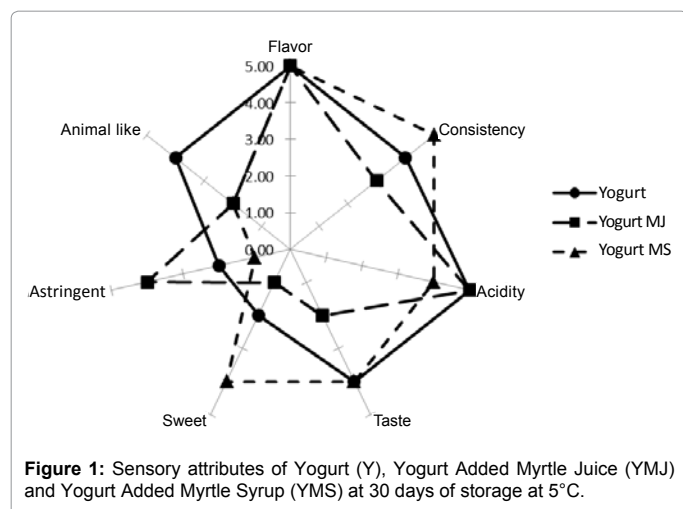


Figure 1: Sensory attributes of Yogurt (Y), Yogurt Added Myrtle Juice (YMJ) and Yogurt Added Myrtle Syrup (YMS) at 30 days of storage at 5°C.

The high number of *L. bulgaricus* in Y and YMJ product could also explain the higher acetaldehyde concentration than YMS. Indeed, between yoghurt micro-organisms *L. bulgaricus* is the major acetaldehyde-producing specie [49,50]. The lower contents of acetaldehyde in the YMS samples can be attributed to the reductase activity of *S. thermophilus* present in high numbers in this type of yogurt [33]. Moreover, the acetaldehyde concentrations of Y and YMS were higher than reported in goat set-up yogurt manufactured by potential probiotic strains [37] and of all secondary aromatic metabolites of *L. bulgaricus* is the most important [47]. FFA content increased considerably during storage in all samples (Table 2) but at the end of storage their amount was significantly higher in YMS. Formisano et al. [51] observed that FFA levels in yoghurt increased over a period of 20 days in cold storage, due in particular to the increase of C14-C18:2 fatty acid [52].

Sensory analysis

The results of the sensory analysis (Figure 1) show that the flavor is well expressed in all products. The consistency is well expressed in Y, decreased with the addition in YMJ and improved with the syrup addition. The acidity perception is probably influenced by the glucose presence, in fact in YMS is less perceived. Parameter astringency due to the presence of tannins [34], is highly expressed in the yogurt with the myrtle juice and is hidden by the presence of sugar in yogurt with syrup. The added of juice and syrup myrtle berries masked the animal like parameter, which is perceived more in natural yogurt. Statistical analysis showed that there are no differences between the panelists and even between sessions.

Conclusion

Our study indicated that myrtle juice and syrup don't affect indigenous LAB fermentative activity and preserve their cells viability in goat's milk yogurt during storage. This behavior is evident in particular for *Lactobacillus bulgaricus* in YMJ as well as *Streptococcus thermophilus* in YMS. Myrtle juice addition has positively influenced the increase of the lactic acid L(+) and acetaldehyde content. These preliminary data indicate that the use of goat's milk, a selected indigenous starter culture and juice myrtle may be suitable for setting up a new yogurt with balanced nutritional characteristics and rich in live Lactic Acid Bacteria, though further research is certainly needed.

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