# Characterization of goat milk from Lebanese Baladi breed and his suitability for setting up a ripened cheese using a selected starter culture

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ABSTRACT

In this work the hygiene and quality parameters of goat milk from Baladi breeds were assess in order to evaluate his suitability for setting up a ripened cheese. Experimental cheese trials were performed on local Lebanese dairy farm using a selected culture starter. Evolution of physicochemical and microbiological features of experimental cheese during ripening were evaluated. Raw milk showed a good microbiological quality meeting the hygiene criteria given by European law on the hygiene of foodstuffs. Mesophilic lactobacilli were found to predominate during cheese fermentation while thermophilic cocci gradually grown and were preponderant during all ripening stage. Non-protein nitrogen and water soluble nitrogen fractions increased gradually over the ripening highlighting good casein primary proteolysis. The Free Fatty Acids (FFA) content increased through the ripening period reaching 7340 mg/100g. Palmitic and oleic acids were the most representative long-chain FFAs at 210 days whereas capric acid was found as a major short-chain FFA. Cheese ripened 90 days, revealed high score for the flavor and taste attributes and good globally acceptance.

Keywords: Baladi goat milk, selected starter cultures, ripened cheese, proteolysis index, FFAs.

## 1. Introduction

Small ruminants in Lebanon contribute to 25% of milk production. Lebanese goat population counts 403.800 animals (Lebanese Ministry of Agriculture, 2010) which most of it (96.8%) is Baladi breed. It is a strong and sturdy breed, well adapted to survive in a harsh environment and in the specific ecologic conditions of the Mediterranean east coast; it tolerates poor nutritional conditions in ranching, prevailing diseases in the region, and drought. The goat milk sector in Lebanon continues to improve since several years. Although the biological, sanitary and socio-economic constraints milk production increased from 21.2 (2008) to 34 (2010) thousand tons (Lebanese Ministry of Agriculture, 2010). It is mainly intended for direct consumption; but it is also processed only into traditional and local dairy product with very short shelf-life as Laban (Tamime et al., 1999), Darfiyeh (Serhan et al., 2009) and Labneh (Aloğlu et al., 2013). These products are much appreciated among Lebanese consumers even though their marketing is limited to small distribution channels and for a limited period of the year. The cheese-making of this milk into ripened cheese could represent an innovation and the qualitative development could have a positive impact on the economic and social development of agricultural regions. In other words, helping the farmer by transferring a technical improvement of the quality of goat cheese and allowing him to offer, all year, a popular cheese will help him meet the ongoing needs of the market and certainly encourage his attachment to his land and village.

The healthy properties of goat milk and dairy products (Slačanac et al., 2010; Yangilar, 2013) as well as the importance of the use of lactic acid bacteria (LAB) in the process, and their optimal technological properties as potential starter cultures in dairy making, are well

established (Alonso-Calleja et al., 2002; Gonzales and Zarate, 2012; Mangia et al., 2014). In particular, in cheese making from sheep milk, *Streptococcus thermophilus* and *Lactococcus lactis* subsp. *lactis* strains showed good growth and acidification activity whereas *Lactobacillus casei* subsp. *casei* strains showed a good proteolytic activity (Madrau et al., 2006; Mangia et al., 2013). Therefore, this study is aimed to produce ripened cheese from Baladi breed goat milk using a selected *Streptococcus thermophilus* SPS1, *Lactococcus lactis* subsp. *lactis* LPS31 and *Lactobacillus casei* subsp. *casei* 3PS103 strains, assuming that LAB strains have a similar behavior in goat and sheep milks than in cow milk (Pappa et al., 2008). This strategy will allow the evaluation of the hygiene and quality parameters of raw milk from Baladi breed. Moreover, on the cheese obtained the microbial and physicochemical parameters during ripening cheese will be evaluated, in particular proteolysis index and free fatty acids content will be analysed. High levels of hygienic and sanitary standards will be applied in order to provide elevate cheese quality.

## 2. Material and Methods

Baladi goats raised on extensive grazing systems in a farm located at medium altitude (500-900m) in the Achkoutregion (Lebanon) where used. Bulk milk sample was collected three times with 5-day intervals from the morning milking during the spring and transported in the laboratory where analysis and experimental cheese-making were performed.

