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Microbial Characteristics of Klila and Jben Traditionnal Moroccan Cheese from Raw Cow's Milk

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Abstract: Two types of some Moroccan local dairy products produced from raw cow's milk dry Klila and Jben beldi were studied. The former is a hard variety cheese made by using the traditional procedures in the home, whithout using a starter culture, by heating a curd of Lben.The latter is a soft variety cheese manufactured by using the végetable rennet. A total of 16 samples of those kinds cheese prepared in the laboratory and purchased from bulks manufactured by different makers from Oujda and its areas were analysed for their microbial profiles. Standard Plate Count (SPC) the indicator organisms (including faecal and total coliforms and Enterococci), Salmonella, spore forming bacteria, Staphylococci, yeast and molds were carried out. The obtained results show that the average counts of aerobic mesophilic flora SPC was: $2.2\ 10^6$ cfu g^{-1}, 8, $64\ 10^3$, $1.2\ 10^4$ cfu g^{-1} $4.4\ 10^4$ and $3.50\ 10^4$ cfu g^{-1} of dry klila 1, klila 2, klila 3, jben 1 and jeben 2 samples respectively. Pathogenic flora as Salmonella, S.aureus, Coliforms and Enterococci were not detected in dry Klila Samples, while Clostridium (Spore forming bacteria) was enumerated in 35% of samples from Oujda and its areas. Lactic acid bacteria were enumerated in all examined samples with an average count of 2.210^3, 5.10^3, 10^4, $6\ 10^2$, 1.510^3 respectively for kila1, klila2, klila3, jben1 and jben 2. Total yeast were detected in all of the analysed samples with and average counts of 2.10^3, 9.10^3,0, 77 and 2.10^4 respectively.

Key words: Klila · Jben · characteristics · végetable rennet

INTRODUCTION

Man has long preserved dairy products during high milk production period (winter).Thus cheese making is a way to preserve milk, creating the potential for trade [1]. Therefore, more than 1000 varieties of cheese are produced around the world [2], Cheese being now processed with modern technology basing on the use starter culture, witch initiate rapid acidification of the raw material, offer more microbial safety, organoleptic [3], had been manufactured through centuries by the traditional procedures by the particular. Then the technology of numeros food was transferred as men moved from one country to another.

The main goal of this procedures is to extend the shelf-life of Milk on one had and to develop a pleasant flavour on the other hand.

In Morocco, Klila and Jben are most popular local dairy products (traditional cheese) and their traditional method of cheese making is still in use. Nowadays industries to make klila or Jben. Nevertheless, there is an increasing demand of the consumers for that kind of cheese, because for its pleasant organoleptic properties, its high protein and calcium content and its low fat content. Also, they are regarded as an important part of human diet. Furthermore, many varieties cheese are well known throughout the world. However, no studies are being focused on Moroccan varieties cheese such Klila and Jben. There is no data on their microbial and biochemical characteristics and on the technological processing.

Klila a hard variety cheese is made from raw cow's milk by heating whey of curd at 50°C to 60°C for 30 minutes without using a starter culture. Then the obtained curd is drained and dried (Fig. 1).

About, Jben a soft variety cheese is produced according to a traditional protocol which includes rennet coagulation of raw whole cow's milk; to which a salt was

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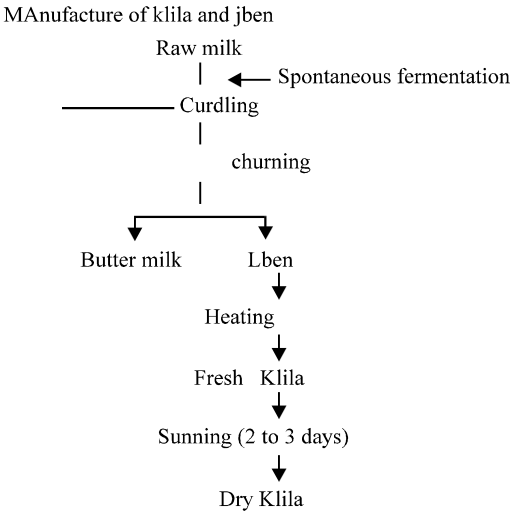

Fig. 1: Flow sheet for manufacture of klila (cheese) in Morocco

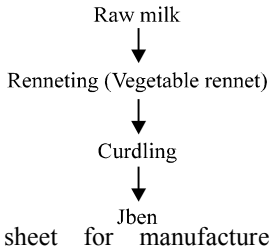

Fig. 2: Flow sheet for manufacture of Moroccan traditional jben (cheese)

added in the proportion of 10-20 Nacl per litre of milk (Fig. 2).

