

Physico-Chemical, Microbial and Sensory Characterisation of Moroccan Klila

^{1,3}Z. Mennane, ³M. Faid, ²M. Lagzouli, ²M. Ouhssine,
²M. Elyachioui, ²E. Berny, ²M. Ennouali and ¹K. Khedid

¹National of Healthy Institute, Avenue Ibn Battouta Rabat Agdal, BP 769, Morocco

²Department of Biology, Faculty of Sciences, Laboratory of Microbial Biotechnology,
Université Ibn Toufail, BP 133 Kénitra, Morocco

³Food Laboratory of Microbiology and Biotechnology Agronomic
Institute and Veterinary Hassan Rabat, Morocco

Abstract: Klila is a Moroccan traditional cheese produced from raw cow's milk, without the addition of starter culture and controlled klila produced in laboratory conditions using mesophilic and thermophilic starter culture were studied to investigate their microbial, physico-chemical and sensory characteristics. Sensorial evaluation of controlled and traditional klila samples was carried out by sensory panel. The controlled klila showed a wide acceptance among sensory panels. The finding of this study suggest that more future research on Moroccan Klila should characterise the changes in microflora, biochemistry, texture during ripening and their correlation with sensorial changes. The results revealed that all samples had an acceptable hygienic and technological quality.

Key words: Klila • traditional • controlled • hygienic and quality

INTRODUCTION

Many varieties of cheese are known throughout the world, more than 1000 varieties of cheese are produced around the world [1]. 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 [2], 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

However, traditional Moroccan varieties have not been studied exhaustively and are still made by the traditional manufacture at the smal scale, certain of varieties have a good quality and are generally appeared in local markers for attributes such texture, aroma, processing properties and pleasant flavour to national and international markets if a large scale manufacture process could been implemented. It is thus, important to have knowledge on the current microbiological and biochemical characteristics change during manufacture and repairing, which determined not only their nutritional value and their organoleptic

properties but also for improvement of traditional, small- scale and household level fermentation process and to preserve their national food heritage. On the other hand, the introduction of appropriate starter toward improved safety, quality and security of traditional product.

The klila is a fresh cheese prepared empirically in certain Moroccan areas by Heating (70°C) of leben until curdling, then the curd was drained (Fig. 1).

In Morocco, Klila is most popular local dairy products (traditional cheese) and their traditional method of cheese making is still in use nowadays industries to make klila. 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 of cheese are well known throughout the world. However, no studies are being focused on Moroccan varieties cheese such Klila. There is no data on their microbial and biochemical characteristics and on the technological processing.

About, controlled klila is produced according to a protocol which using starter culture (Fig. 2).

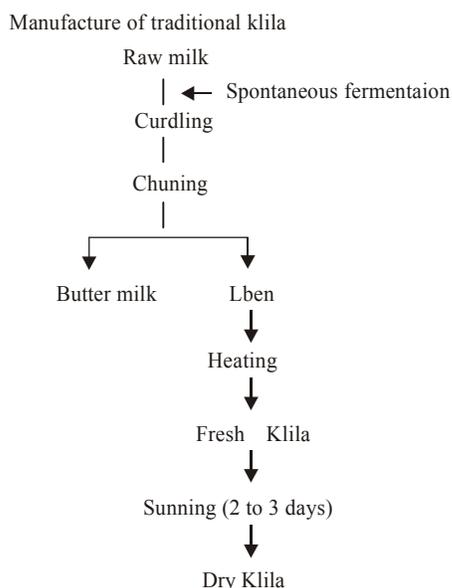


Fig. 1: Flow sheet for manufacture of traditional klila (Moroccan cheese)

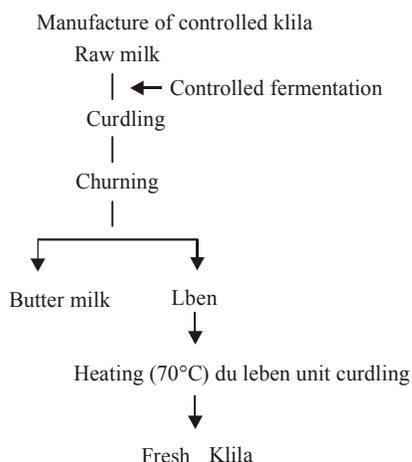


Fig. 2: Flow sheet for manufacture of controlled klila (Moroccan cheese)

In present work, we have investigated the microbiological and physico-chemical characteristics of two Moroccan varieties cheese traditional Klila and controlled klila prepared in laboratory conditions.

MATERIALS AND METHODS

1. 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 tree of klila 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.