### *2.1. Starter culture and experimental cheese manufacturing*

For starter culture preparation, lactobacilli were grown overnight in MRS (Himedia, Mumbia, India) broth and streptococci in M17 (Himedia) and centrifuged at 10,000g for 20 min. The pellets were re-suspended in 10 ml of pasteurized goat milk and incubated at 37 °C for 12 h before use. Lactic streptococci and lactobacilli were then added to the milk to achieve $10^7$ and $10^6$ CFU/g, respectively.

Raw milk was pasteurized to 72 ˚C for 15 seconds and then cooled to 40 ˚C and inoculated with 1% of the selected starter culture. Coagulation was done using 3–4 mL of commercial liquid calf rennet (Chr. Hansen, Denmark; strength 1:10.000) for 100 L of milk. When gel firmness was judged to be adequate (by empirical evaluation), the curd was cut into grains of about 0.5 cm so that it retains greater amount of serum and cooked to about 42 °C for 10 minutes. The product was transported into molds and reversed every 30 minutes for 3, 4 times. Salting was performed after 5 hours of molding and cheese samples were left to dry for 1 hour before conservation. Cheese molds were reversed 2 times a day for 3, 4 days and left in a cold room at a constant temperature of 10˚C and a constant relative humidity of 85% for ripening.

96 *2.2. Sampling and microbiological analysis*

97 Milk samples were analysed before pasteurization, while cheese samples at 1, 30, 60,
98 90 days of ripening. Both milk (10 mL) and cheese (10g) samples were diluted in 90 mL of
99 physiological sterile Ringer's solution (Himedia, Mumbia, India) for 2 min in a Stomacher
100 Lab Blender 80 (PBI, Milan, Italy). Samples were 10-fold diluted in Ringer's solution and
101 plated on the specific media of different microbial groups.

102 Total mesophilic count was counted on Plate Count agar (PCA, Himedia) plates after 48h
103 of incubation at 32 °C, while coliforms were counted on MacConkey agar (MC, Himedia)
104 after 48h at 30 °C. Coagulase positive staphylococci (CPS) were counted on Baird Parker
105 agar (BP, Himedia) supplemented with Egg Yolk Tellurite Emulsion (Himedia) after 48 h
106 at 37 °C and typical colonies were assayed for coagulase activity using the Staphylase test
107 (Himedia). Lactobacilli counts were done on MRS agar (Himedia) after 48h of incubation
108 at 22°C and mesophilic and thermophilic cocci were counted on M17 agar (Himedia) after
109 incubation of 48h at 22 °C and 42 °C respectively.

110 Milk samples were also subject of *Brucella* control using the Milk Ring test (MRT)
111 (Cadmus et al., 2008), *Salmonella* spp. was determined using a Salmonella Rapid Test
112 Salmonella tests (Oxoid, Milan, Italy).

113 *2.3. Physicochemical analysis*

114 On milk and cheese samples at different ripening time the most important
115 physicochemical parameters were determined: pH value was measured using a pH meter
116 (Thermo Orion 3 Star pH Benchtop) directly after sampling; titratable acidity determination
117 was carried out in 10g of milk/cheese titrated with 0.l N NaOH, phenolphthalein was used
118 as indicator and acidity was expressed as percentage of lactic acid; lactose, was quantified

using enzymatic assays (Boehringer Mannheim, R-Biopharm, Germany); total solids (IDF, 1982), ash (IDF, 1964) and fat (IDF, 1986) were determined following the IDF Standard Methods. Total nitrogen (TN), non-casein nitrogen (NCN), non-protein nitrogen (NPN) and water soluble nitrogen (WSN) amounts were calculated following the Kjeldahl method according to Bütikofer et al. (1993). Based on the data generated, two "proteolytic indexes" were calculated as ratios WSN/TN and NPN/TN. Free fatty acids (FFAs) were extracted from cheeses and determined by gas chromatography according to the method of de Jong and Badings (1990) with some minor modifications reported by Mangia et al. (2013).

