

Full Length Research Paper

Use of lactic strains isolated from Algerian ewe's milk in the manufacture of a natural yogurt

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Fifty three strains of thermophilic and mesophilic lactic acid bacteria were isolated from the ewe's milk. Identification reveals the presence of nineteen strains (36%) of *Lactobacillus* sp., seventeen strains (32%) of *Lactococcus* sp., nine strains (17%) of *Streptococcus thermophilus* and eight strains (15%) of *Leuconostoc* sp. The strains were characterized for their technological properties. A high diversity of properties among the studied strains was demonstrated. On the basis of technological characteristics, two strains (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) were screened with respect to their acid and flavour production for the preparation of a natural yogurt and compared to a commercial starter cultures. Sensorial analyses revealed that the product manufactured on the basis of the isolated strains have a cohesiveness and adhesiveness corresponding to standard products. The pH and the acidity recorded are also within accepted levels during all the period of conservation.

Key words: *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, yoghurt, cohesiveness, adhesiveness, Algerian ewe's milk.

INTRODUCTION

Ewe's milk plays an important role in certain parts of the world. In Algeria, much of dairy products are manufactured by traditional methods, using raw cows or goats and also ewe's milk. El-Klila, a traditional cheese, is made from the raw cow or goat's milk (Boubekri and Otha, 1996; Cheriguene et al., 2007).

In the dairy industry the selection criteria for lactic acid bacteria depends on the type and the desired characteristics of the final product, the desired metabolic activities, the characteristics of the raw materials and the applied technology (Buckeniiskes, 1993). Today, the spontaneous fermentations are replaced by the controlled fermentations using starter cultures containing several microorganisms. The lactic acid bacteria used in commercial starter cultures possesses numerous metabolic characteristics such as acidifying activity, proteolytic activity, synthesis of bacteriocin, resistance to bacteriophage and production of exopolysaccharides. All of these important activities contribute to the flavour, texture and

frequently the nutritional attributes of the products (Ayad et al., 2004).

Yogurt is a fermented milk product made with *Streptococcus salivarius* ssp. *thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus* (Tamime and Marshal, 1997). These two organisms have a symbiotic relationship during the manufacture of yogurt (Amoroso et al., 1989).

The aim of this work is to determine the characteristics of yogurt manufactured with *Streptococcus thermophilus* and *Lactobacillus bulgaricus* isolated from Algerian ewe's milk and to carry out a comparative study with the results of the utilisation of a *S. thermophilus* and *L. bulgaricus* (a commercial mixed culture from CHR Hansen Denmark).

MATERIAL AND METHODS

Microorganisms

Lactic acid bacteria isolates were selected from MRS and M17-agar plates of the highest dilutions. The isolates were subcultured in MRS and M17-broth and streaked onto MRS-agar until pure cultures were obtained. The isolates were examined for cell morphology, Gram reaction, catalase production, growth at 10 and 45°C (cocci) or 15 and 45°C (rods), and gas production from MRS-broth (Falsen

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et al., 1999 and Klein 2001). Lactic acid bacteria isolates were further characterized by their carbohydrate fermentation pattern according to the method described by Schillinger and Lücke (1987) using the miniplate method described by Jayne-Williams (1975) with bromocresol purple as an indicator. The identified strains were tested for their technological properties such as acidifying activity, proteolytic properties and polysaccharides production (Ayad et al., 2004).

Selected cultures (*S. thermophilus* and *L. bulgaricus*) having a fast acidification activity and producing exopolysaccharides were then evaluated as starter cultures for the manufacture of yogurt. A mixture of commercial starter cultures of the two strains (*S. thermophilus* and *L. bulgaricus* YF-L811) (CHR Hansen Denmark) were used for comparison.

Yoghurt preparation

Yoghurt was prepared using ewe's milk. A sample control of yogurt was prepared using reconstituted skim-milk powder 13% (w/v). After standardization and pasteurisation at 90 - 95°C for 3 min, the product is homogenized at 85°C. The mixture is cooled to 45°C, then 0.5 g of strains (*L. bulgaricus* and *S. thermophilus*) (CHR Hansen, Denmark) are inoculated. The inoculated mixture was incubated for 4 h at 45°C.