That kind of cheese are very esteemed by consumers and could be promoted nationally and internationally, if it will be manufactured on a large scale respecting their organoleptic characteristics, because it has a salty, slightly acid taste and pleasant oraganoleptic properties.

In presnt work, we sought to investigate the microbiological characteristics of two Moroccan varieties cheese made in the home with samples processed in laboratory conditions. Also we sought to investigate, the processed dry klila and jben in laboratory conditions and its comparison to their tradiotional products.

MATERIAL AND METHODS

1. Samples collection: The various samples of dry klila processed by traditional procedure in the home were collected from the retail market.They were transported to the laboratory under refrigeration (4°C) and analysed immediately. The samples of klila and jben produced in laboratory conditions were analysed after their production.

2. Manufacture of jeben and klila in laboratory conditions

2-1) Klila: raw whole cow's milk submitted at spontaneous fermentation at room temperature followed by a manual curd churning followed which lead at butter and lben. Then, the klila was obtained by a heating lben at 72°C for 15 secondes until coagulation.

2-2 Jben: Whole raw cow's milk was pasteurised at 65°C/30mn, then, it was curdled at 45°C by adding vegetable rennet. Which is obtained by extract in water diffusion of artichoke flowers at 5% (5 ml of extract per 100 ml of milk). The curd was placed in perforated moulds for draining. Also, we have prepared the jeben using thermophilic lactic leaven and extraxt of artichoke flower.

3. Microbiological analysis: Viable cell counts were performed by the standard pour-plate method after serial dilution in saline solution (0.85% w/v). After incubation plates with 30-300 colonies were counted.

Twenty five grams of each klila and jben samples were taken aseptically after discarding the surface layers about 5 mm in thickness the rind of saline sterile water. Tenfold dilutions were made in the same diluent.

3.1. Aerobic mesophilic bacteria: Total bacterial number of sample were determined on standard plate count agar (SPC, Oxoid, England), after incubation at 30°C for 48h according to the standard method for examination of dairy products [4].

3.2 Coliform count: Coliform bacteria were enumerated on Desoxycholate Agar (DL, Oxoid, England) after incubation at 37°C for 24h for Total Coliforms (TC) and at 44°C for fécal (FC) coliforms. Counting of red colonies was done after 24 h of incubation.

3.3 Enterococci: Enterococci were estimated by using Bile Aesulin Azide Agar (BEA, Oxoid, England).Colonies surrounded by a black halo after 24h of incubation at 37°C were counted.

3.4 S. aureus: Enumeration of S. aureus was performed on Mannitol Salt Agar (MSA, Merck, Germany).Yellow colonies were counted after 24h of incubation at 37°C.

3.5 Yeast: Number of yeast was determined on acidified Potato Dextrose Agar (PDA, Merck, Germany). at pH 3.5 using acid tartaric at 10%. The plates were incubated at 25°C for 3 days.

3.6 Lactic Acid Bacteria (LAB): LAB counting were performed on MRS agar at pH 6.2 [5] incubated at 30°C for 48h.

3.7 Spore-forming bacteria: The initial dilution was exposed to 80°C for 10 min to destroy vegetative cells, then 2, 1 and 0.5 ml of this heat actived (dilution) were transferred to SPS Agar in tubes and incubate at 30°C for 24 h. Dark colonies were counted.

RESULTS

1. Klila: The microbial characteristics of the investigated products were summarised in Table 1 to 5 respectively for klila1, klila2, klila3, jben and jben2.

The SPC count in dry klila samples purchased from different maker (or retail)was ranged from 410^3 to $4\ 10^6$ cfu g^{-1} with an average of $2.2\ 10^4$cfu g^{-1} (Table 1). Indicator (Coliforms,and Entrococci) and pathogenic (*S.aureus*, *Salmonella* and anaerobic, spore forming) microorganisms were not detected,

Among ASF organisms 35% of the analysed Klila1 samples were contaminated (Table 1).

Lactic acid bacteria are by far the major microbial group in klila products, showing counts equal to or even lower than those of the SPC.

Yeast was present in klila 1 and klila2 at the level of LAB. However, this group was absent in klila3 produced with pasteurised Lben.

The hygienic quality of klila3 made by industrial (pasteurized) Lben was best.

2. Jben: In the traditional jben (jben1) that use vegetable rennet, the average content of aerobic mesophilic flora, coliforms and faecal coliforms was higher than in controlled jben (jben2) produced by using starters culture.