*2.4. Acceptance test*

Assuming that the ripened cheese produced from Baladi goat milk is a new product for the Lebanese market, we decided to make a preliminary acceptance test to provide essential information of the "Baladi ripened cheese". A nine-point structured hedonic scale (1=disliked and 9=liked extremely), was used for the evaluation of the appearance, flavor, taste and global acceptability. In addition, each assessor should indicate buying intention using a 5-point hedonic scale (1=certainly would not buy and 5=certainly would buy).

The test was conducted by regular cheese consumers (n=52) of both sexes, students and staff of the University.

*2.5. Data analysis*

For each cheese making trial (n=3) all the microbial and physicochemical determinations were carried out in triplicate on each sample. The results are expressed as mean values ± standard deviation.

## 3. Results and Discussion

### *3.1. Composition of raw goat milk from Baladi breed*

Mean values of the physicochemical parameters of raw goat milk used in the cheese making trials were the following: pH 6.65±0.20; fat 4.31±0.18; protein 4.00±0.06; lactose 4.05±0.24; total solids 14.08 ± 0.21; ash 0.72±0.04. Goat milk showed higher fat and protein content than that reported for Saanen (Nudda et al., 2013), Damascus (Keskün et al., 2004) and Alpine (Costa et al., 2014) as well as other goat breeds (Tamime et al., 2011; Park et al., 2007). Instead, similar overall composition was reported by Guler et al. (2007). In the same samples the total microbial count (TMC) was 6.92±0.71 ufc $mL^{-1}$, coliformes 4.72±2.09 ufc $mL^{-1}$, coagulase negative staphylococci 4.82±0.34 ufc $mL^{-1}$; coagulase positive staphylococci and *Salmonella* were absent as well as *Brucella* test was negative in all samples. The results of microbiological analysis meet the hygiene criteria given by European law on the hygiene of foodstuffs (Regulation EC no. 853/2004/EC). Among other criteria, European Regulation reports that goat milk must belong to a breed free of brucellosis and TMC value must be less of 1.5 million CFU/ml. Evidently milk production was performed applying good hygiene practice that avoid the common factors responsible for milk contaminations and cross-contamination (Suguna et al., 2012).

### *3.2. Cheese microbiological features*

Enumerations of microbial groups during the ripening process are shown in Table 1. In general, LAB rates were great during all the ripening period with the highest values at 1 day (about 9 log cfu $g^{-1}$) highlighting a good activity of the starter culture in the fermentation period, also evident from pH value (Table 2).

In particular, mesophilic lactobacilli were found to predominate during cheese fermentation, than declined, as well as mesophilic lactococci group. This behavior is in contrast to that observed in other goat cheese (Caridi et al., 2003; Di Cagno et al., 2007), where prevalence of mesophilic lactobacilli at the end of the ripening period has been reported.

*Lactobacillus casei* 3PS103 strains when used in Pecorino cheese manufacturing showed good growth ability in all 240 day of ripening (Madrau et al., 2006). Our result could be explained with a partial inadequacy to goat milk of *Lactobacillus* strain used as culture starter, or alternatively the synergic effect among mesophilic LAB, as observed by Ortigosa et al. (2006) and Mangia et al. (2008), did not occur. Thermophilic cocci grew gradually during the first 30 days (9.3 $\log_{10}$ cfu/g) and roughly maintained constant during 90 days of ripening, predominating over the other LAB groups. Thermophilic cocci number is consistent with Asteri et al. (2010) reported values of 9.25 $\log_{10}$ cfu/g in goat soft cheese after 30 days of ripening. In our case, the high number of viable thermophilic cocci is certainly due to *Streptococcus thermophilus* added as starter but we cannot exclude the Enterococci group, considering the low selectivity of the M17 medium used during analysis (Caridi et al., 2003). The presence of enterococci in goat milk is widely reported (Asteri et al., 2010; Oliszewski et al., 2013) and does not always appear to be associated directly with faecal contamination or poor hygiene (Foschino et al., 2002). *Enterococcus* strains isolated from raw goat milk showed high ability to produce metabolites as bacteriocins (Cocolin et al., 2007) with good prospects of using them in food industry (Schirru et al., 2014). Although contaminating microorganisms were present in the raw milk, total coliforms and *Staphylococcus* were absent in all cheese; this could be due to milk pasteurization process,

188 and/or to the addition of starter culture that inhibited the growth of undesired
189 microorganisms (Martley and Crow, 1993). Anyway, their absence highlights greater
190 attention of hygiene practice used during cheese-making procedures.