The prepared milk is distributed in the plastic sterile pots at the rate of 100 ml for every pot. The inoculation is achieved in aseptic conditions in pots with amounts of 1.5, 2.5, 3 and 3.5% (lot 2, 3, 4 and 5 respectively) of starter cultures with a rate of 2S/L (*Streptococcus/Lactobacillus*) for test lots and 2.5% for the control lot (1). The same principle of yoghurt preparation was applied for the commercial strains. The length of fermentation ranges from 2 to 5 h for the isolated strains, and from 2 to 4 h for the commercial strains.

Conservation was followed with a cooling to 6°C in order to stop the acidification at the end of fermentation, which slows down the bacterial action and assures a conserved product of about one month.

Acidity

The acidity was measured by titration of a sample of 10 ml of yoghurt with 0.1 N NaOH using phenolphthalein solution as colour indicator (Dornic method, Tamime and Robinson, 1985). pH measurements were carried out using a pH meter (HANNA instruments HI8519N) (AOAC, 1995).

Viscosity

The viscosity was measured with a viscosimeter model HAAKE Viscosimeter (Mess Technik GmbH) using a glass tube and a normalized ball equipped with a chronometer at 25°C and was expressed as mPas. Every experiment was repeated 3 at 5 times to have some meaningful results after a statistical analysis.

Sensorial analysis

A descriptive sensory analysis was performed by a trained panel consisting of 10 subjects. During training, panelists were asked to identify and define the taste. A 7 point rating scale was used with 1 = very bad, 2 = bad, 3 = nor bad nor good, 4 = enough good, 5 = good, 6 = very good, and 7 = excellent (Moraes, 1985). Samples were then rated according to 2 texture attributes; cohesiveness (the strength of the internal bonds making up the body of the product) and adhesiveness (work necessary to overcome the attractive forces between the surface of the food comes in contact) (Szczesniak, 1987).

Enumeration of microorganisms

Lactic acid bacteria counts were determined on MRS and M17 incubated at 42°C for 3 days. Cultures were activated by three successive transfers in nutrient medium before enumeration. 1 ml of each culture was 10-fold serially diluted (10^{-1} to 10^{-5}) in sterile distilled water diluents solution. Enumeration was carried out using the plate technique. The cultures were cultivated in the following media: MRS (De Man et al., 1960) and incubation at 42°C during 48 h for the enumeration of *L. bulgaricus*; M17 (Terzaghi and Sandine, 1975) (Biolife, Milano, Italy) and incubation at 30°C for 48 h to count *S. thermophilus*.

Plates containing 30 to 300 colonies were enumerated and recorded as colony forming units (cfu) per ml of the product or culture. All experiments and analyses were repeated at least twice. The results presented are averages of at least two replicates.

RESULTS AND DISCUSSION

Physico-chemicals analysis

Changes in the pH of yoghurts from control and acidified samples were studied. During the period of fermentation we recorded a remarkable decrease in the pH for both yoghurt manufactured with our local isolated strains as those manufactured using commercial strains. The values of pH recorded are inversely proportional to the inoculating amount of strains and according to period of fermentation, we remarked that the values decrease when the amount of strains increases (Tables 1 and 2).

Results obtained in this study of lactic acid assays are in keeping with the data reported by Tamime and Robinson (1985) who found over a five-fold increase in the content of lactic acid found after 3 h and 30 min of incubation of milk containing 0.15% lactic acid and 12% dry matter, i.e. a value similar to the dry matter content in the experimental yoghurt and with those reported by Sokolinska et al. (2004) which indicates that the pH values of milk under processing, from the time it was inoculated with bacterial cultures to the time the yoghurt was manufactured, decreased from 6.70 to 4.34. The same decrease was observed by O'neil et al. (1979).

According to other authors (Luquet, 1990), lactic strains have the ability to ferment lactose into lactic acid, with an increase in the acidity and lower pH fermented milk; this reveals the influence on the composition of the inoculum on the rate of growth of bacteria including *Streptococcus*. Indeed, the development of these germs appears to be proportional to the rate of protein (and therefore certainly rates amino acids) in the medium. In addition, during the total experimental duration values of acidity are even higher. Analysis of variance revealed that the period of fermentation and gradual concentration have a significant effect on decreasing pH.

Acidity of sheep's milk yoghurt during fermentation

There were significant differences among the titratable acidities of the various treatments. The acidity of the yo-

Table 1. Mean values for pH in yoghurts made from sheep's milk using commercial starter cultures during fermentation and post-fermentation.