About spoilage microorganisms the results showed that the content of yeast was an average of 77 cfu g^{-1} and $2\ 10^4$ cfu g^{-1} respectively for Jben1 and jeben2 (Table 4 and 5). As expected, controlled jeben (jeben2) had yeast content higher than the traditional jeben (jeben1),

The hygienic quality of jeben2 was best than jeben1 due to use of starter culture that ferment lactose to acid lactic which prevent the growth microorganisms. 610^2, 1.510^3

The number of LAB was an average of 610^2cfu g^{-1} and $1.5\ 10^3$ cfu g^{-1} respectively for jben1 and jben 2 (Table 4 and 5).

Table 1: Microbial profiles of dry klila samples produced by manner home

Sample of klila1	SPC cfu/g	TC cfu/g	FC cfu/g	Staph cfu/g	Salm cfu/g	ASF cfu/g	LAB cfu/g	yeast cfu/g
Min	$403\ 10^3$	0	0	0	0	10	$2.6\ 10^2$	$2\ 10^2$
Average	$22\ 10^5$	0	0	0	0		$2.2\ 10^3$	$2\ 10^3$
Max	410^6	0	0	0	0	10^3	$4\ 10^3$	$6\ 10^4$
% of contam	100	0	0	0	0	35.7	100	100

Table 2: Microbial profiles of dry klila manufactured in Laboratory conditions from traditional Lben

Sample of klila2	SPC cfu/g	TC cfu/g	FC cfu/g	Stap cfu/g	Salm cfu/g	ASF cfu/g	LAB cfu/g	yeast cfu/g
Min	0	0	0	0	0	0		0
Average	$8.6\ 10^3$	0	0	0	0	0	$5\ 10^3$	$9\ 10^3$
Max	5 104	0	0	0	0	0		17.210^3
% of contam	0	0	0	0	0	Nd		

Table 3: Microbial profiles of dry klila manufactured in Laboratory conditions from industrial Lben

Sample of klila3	SPC cfu/g	TC cfu/g	FC cfu/g	Stap cfu/g	Salm cfu/g	ASF cfu/g	LAB cfu/g	yeast cfu/g
Min	0	0	0	0	0	0		0
Average	$1.2\ 10^4$	0	0	0	0	0	410^4	0
Max	$4.5\ 10^4$	0	0	0	0	0		0
% of contam	*	0	0	0	0	0	*	0

Table 4: Microbial profiles of Jben prepared by using vegetable rennet

Sample of jben1	SPC cfu/g	TC cfu/g	FC cfu/g	Staph cfu/g	Salm cfu/g	ASF cfu/g	LAB cfu/g	yeast cfu/g
Min	$2.5\ 10^3$	0	0	0	0	0	0	0
Average	$4.4\ 10^4$	$2\ 10^3$	8.210^4	0	0	0	610^2	77
Max	$7.5\ 10^4$	2.210^3		$2\ 10^3$	0	0	0	$4.5\ 10^2$
% of contam	100	12.5	25	0	0	0	*	*

Table 5: Microbial profiles of Jben prepared by using vegetable rennet and lactic leaven

Sample of jben2	SPC ufc/ml	TC cfu/g	FC cfu/g	Staph cfu/g	Salm cfu/g	ASF cfu/g	LAB cfu/g	yeast cfu/g
Min	$1.2\ 10^4$	0	0	0	0	0	0	0
Average	3.510^4	2.510^2	410^2	0	0	0	$1.5\ 10^3$	$2\ 10^4$
Max	10^5	310^4	10^3	0	0	0	$3\ 10^3$	$3\ 10^4$
% of contam	100	27	25	0	0	0	*	22

The hygienic quality of jben 2 was best than jeben1, this is due to effects of starter culture that produce lactic acid, which in turn manufacture affects several aspects of cheeses manufacture, including coagulant activity, retention of coagulant in the crud, rate of proteolysis

during storage, cheese yields, cheese moisture and rate of pH decline in the cheese [6]. All those factors help in the preservertion and stability of the product.
The pathogenic flora was absent in jben.

DISCUSSION

1-Klila: The SPC count in dry klila samples purchased from different maker were higher than recorded in klila2 and klila3 prepared in laboratory. However, these counts are normal in klila produced with raw Lben and they are very inferior to those abtained in jben analysed by other authors (hamama.....Aboulala) and including in stanadard limits accepted in Morocco for raw cheese. According to Benkerroum *et al*. [7] raw Lben had a standard plate count ranging from 1.6 to 6.8 10^6 ufc/ml.

Indicator and pathogenic microorganisms were not detected relevant data will be due to the combination of these factors: heating, low aw reached by sun drying of the curd, as well as the nature of the Lben used for making klila, which is reported to have pH ranged from 3.8 to 4.7, titrable acidity ranged from 63 to 110°D [8]. These circumstances, prevent the microbial growth of undesirable microorganis and/or more contamination. So that the value obtained in klila told about an improving of bacteriological safety of klila.