1.1. Aerobic mesophilic bacteria: Total bacterial number of sample were determined on standard plate count agar (SPC, Oxoid, England), after incubation at 30°C for 48 h according to the standard method for examination of dairy products [3].

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

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

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

1.5 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.

1.6 Lactic acid bacteria (LAB): LAB counting were performed on MRS agar at pH 6.2 [4] incubated at 30°C for 48h.

1.7 Spore-forming bacteria: To enumerate the spores of spore forming micro-organisms, the samples were

2 Chemical characteristic

pH: Were measured by using an apparatus with digital display (consort).

Acidity: 10 g of sample is titrated by soda (NaOH N/9) in presence of phenolphthalein until the turn of colouring. The results are expressed in dornic degree (1D° corresponds to 1 dg/L of lactic acid).

Total solid (TS): Obtained by staving of a mass of cheese at 105°C/24 h.

Ashes: Total solid is precalcined and incinerated in an oven electric with 500°C during 6 h.

Output: Quantity of cheese obtained from a given quantity of milk

Total nitrogen: Is determined by the method of Kjeldal.

Determination of the fat content: It is given by using the methods of soxhlet.

3 Sensory evaluations: Sensory evaluation of klila samples was performed by a sensory panel composed of 10 members. Prior degrees and special attention was given to the taste, flavour, odour, colour and texture.

RESULTS

The microbial characteristics of the investigated products were summarised in Table 1 and 2 for traditional klila and in Table 3 and 4 for controlled klila (klila1, klila2, klila3, klila4, klila5 and klila6, respectively).

Physico-chemical and microbiological analysis Traditional klila: The physicochemical analysis show (Table 1) an average of pH about 4.14 with an average output of cheese about 16.85%. Indicator (Coliforms and

Enterococci) and pathogenic microorganisms (S.aureus, Salmonella and anaerobic spore forming) were not detected, Lactic acid bacteria are by far the major microbial group in traditional klila products,.

Physico-chemical and microbiological analysis of controlled fresh cheese (klila) obtained by using thermophilic starter culture: Physicochemical Results (Table 3) showed an average of acidity, 118D° for klila2 and 108D° for klila1.

The microbiological analysis (Table 4) showed an average of SPS about 18 10⁴, 10⁶, 14.3 10⁴ and 82.5 10⁴ cfu/ml respectively in klila1, 2, 3 and 4. However, the enterococci and staphylococci were not detected; the total and fecal coliform were found only in klila3.

Physico-chemical and microbiological analysis of controlled fresh cheese (klila) obtained by using the mesophilic starter culture: The results (Table 3) an average of proteins (10.38% for klila5 and 11.76% for klila6), an average of fat (12.65% for the klila5 and 7.7%

Table 1: results of physicochemical characteristics of traditional klila

Klila	No. Samples	output %	pH	AcidityD°	TS	Ashes
cheese	7	16.85	4.14	73	28.14	*
whely	7	*	4.17	62.14	8.01	0.62

* TS: Total solid, TN: total nitrogen

Table 2: Results of Microbiological analysis of traditional klila

Klila	No. Samples	SPC 10 ⁶ cfu/ml	TC 10 ³ cfu/ml	FC 10 ³ cfu/ml	Enté 10 ⁴ cfu/ml	Heat-resist 10 ³ cfu/ml	ASF cfu/ml	Stap 10 ⁴ cfu/ml	Salm cfu/ml	LAB 10 ⁶ cfu/ml	yeast 10 ⁶ cfu/ml	Mould 10 ⁶ cfu/ml
tradition Klila	7	5.1	---	0	0	5.8	-	0	0	3.9	4.35	2.1

SPC: Standard Plate Count. ASF: Anaerobic Spore Forming TC: Total Coliforms LAB: Lactic Acid Bacteria FC: Fecal Coliforms Salm: Salmonella Stap: Staphylococci Yeast: Yeast and moulds

Table 3: Results of physicochemical characteristics of controlled klila

Klila	No. samples	Type of leaven	output %	pH cheese	pH whey	Acidity cheese D°	Acidity whey D°	Tscheese D°	TS whey D°	Fat %	TN %
Klila1	6	ThermLv1	20	4.64	4.76	108	60.25	27.5	6.7	8.22	14.58
Klila2	8	ThermLv2	21	4.7	4.8	118	64.3	35	7.5	9.2	16.04
Klila3	3	ThermLv3	18.7	4.43	4.36	72	56	33.33	8	9.48	16.02
Klila4	2	ThermLv4	18	4.58	4.4	45	43.5	28	7	10.07	14.6
Klila5	3	MesoLvm1	20	4.28	4.26	75	57	23.7	6.7	12.68	10.38
Klila6	4	MesoLvm2	22	3.8	3.98	118.5	83.3	25.3	7	7.7	11.76