Period		Control	1.5%	2.5%	3%	3.5%	Analysis of variance
Period of fermentation	2 h	5.42±0.115c	5.88±0.00b	6.10±0.012a	6.10±0.017	5.86±0.153b	HS
	4 h	4.47±0.06b	5.75±0.072a	5.60±0.01a	5.48±0.409a	5.35±0.121a	HS
Period of post-fermentation	7 days	4.65±0.05c	5.60±0.1a	5.56±0.113a	5.33±0.082b	5.27±0.075b	HS
	14 days	4.55±0.05c	5.45±0.089a	5.32±0.02ab	5.27±0.061b	5.22±0.111b	HS
	21 days	4.49±0.039b	5.05±0.066a	5.26±0.156a	5.11±0.18a	5.19±0.078a	HS

HS: Highly significant.

Table 2. Mean values for pH in yoghurts made from sheep's milk using wild strains during fermentation and post-fermentation.

Lots		Control	1.5%	2.5%	3%	3.5%	Analysis of variance
Period of fermentation	2h	5.747 ± 0.006a	5.730 ± 0.02a	5.600 ± 0.026b	5.523 ± 0.006c	5.507 ± 0.006c	S
	5h	5.283 ± 0.047a	5.373 ± 0.015a	4.957 ± 0.286b	4.873 ± 0.110b	4.683 ± 0.055b	HS
	7 day	4.920 ± 0.114a	4.820 ± 0.125a	4.580 ± 0.053b	4.503 ± 0.055b	4.320 ± 0.030c	HS
	14 day	4.613 ± 0.340a	4.577 ± 0.081a	4.420 ± 0.075ab	4.110 ± 0.135bc	3.963 ± 0.201c	HS
	21 day	4.440 ± 0.135a	4.327 ± 0.040ab	4.180 ± 0.090b	3.997 ± 0.050c	3.903 ± 0.061c	S

HS: Highly significant; S : Significant.

ghurt made using commercial strains is marked with an increase during the fermentation and post-fermentation. Values recorded ranges from 48.33°D after 2 h of fermentation to 66.34°D after 4 h and from 88.00°D the 7th day to 104.00°D the 14th day and reached 121.67°D the 21st day in average for a concentration of 2.5% of strains. It appears that there is a proportional relationship between inoculating amount of strains and acidity; the inoculating amount increases proportionally to the degree of acidity. The results showed that the type of the bacterial culture applied during milk inoculation had a significant impact on the titratable acidity of the obtained curd as was previously reported by Salji and Ismail (1983) and Sokolinska et al. (2004).

Analysis of variance revealed that the duration of fermentation and gradual concentration have a significant effect on the increase in acidity. In addition, we recorded values of acidity less important for yoghurt made with locally isolated strains during fermentation. Nevertheless, during the period of post-fermentation, recorded values are in the order of 113°D for the control and 121°D for lot 3 and 132.34°D for lot 5.

Viscosity of ewe's milk during fermentation

The viscosity of the yoghurt change as a function of time; it increased from 0.32 mps after 2 h at 0.74 mps after 5 h in the control lot, against 0.950 and 1.277 mps in average in lot 2 (1.5%). This increase of values of viscosity has also been noted in lots 3 (2.5%), 4 (3%) and 5 (3.5%).

However, after 5 h of fermentation until the 21st day, the viscosity decreases from 0.74 to 0.19 mps at the control lot and from 1.277 to 0.787 mps in lot 2. This decrease of values has also been observed in lots 3, 4 and 5 (Table 3). Presumably, the viscosity increases proportionally with the amount of inoculated strains. We have recorded values of 0.23, 0.28, 0.31 and 0.38 with inoculating rate of 1.5, 2.5, 3 and 3.5%, respectively.

These results may be explained by the fact that the specific strains of yoghurt including *S. thermophilus*, are responsible for the start-up of lactic fermentation, while *L. bulgaricus* complete it and are the most active on post-fermentation period when the medium pH is acidic (Rasic and Kurmann, 1978). According to Rawson and Marshall (1997), *S. thermophilus* germs are most involved in the production of exopolysaccharides likely to interact with the substance of milk protein and increase the viscosity hence the rheological quality of products.

During the period of post-fermentation, the activity of *S. thermophilus* is not completely stopped but it is less significant compared to that of *Lactobacillus* which produces not only lactic acid but possibly a slight amount of texturing agents (Luquet, 1994).

In contrast, the decreases of viscosity observed during this period at all levels of field trials are possibly linked to an action of a hydrolytic gel exopolysaccharidic protein by some enzymes secreted by the lactic acid bacteria inoculated during their growth.