191 *3.3. Cheese physicochemical features*

192 Cheese after 1 day ripening showed a pH and lactic acid values of 5.22 and 1.25 %
193 respectively, both values first increase and then after 30 days decrease through ripening
194 time (Table 2). Similar pH and acid lactic values in the first days were determined, at the
195 same time in Tenerife goat cheese and in Pecorino cheese (Gonzales and Zarate, 2012;
196 Mangia et al., 2013) whose technological process is similar to that applied by us
197 (pasteurized milk, culture starter added, type of rennet, curd heating). This leads us to
198 assume that pH and lactic acid values, as well as the lack of lactose are indicators of
199 successful cheese acidification due to the lactic microbiota activity, found in high number
200 at the same period.

201 Total solid, fat and proteins content increased until day 90 of ripening due to a decrease
202 of the $a_w$. Remarkably, high TS content (about 80%) was measured at the end of ripening,
203 even if similar value was detected in Spanish and Greek goat cheese (Calvo et al., 2007;
204 Bontinis et al., 2008) manufactured with different technologies. The authors justified the
205 high loss of moisture during aging due to the great ability of goat milk to undergo
206 acidification and the low relative humidity of ripening room. in our study, also the high
207 temperature (15 °C) and relative humidity value (~ 68-70%) of the ripening room, where
208 the cheese have been kept for 90 days, may have contributed.

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211 *3.3.1. Proteolytic activity*

212 NPN and WSN nitrogen fractions increased gradually over 90 days of ripening,
213 highlighting a good process of casein primary proteolysis (Table 3) which is caused by
214 enzymes from the rennet (Oliszewski et al., 2013), although can not be excluded the
215 enzymatic activity of autochthonous LAB (Gonzales and Zàrate, 2012). NCN fraction also
216 increased through ripening time, indicating the production of small peptides and free amino
217 acids, caused by the microbial proteinases and peptidases (McSweeney and Sousa, 2000).
218 The NPN/TN ratio increased during ripening process reaching a value of 16.24% at 90
219 days. This value resulted higher than those found in raw goat milk cheese (Delgado et al.,
220 2011b) at the same ripening period. Similar evolution of the WSN/TN has been reported for
221 Tenerife, a pasteurized goat milk cheese (Gonzales and Zàrate, 2012). The increase in the
222 NPN/TN and WSN/TN ratios during cheese ripening is indicative of the development of a
223 variety of nitrogenous compounds deriving from proteolysis which first leads to the
224 formation of polypeptides and subsequently to small- and medium-size peptides and
225 eventually to free amino acids (Tavaria et al., 2003).

226 *3.3.2. Lipolysis and free fatty acid evolution*

227 Table 4 shows the concentrations of individual FFA throughout 90 days of ripening.
228 Generally, FFA content increased through all ripening time reaching 7340 mg/100g after 90
229 days of ripening. Surprisingly, these values were much higher than those documented in
230 other papers related to cheeses made from goat or sheep pasteurized milk (Atasoy et al.,
231 2009; Mangia et al., 2013) and in Queso Ibores, a Spanish cheese manufactured with raw
232 goat milk (Delgado et al., 2011a).

