RESEARCH ARTICLE Asian Journal of Dairy and Food Research, Volume 40 Issue 2: 157-161 (June 2021)

Physico-chemical Parameters with Direct Influence on the Dynamism of the Indigenous Microflora of the Traditional Cheese "J'ben *Elgafs*"

Z. Saidane, A.A. Dahou, H. Tahlaiti, M. Daoudi, K. Doukani, A. Homrani

10.18805/ajdfr.DR-224

ABSTRACT

Background: The microbial evolution of the J'ben *Elgafs* prepared with raw milk from local cows, was studied during the manufacturing and maturating process in order to characterize this variety of cheese from the Algerian terroir.

Methods: The microbial activity and physical-chemical parameters were tested during the three dairy seasons of the year. Total, lactics and alteration floras were counted on their selective environments.

Result: Lactic germs multiply considerably during the first days and only stabilize towards the end of maturation. The low presence of alteration floras is the result of the continuous modification of the physico-chemical parameters of Aw and pH from one stage to the other of the j'ben production and the respect of good processing practices. These different proportions of variation are induced by the biochemical reactions and microbial interactions that take place responsible for the sequential growth of one microbial group compared to another.

Key words: J'ben *Elgafs*, Maturation, Microbial activity, Physico-chemical parameters.

INTRODUCTION

Any traditional foods reflect a symbiotic relationship between the physical characteristics of the region and the culture of the rural community (Licitra, 2010). The cheeses are classified according to their typicality, derived from the characteristics of their land of origin (Dahou *et al.,* 2021; Delfosse, 2006; Montel *et al.,* 2014).

J'ben *Elgafs* is one of them, a local dairy product, originating from Boussaâda, a town in northeastern Algeria. It is classified as a mature soft cheese, prepared with raw milk, under the fermentative action of the original microbial flora and consumed after 15 days of ripening (Meribai *et al.,* 2017).

Due to its empirical nature and low level of uniformity and sanitary guarantee, the traditional technology of making j'ben *Elgafs remains* local and unknown.

Our objective is the understanding the most representative microbial dynamics during the manufacture and maturation of the j'ben *Elgafs* and its correlation with some of the most relevant physico-chemical parameters and beyond, to know the maturation phenomena responsible for the development of the organoleptic characteristics of j'ben that take place.

MATERIALS AND METHODS

Sampling

The milk was collected simultaneously from three farms operating local cows in an area of western Algeria, for a total of nine samples. The sampling was carried out during three milk production seasons in 2019-2020. In the summer and fall of 2019 "middle season," in winter "low season" and in spring 2020 "high season."

Laboratory of Sciences and Technics of Animal Production, Abdelhamid Ibn Badis University, Mostaganem-Algeria.

Corresponding Author: Z. Saidane, Laboratory of Sciences and Technics of Animal Production, Abdelhamid Ibn Badis University, Mostaganem-Algeria. Email: zohra.saidane.etu@univ-mosta.dz

How to cite this article: Saidane, Z., Dahou, A.A., Tahlaiti, H., Daoudi, M., Doukani, K. and Homrani, A. (2021). Physico-chemical Parameters with Direct Influence on the Dynamism of the Indigenous Microflora of the Traditional Cheese "J'ben *Elgafs*". Asian Journal of Dairy and Food Research. 40(2): 157-161. DOI: 10.18805/ajdfr.DR-224.

Submitted: 29-01-2021 **Accepted:** 14-04-2021 **Online:** 29-04-2021

The milk was transported to the laboratory in sterile and refrigerated containers (4°C). The prepared j'bens were sampled at the demoulding of the coagulum, after 10 and 15 days of maturation. Each sample was subjected to physico-chemical and microbiological analyses.

The cows are raised in extensive breeding, whose health and performance remain dependent on fodder and weather hazards.

Handerafted manufacture of j'ben *Elgafs*

After collection, the milk mixture is placed in a basin and then delivered to itself without the addition of rennet. 24 to 72 hours later, the fermented milk is gently heated to 35°C and then drained. The draining time depends on the consistency of the desired paste. The moulding of the coagulum is done manually to obtain an ovoid shape and then deposited on the stems of a plant called "El halfa"*(Stipa tenacissima*) softened beforehand (Fig 1). These stems are

shaped like a cage, hence the name *Elgafs.* The cage is directly closed on the curd.