According to Vlahopoulou and Bell (1990), yoghurts containing traditional cultures in which the *L. delbrueckii*

Table 3. Viscosity evolution of ewe's milk during fermentation.

Lots		Control Lots 1	Lots 2 1.5 %	Lots 3 2.5%	Lots 4 3 %	Lots 5 3.5 %	Analysis of variance
Period of fermentation	2h	0.320 ± 0.010	0.950 ± 1.152	1.067 ± 1.242	0.997 ± 1.164	0.287 ± 0.081	NS
	5h	0.740 ± 0.020	1.277 ± 1.347	1.580 ± 1.190	1.617 ± 1.207	0.723 ± 0.431	NS
	7 day	0.250 ± 0.010	0.980 ± 1.101	0.920 ± 1.136	0.915 ± 1.149	0.310 ± 0.095	NS
	14 day	0.240 ± 0.010	0.870 ± 0.971	0.827 ± 1.044	0.817 ± 1.035	0.300 ± 0.130	NS
	21 day	0.189 ± 0.010	0.787 ± 0.870	0.711 ± 0.946	0.703 ± 0.926	0.253 ± 0.142	NS

NS: Not significant; H: Hour; D: Day.

Table 4. Chesiveness (Σ ranks) for experimental yoghurts during fermentation period.

Lot	Period (days)			
	1 d	7 d	14 d	21 d
Lot 1	47	15.5	19	31
Lot 2	26.5	37.5	41	29.5
Lot 3	21	39	37	34
Lot 4	35.5	33	28.5	29.5
Lot 5	20	25	24.5	26

subsp. *bulgaricus* and *S. thermophilus* strains occur at 1:1 ratio are characterized by a lower viscosity than those with the traditional, high-aromatizing culture with the domination of the *L. delbrueckii* subsp. *bulgaricus* strain. The above mentioned researchers reported a 26.3% lower viscosity of yoghurts manufactured from milk inoculated with the traditional, low-aromatizing culture in comparison with the viscosity of yoghurts manufactured using milk inoculated with the traditional high-aromatizing culture. In addition, *L. delbrueckii* subsp. *bulgaricus* bacteria stimulates increase in yoghurt viscosity by producing special mucous substances of polysaccharide nature (Ariga et al., 1992; Brandt, 1997). These substances form, primarily with casein, permanent aggregates of high resistance to mechanical action and, hence, of high resistance to syneresis (Gruter et al., 1993).

Sensory evaluation of yoghurt

Panelists scored the taste of yoghurt prepared with commercial strains using ewe's milk inoculated with an amount of 1.5, 2.5, 3 and 3.5% of starter culture as the best compared to the control (prepared using powder milk). This improvement of taste results probably from fermentation of lactose present in the medium of lactic acid bacteria (Bourgeois and Larpent, 1989). However, the tasting panel described the taste of experimental yoghurt prepared using wild strains and made using ewe's milk more acceptable. After the 21st day of fermentation, a slight elevation of taste ranging from 13 to 19.5 points on average in the control lot in relation to tested

lots where a slight decrease in values was recorded. But this change does not affect the tasting properties (organoleptic quality preserved). In terms of statistical analysis, the two factors do not have a significant effect on this parameter.

Anifantakis et al. (1980) observed that yoghurt made from stored frozen sheep's milk was generally of good quality. On the other hand, these authors also reported that their consumer-type panel, in most cases, was rather insensitive to the oxidized flavour of yoghurt and did not complain about it. Small differences in the sensory attributes of yoghurts prepared from stored frozen sheep's milk were observed by Katsiari et al. (2002) between 1 and 14 days of cold storage. The body and texture, the flavour and the overall acceptability scores of all yoghurts increased slightly during their cold storage for 14 days. A similar trend was observed by Abrahamsen and Holmen (1981) for control cow's milk yoghurt. Generally, cohesiveness is more satisfying when the amount of inoculated trade strains is important, with means of 25.08, 30.93, 36.27 and 41.22 for variable rates of 1.5, 2.5, 3 and 3.5% of starter culture, respectively. For milk inoculated with wild strains, during all the period of fermentation, cohesiveness of experimental yoghurt is 34 at the dose of 2.5%. Meanwhile, at doses of 1.5, 3 and 3.5% we recorded values of 29.5, 29.5 and 26, respectively on average (Table 4). According to Rawsan and Marshall (1997), this appreciation of rheological characteristics of yoghurts (adhesiveness and cohesiveness) is probably linked to exopolysaccharides produced by specific strains of yoghurt *S. thermophilus* and *L. bulgaricus*. Analysis of variance showed significant effect of the two factors (duration of fermentation and rate of addition of inoculum) on the development of cohesiveness yoghurts tested.