233 FFA composition showed that palmitic (C16), oleic (C18:1), stearic (C18:0) and myristic
234 (C14:0) acids were the highest FFA found during all ripening stages, representing more
235 than 80% of total FFAs at 90 days ripening. The same results were obtained in different
236 Spanish goat cheese from pasteurized milk (Poveda and Cabezas, 2006). Among short-
237 chain fatty acids (C4-C10) capric acid (C10:0) was the main FFA detected while acid
238 butyric (C4:0) resulted the lowest as reported for Xinotyri (Bontinis et al., 2012) and
239 Pecorino cheese (Mangia et al., 2013) but in contrast to what determined in Ibores PDO
240 cheese (Delgado et al., 2011) where butyric acid content resulted the highest. Linoleic acid
241 (C18:2) content was consistent with those reported in several Spanish goat cheeses by
242 Poveda and Capezas (2006). Considering that lipase from milk are sensible to
243 pasteurization treatment (Driessen, 1989; Atasoy et al., 2009) and calf rennet used was
244 without lipolytic enzyme, we can deduce that the high content of FFA is due to the LAB
245 enzymatic activity, that although are defined "a low lipolytic activity", are a source of
246 esterase and lipases (McSweeney and Sousa, 2000; Esteban-Torres et al., 2014). The
247 cheeses obtained using mesophilic lactobacilli as adjunct cultures have a higher
248 concentration of FFA than "control" cheese (Kondyli et al., 2003; Di cagno et al., 2006) as
249 well as *Lactococcus* sp. may be responsible for the liberation of quite high levels of FFA
250 (McSweeney and Sousa, 2000).

251 *3.4. Acceptance test*

252 Overall, Baladi cheese ripened 90 days, revealed high score for the flavor and taste
253 attributes and good globally acceptance (Table 5). The preliminary results are encouraging,
254 since the major part of the panellists would probably buy the cheeses.

255

## 4. Conclusions

This work has revealed d that the production of ripened cheese from Baladi goat milk is possible through the application of the right techniques and the best choice of starter culture. The experimental cheeses obtained for the first time in Lebanon, were characterized by good fermentation and ripening process due to raw milk quality and the starter culture used. Such production could significantly affect both Lebanese dairy sector and local farmers, certainly more research and market studies are required before large scale applications.


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393 **Table 1**

394 Microbial groups evolution ($log_{10}$ cfu/g of cheese) during ripening cheese.

| | Ripening time (days) | | | |
|---|---|---|---|---|
| Microbial groups | 1 | 30 | 60 | 90 |
| Total Microbial Count (PCA, 32 °C | 8.1 ± 9.1 | 7.1 ± 7.0 | 6.7 ± 6.4 | 6.4 ± 6.5 |
| Thermophilic cocci (M17, 45 °C) | 8.5 ± 0.7 | 9.3 ± 1.1 | 9.5 ± 1.0 | 9.7 ± 1.1 |
| Mesophilic lactococci (M17, 22 °C) | 8.4 ± 0.8 | 7.7 ± 0.5 | 6.3 ± 0.6 | 5.9 ± 0.8 |
| Mesophilic lactobacilli (MRS, 22 °C) | 9.3 ± 0.8 | 7.9 ± 0.9 | 6.7 ± 1.3 | 6.6 ± 1.7 |

395 Values are means ±SD from three batches.

396

**Table 2**

Change of physicochemical parameters during ripening cheese.

| | Ripening time (days) | | | |
|---|---|---|---|---|
| | 1 | 30 | 60 | 90 |
| pH | 5.22 ± 3.12 | 5.47 ± 0.42 | 5.34 ± 0.04 | 5.31 ± 0.38 |
| Lactic acid | 1.25 ± 0.12 | 1.31 ± 0.27 | 1.29 ± 0.30 | 1.26 ± 0.38 |
| Lactose | 0.00 | 0.00 | 0.00 | 0.00 |
| Total solids | 38.23 ± 19.91 | 62.87 ± 1.46 | 76.25 ± 1.97 | 81.00 ± 1.95 |
| Proteins[a] | 19.97 ± 0.56 | 28.71 ± 0.26 | 31.58 ± 0.86 | 33.05 ± 0.86 |
| Fat | 14.08 ± 7.1 | 30.49 ± 6.7 | 37.91 ± 3.2 | 57.04 ± 3.0 |

Values are means ±SD from three batches. Except for pH value are expressed as %.

[a]TN x 6.38.