TS: Total solid, TN: total nitrogen

Table 4: results of Microbiological analysis of controlled klila

Klila	No.	Type of	SPC	TC	FC	LAB		yeast	
	Samples	starter culture	10 ⁴ cfu/ml	10 ³ ucfu/ml	cfu/ml 10 ³	Entéroccfu/ml 10 ³	Stap	10 ³ cfu/ml	10 ⁴ cfu/ml
Klila1	6	ThermLv1	18	0	0	0	0	12	87
Klila2	8	ThermLv2	101	0	0	0	0	1	79.4
Klila3	3	ThermLv3	14.3	11	5.3	0	0	0	17.3
Klila4	2	ThermLv4	82.5	0	10	0	0	2	6
Klila5	3	MesoLvm1	15	0	0	0	0	0	1
Klila6	4	MesoLvm2	78	0	0	0	0	1	31

SPC: Standard Plate Count. ASF: Anaerobic Spore forming TC: Total coliforms LAB:lactic acid bacteria FC: Fecal coliforms Salm: Salmonella Stap: staphylococci Yeast: Yeast and moulds

for klila6), an average of pH (4.26 and 3.8 respectively for klila5 and 6), an average of acidity (75D° for klila5 and 108 D° for the klila 6) and an average of total solid (6.7%).

The microbiological analysis showed different concentration of SPS: 15.10⁴ for klila5 et 6.8 10⁴ cfu/g for klila6. Average of yeast reached was 3.1 10⁵ in the klila6. Also, we noted the absence of the pathogenic micro-organisms (Staphylococci) and the indicators of contamination (coliforms and the enterococci).

DISCUSSION

Physico-chemical and microbiological analysis

Traditional klila: The physicochemical analysis show (Table 1) an average of pH about 4.14 with an average output cheese of 16.85%. The low value of the output of cheese suppose a possible water addition of the leben. Also Fat content was low, because process Lben required the separation of fat after churning

The SPC count in traditional klila samples shows an average of 5.1 10⁶ cfu/g (Table 1). This value was inferior to that obtained in others Moroccan traditional cheese such jben hamama [5], Aboulala [6]. This result is included in standard limits, accepted in Morocco for raw cheese. According to Benkerroum [7] raw Lben had a standard plate count ranging from 1.6 to 6.8 10⁶ cfu/g. Indicator (Coliforms and Entrococci) and pathogenic microorganisms (S.aureus, Salmonella and anaerobic spore forming) were not detected, because of the heating, 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 growth of micro-organism and/or more contamination. The value obtained in klila told about an improving of bacteriological safety of klila.

Lactic acid bacteria are by far the major microbial group in traditional klila products, showing counts equal

to or even lower than those of the SPC. 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 flavour 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.

These microbiological values are relatively reduced compared to others works mainly those reported on Italian cheese "Fossa"[10], the Mexican cheese "fresco" [11], Moroccan cheeses [4] and the Turkish cheese "Beyaz peynir" [12].

Physico-chemical and microbiological analysis of controlled fresh cheese (klila) obtained by using thermophilic starter culture:

Physicochemical Results (Table 3) showed high values of acidity, 118D° for klila2 and 108D° for klila1. This can be explained by the strong activity of the lactic acid bacteria of the leaven used whereas the values of acidity of klila 3 and the klila4 are low because of the insufficient activity of starter culture used or because of a complete coagulation of milk. In addition, they have a low fat content (8.22% for klila1, 9.2% for klila 2, 10.07% for klila 3 and 9.48% for klila4) because the process eliminates the fat with the whey.

The product is also characterized by an important total solid and an important output of 21, 20, 18.7 and 18% respectively for the klila 1, klila2, klila3 and klila4 (Table 3) the product showed a normal and acceptable contents of protein (14.58, 16%, 16.02 and 14.6% respectively).

The result of titrable acidity are higher than those found by Hamama [4], Aboulala *et al.* [5] and Mahi [13] whereas the contents of fatty content is lower.

The microbiological analysis (Table 4) showed a low number of SPS about 18 10⁴, 10⁶, 14.3 10⁴ and 82.5 10⁴ cfu/ml respectively in klila1, 2, 3 and 4.

However, the enterococci and staphylococci were not detected; the total and fecal coliform were found only in klila3.