Adhesiveness of ewe's milk yogurts during fermentation

At the first day of fermentation, the value of the adhesiveness noted is 47 on average for the control lot. This value is superior to those of the tested lots (Table 5). After 7 days of fermentation, an important decrease of this parameter has been observed in the control lot; wherever, in

Table 5. Adhesiveness (Σ ranks) of ewe's milk yogurts during fermentation.

Lot	Period (days)			
	1 d	7 d	14 d	21 d
Lot 1	47	16	17	27
Lot 2	23.5	35	39.5	37
Lot 3	22	35.5	38	34
Lot 4	35.5	32	31.5	22
Lot 5	22	30.5	24	27

lots 2, 3 and 5, this parameter increased. At the end of the experimentation, the adhesiveness increased in lots 2 and 3; on the other hand, values were lower in relation to the control. The same remarks was reported by Rawsan and Marshall (1997) and Katsiari et al. (2002) who indicated that adhesiveness increase in sheep's milk yoghurt during storage.

Changes in the amount of *L. bulgaricus* and *S. thermophilus* during fermentation

During the period of fermentation from the 2nd h until the 14th day using local isolated strains, we have noted a significantly increase in the number of microorganisms from 43×10^5 to 83×10^5 cfu/ml, 52×10^5 to 88×10^5 cfu/ml 54×10^5 to 91×10^5 cfu/ml and 75×10^5 to 94×10^5 cfu/ml on average for 2, 2.5, 3 and 3.5% starter cultures, respectively. Conversely, after the 14th until the 21st day, we noted a relative decrease in the number of microorganisms in the experimental yoghurt; we recorded at the end of fermentation the values 61, 67.73 and 86×10^5 cfu/ml on average for 2, 2.5, 3 and 3.5% starter cultures, respectively

With regards to *L. bulgaricus* of yoghurts made with ewe's milk during fermentation using the same wild strains we noticed at the beginning an increase from 28×10^5 to 72×10^5 cfu/ml, between 2 h and the 14th day for 2.5% starter culture. During the fermentation process, the number of *S. thermophilus* (18.3×10^5 cfu/ml after 2 h and 25.10^5 cfu/ml after 4 h on average) is relatively higher than those of *L. bulgaricus* ($10.3.10^5$ cfu/ml and $13.3.10^5$ cfu /ml) at the same periods. However, this trend is reversed during the post-fermentation, i. e. the number of *L. bulgaricus* compared to *S. thermophilus* is 71×10^5 cfu/ml against 60×10^5 cfu/ml on the 7th day and 73×10^5 cfu/ml against 54×10^5 cfu/ml the 14th day, and then 76×10^5 cfu/ml against 54.4×10^5 cfu/ml the 21st day. Sokolinska et al. (2004) reported that the number of bacteria of the *Lactobacillus* and *Streptococcus* genera in the yoghurt increased immediately after its manufacture.

According to Zbikowski (1997), after 21 days of storage, of the all yoghurt microflora, the total of 45% of bacteria undergo quantitative changes and in the case of yoghurts manufactured using probiotic cultures, the value

is 41.1%. The author showed that during the same period of storage of yoghurt manufactured from milk inoculated with a monoculture, the survival of *L. delbrueckii* subsp. *bulgaricus* bacteria was lower than that of *S. thermophilus*. Investigations carried out by Beal et al. (1999) revealed that the survival of milk fermentation bacteria could fluctuate in the range of 40 to 75% and, as a rule; more bacteria from the *Lactobacillus* genus survive than those from the *Streptococcus* genus.

According to Beal et al. (1999), *S. thermophilus* causes the start-up of yoghurt lactic fermentation; their growth is stimulated by amino acids released following the proteolytic activity of *Lactobacillus*. Then, during the post-fermentation when the environment becomes more or less acidic, the growth of *Streptococcus* germs is relatively restrained, while *Lactobacillus* which are more resistant to acid pH continue to grow. In terms of the analysis of variance, no significant effect of the two factors (period of fermentation and concentration of inoculum) was observed in the evolution of this parameter.

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