In fact, removal of water drying has been used to preserve foods since antiquity by lowering the aw, which prevent microbial growth, this methods is easily adapted in devoloping/tropical countries where refrigeration facilities are lacking.

Klila1 samples were contaminated (Table 1). These results might be due to the poor sanitary conditions during klila processing. In fact, dry klila usually produced under traditional conditions and is handled at various stages, thus, various types of microorganismes may enter during klila making and subsequent hadling on the other hand, ASF are not sensitive to heat-treatment, Their presence (ASF) can lead to food poisoning.

The predominance of LAB in dairy products is a reassuring factor and has a long and safe history of use as preservatives especially in cheese making [9].

Because, LAB have been use for centuries in the fermentation of foods, not only for flavor and texture development but also for their ability to produce antimicrobial compounds such organic acid, Hydrogen peroxide and bacteriocin, which prevent the growth of spoilage an pathogenic bacteria.

Yeast was present in klila 1 and klila2 at the level of LAB because the fungic flora is sensitive to heat-treatment. The high counts of the fungic flora are related to their h igh resistance to low values of the aw or possibly owning to the lesser environmental contamination in the production and ripening rooms at ambient temperature.

The co-existence of yeast and LAB may occur in a balanced-system in those klila products which were kept for a long time. In fact, LAB and yeast are common in a wide range of African traditional foods and beverage fermentation and their interaction may constitue an important factor for the flavors and the texture of the klila. Also, yeast is not sensitive to producing LAB acidity.

Quality of klila3 made by industrial Lben was best. For possible industrial of those kind of cheese, the Lben must be controlled by pasteurization. In dairy products many methods are applied in order to extend their shelf-life and to improve their organoleptic quality such aw decrease by salting and/or drying and pH slow down by lactic acid fermentation. In klila technology, prevention of microbial growth and/or more contamination is achieved by using Lben with a low pH o, heat-treatment and by sun drying curd.

2. Jben: Traditional jben (jben1) that use vegetable rennet, the average content of aerobic mesophilic flora, coliforms and faecal coliforms was higher than in controlled jben (jben2) our results were lower that those found in others varieties of soft cheese like Egyptian karish [10], Mexican Fresco cheese [11], Turkish white cheese [12] and Moroccan cheese (Jben) [13-15] and was inferior to the maximum stipulated by the Moroccan legislative standards [16]. Indicating the effecteness of heat treatment and/or inhibition by the highly competitive LAB which it has been reported to have an intensive antagonistic activity against *E. coli* [17].

Controlled jeben (jeben2) had yeast content higher than the traditional jeben (jeben1), which may be present in milk or contaminate the milk after pasteurisation or possibly owning to deficient hygienic conditions maintained during the cheese manufacture. Since they are tolerant to the hostile environement such decrease of pH, low aw and the decrease of titrable acidity. It suggested that pasteurisation of milk and good hygiene during cheese making could prevent yeast growth, because they were able to support acidity and hight salt concentration. However these results were inferior to those found by Benkerroum and Tamime [18], which reported were an average superior to 10^6 cfu g^{-1}. It has been reported that presence of yeast and molds at high level can metabolize lactic acid, as well as liberate alkaline coumpounds during

breakdown of the proteins, resulting in pH values near neutral. this exposes curd to the risk of pathogen growth and to main faults in the products such as slimy appearance, discoloration, alayer surface growth and strong alcohol odour [18] but in our case, the yeast were found at moderate level which may be contribute to cheese flavours.

Quality of jeben2 was best than jeben1 due to use of starter culture. This gives that LAB was by Far the most major microbial group in the production of jben2, showing counts nearly equal to the use of the total aerobic mesophilic flora. This finding is in agreement with the result of Hamama [19] which has reported that the microflora of jben was dominated by LAB (10^8-10^9 cfu g^{-1}). The predominance of LAB in jeben2 is resulting to use of starter culture and to a weak amount of rennet, adding to a long period of coagulation [20].

The Absence of pathogenic flora in jben and klila indicated the maintain of proper sanitary procedures during production, handling and cheese processing and the effect of pasteurization of cheese milk and the use of starter culture.

CONCLUSION

Compared to jben that may be vehicles for toxigenic *E. coli* the hygienic risk from klila may be lower because of the intrinsic characteristic that depend on the technology used in each product. In klila, preservation is aided by heat-treatement and drying, however in jben prevention of microbial growth and/or more contamination is resulting of both heat-treatement and use of starter culture and/or vegetable rennet. In further studies we hope to tell more about LAB sauvery, biochemical properties and microbial profiles during fermentation of klila and jben.

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