Number of yeast are $8.7 \cdot 10^4$ cfu/g, $8 \cdot 10^5$ cfu/g, $1.7 \cdot 10^5$ cfu/g and $6 \cdot 10^4$ cfu/g respectively in klila1, 2, 3 and 4.

Physico-chemical and microbiological analysis of controlled fresh cheese (klila) obtained by using the mesophilic starter culture: The results (Table 3) showed low values of proteins (10.38% for klila5 and 11.76% for klila6), low content of fat (12.65% for the klila5 and 7.7% for klila6), low values of pH (4.26 and 3.8 respectively for klila 5 and 6), high acidity ($75D^\circ$ for klila5 and $108 D^\circ$ for the klila 6) and an important total solid (6.7%) due to the fat content diffused in the whey during the process. But the fat content remains lower than those reported by Mahi *et al.* [12].

The microbiological analysis showed different concentration of SPS: $15 \cdot 10^4$ for klila 5 et $6.8 \cdot 10^4$ cfu/g for klila 6. Average of yeast reached was $3.1 \cdot 10^5$ cfu/g in the klila 6. Also, we noted the absence of the pathogenic micro-organisms (Staphylococci) and the indicators of contamination (coliforms and the enterococci).

CONCLUSIONS

After this work we retained the following conclusions:

- The klila showed a satisfactory hygienic, nutritional and technological quality (low in fat content, satisfactory content of protein). Which is conform to standards.
- The cheese klila prepared by controlled fermentation presents a microbiological and physico-chemical characteristics more important than those of traditional klila.
- The fresh cheese klila prepared by the thermophilous starter culture Therm Lv2 comes in first row because it presents 21%, of output, 16.02% of proteins, $118D^\circ$ of acidity, a satisfactory hygienic quality (absence of the pathogenic micro-organisms and contaminations) and an important organoleptic characteristics.

The hygienic quality of the klila is better than the cheese "jben" analyzed by other Moroccan authors.

Some Strains of lactic acid bacteria, used in this studie, could be selected because of their desirable acidifying capacity. You want to tell about the changes of physicochemical and microbiological characteristic of klila during its storage (refining).

REFERENCE

1. Hayaloglu, A.A., M. Guven and P.F. Fox, 2002. Microbiological, biochemical and technological properties of turkish white cheese «Beyaz Peynir». In. Dairy Journal, 12: 635-648.
2. De Vuyst., 2004. Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends in Food Science and Technology, 15: 67-78.
3. A.P.H.A., 1985. Chemical and physical methods, In G. H. Richardson (ed), Standard Methods for the Examination of Dairv Products, 15th ed. Amer. Pub. Hlth. Assoc., Washington, D.C., pp: 327-404.
4. Reuter, M., 1985. Chiral anomalies and zeta-function regularization. Phys. Rev., D 31: 1374-1385.
5. Hamama Abed, 1989. Stadies of the hygienic quality of Moroccan traditional dairy products. Ph.D. thesis university of Minnesota. USA., pp: 1-35.
6. Aboulala Fouzia, Hamama Abed, Zahar Mohamed, El Marrakchi Abelhaq et Bent Mohamed Aberrahman Meriem, 1995. Actes Institut Agronomique et Vétérinaire Maroc, 15(4): 21-26.
7. Benkerroum, N. and A.Y. Tamime, 2004. Technology transfer of some Moroccan traditional dairy products (lben, jben and smen) to small industrial scale: A review. Food Microbiology, 21(4): 399-413.
8. Boubekri, C., A. Tantaouin Elaraki, M. Berrad and N. Benkerroum, 1984, Caracterisation physico-chimique du Leben marocain. Le lait, 64: 436-447.
9. Sullivan, L.O., R. Rosi and C. Phill, 2002. Potencial of bacteriocin lactic acid bacteria conprovements in food safety and quality. Biochimie. 84: 593-604. Direction d'élevage, Réglementation du lait et produits laitiers
10. Gobbetti, M., B. Folkertsma, P.F. Fox, A. Corsetti, E. Smachi, M. DeAngelis, J. Rossi, K. Kilcawley and M. Cortini, 1999. Microbiology and biochemistry of Fossa (Pit) chesse. International Dairy Journal.
11. Toress Llanez, M.J. and B. Vallejocordoba, 2004. Characterisation of the natural microflora of artisanal Mexican Fresco cheese.
12. Turantas, F., A. Unluturk and D. Goktan, 1989. Microbiological and compositional. Journal of Food Microbiology, 8(1): 19-24.
13. Mahi Nouredine, Hamama Abed, et Abouddrar Wafae 1995. Actes Institut Agronomique et Vétérinaire Maroc, Vol. 15(3): 27-32.