403

404 **Table 3**

405 Proteolysis behaviour during ripening cheese.

| | Ripening time (days) | | | |
|---|---|---|---|---|
| | 1 | 30 | 60 | 90 |
| NPN[a] | 0.28 ± 0.56 | 0.41 ± 0.15 | 0.61 ± 0.25 | 0.84 ± 0.06 |
| WSN[a] | 0.39 ± 0.09 | 0.95 ± 0.20 | 1.01 ± 0.20 | 1.14 ± 0.37 |
| NCN[a] | 0.15 ± 0.10 | 0.19 ± 0.09 | 0.38 ± 0.10 | 0.68 ± 0.10 |
| NPN/TN (%) | 9.00 ± 0.35 | 9.16 ± 0.18 | 12.41 ± 0.33 | 16.24 ± 0.38 |
| WSN/TN (%) | 12.74 ± 0.19 | 21.42 ± 0.40 | 20.47 ± 0.20 | 22.07 ± 0.31 |

406 Values are means ±SD from three batches.

407 NPN: non-protéin nitrogen; WSN: water-soluble nitrogen; NCN: non-casein nitrogen: TN: total nitrogen

408 [a] g/100 g of cheese.

409

**Table 4**

Free Fatty Acids (FFA) content (mg/100 g of cheese) during ripening cheese.

| | Ripening time (days) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FFAs | 1 | | | 30 | | | 60 | | | 90 | | |
| C4:0 | 22.5 | ± | 28.87 | 39.98 | ± | 15.7 | 58.69 | ± | 19.6 | 61.78 | ± | 7.9 |
| C6:0 | 28.1 | ± | 21.67 | 41.08 | ± | 25.5 | 65.44 | ± | 22.2 | 78.03 | ± | 31.7 |
| C8:0 | 36.9 | ± | 11.2 | 76.12 | ± | 27.5 | 97.26 | ± | 31.2 | 142.93 | ± | 26.9 |
| C10:0 | 150.1 | ± | 71.1 | 249.72 | ± | 138.0 | 381.28 | ± | 87.9 | 620.17 | ± | 42.6 |
| C12:0 | 54.9 | ± | 30.5 | 86.77 | ± | 46.0 | 141.95 | ± | 21.5 | 228.29 | ± | 17.8 |
| C14:0 | 181.6 | ± | 112.4 | 403.85 | ± | 126.1 | 465.53 | ± | 62.5 | 721.42 | ± | 18.1 |
| C16:0 | 562.0 | ± | 331.7 | 1250.10 | ± | 334.8 | 1493.02 | ± | 175.2 | 2286.97 | ± | 63.5 |
| C18:0 | 301.6 | ± | 82.8 | 542.52 | ± | 70.3 | 677.68 | ± | 47.8 | 1029.09 | ± | 142.8 |
| C18:1 | 451.1 | ± | 206.9 | 1071.85 | ± | 231.1 | 1305.31 | ± | 55.2 | 1914.66 | ± | 202.8 |
| C18:2 | 52.5 | ± | 25.8 | 125.29 | ± | 31.1 | 149.10 | ± | 26.4 | 191.88 | ± | 26.6 |
| C18:3 | 11.7 | ± | 17.0 | 42.26 | ± | 8.2 | 22.86 | ± | 27.6 | 65.21 | ± | 29.0 |
| TFFAs | 1852.83 | ± | 939.83 | 3929.52 | ± | 1054.32 | 4858.10 | ± | 577.14 | 7340.43 | ± | 609.74 |

Values are means ±SD from three batches.

C4:0, butyric acid; C6:0, caproic acid; C8:0, caprylic acid; C10:0, capric acid; C12:0, lauric acid; C14:0, myristic acid; C16:0, palmitic acid; C18:0, stearic acid; C18:1, oleic; acid; C18:2, linoleic acid; C18:3, linolenic acid. TFFAs, total free fatty acids.

**Table 5**

Acceptance test of Baladi goat cheese ripened 90 days.

| Cheese ripened | Appearence | Flavour | Taste | Global acceptance | Buying intention |
|---|---|---|---|---|---|
| 90 days | 6.14±0.21 | 7.28±0.36 | 7.47±0.20 | 7.22±0.19 | 3.82±0.15 |

Data are mean ± standard deviation.