Finally, the j'ben *Elgafs* (Fig 2) is placed in a room where the temperature is between 12 and 15°C and the humidity level is between 90 and 95% during 15 days of maturation.

The samples of j'ben were transported to the laboratory and analyzed as soon as they arrived. The physico-chemical analyses were determined in accordance with the I.D.F standard (ISO 707/ I.D.F 2018).

Microbiological analysis

This study was carried out at the Laboratory of Sciences and Technics of Animal Production of Mostaganem University, Algeria.

For each sample, 1 ml of milk or 1 g of cheese sample to be analyzed was added to 9 ml of sterile physiological water. A 10^{-1} stock dilution was thus obtained, from which decimal dilutions up to 10^{-10} were made. 1 ml volumes of each dilution were inoculated in depth with media specific to each microbial flora sought (Benamara *et al.* 2016, Benlahcen *et al.,* 2017).

The culture media used and incubation conditions for the isolation of microbial flora (Table 1) were recommented by Guiraud (2003) and Idoui and Karam (2008).

Statistical analysis

The statistical correlations between the microbial group counts and the studied physico-chemical parameters were achieved by the Pearson correlation coefficient, using the R software, version 4.0.3, 2020. The threshold of statistical significance is estimated at P< 0.05.



Fig 1: J'ben *Elgafs* maturing in his El halfa cage *"Stipa tenacissima."*



Fig 2: J'ben *Elgafs* at the end of maturation.

RESULTS AND DISCUSSION

Bacterial counts performed over the 03 lactation periods show excellent repeatability for the 03 flora studied with an average incidence as shown in Table 2. For the total flora on PCA medium, the average log count is 5.16 cfu.g^{-1} at demoulding versus 5.08 cfu.g^{-1} at the end of ripening.

The lactic flora recorded gave the following results: On the MRS medium; the average count of logarithms is 5.4 cfu.g^{-1} at demoulding against 6.34 log cfu.g^{-1} at the end of maturation, on the M17; the average count of logarithms is 7.29 log cfu.g^{-1} at demoulding against 6.05 log cfu.g^{-1} at the end of maturation and finally on the MSE culture medium; 5.44 log cfu.g^{-1} at demoulding against 6.32 log cfu.g^{-1} at the end of maturation.

The alteration flora represented the following as an average logarithmic count: on the VRBG 4.13 log cfu.g^{-1} at demoulding versus 1.3 log cfu.g^{-1} at the end of maturation. For the fungal flora on the OGA, the average log count 3.83 cfu.g^{-1} during demoulding and represents 5.07 log cfu.g^{-1} at the end of maturation of the j'ben *Elgafs*. Fungal flora is indefinite on D+9 because during this phase of maturation, yeasts and moulds are abundant and tend to consume the lactic acid released by acidifying flora to allow other microbial floras not tolerant to acidic environments to proliferate with a triggering of the lipolytic and proteolytic activities necessary for the refining of the J'ben (Caridi *et al.,* 2003).

The population levels achieved are comparable to those observed on similar cheeses made from natural cow's milk (Benamara *et al.,* 2016, Benlahcen *et al.,* 2017) and much more than those provided by Dahou *et al.,* 2020 and Montel *et al.,* 2014.

Based on the results obtained for the three periods of high, medium and low lactation and compared to those obtained on similar cheeses by Benyagoub *et al.* (2016); it appears that the dominance of microbial species varies with the maturation time; the specific media used allowed the isolation of 05 flora; the MRS medium determined a lactic flora presumed to lactobacilli, the M17 medium characterized a lactic flora presumed to contain lactococci, the MSE medium isolated the leuconostocs, the VRBG medium ensured the enumeration of an alteration flora essentially constituted by enterobacteriaceae and the OGA medium was specific for the fungal flora of the ripening (Fig 3).

These dominance proportions are different throughout maturation process because of the biochemical reactions and bacterial interactions that occur responsible for the sequential growth of some groups compared to others.

Monitoring of the average evolution of the microbial flora during the three phases of lactation has revealed that *Lactococcus* was in the majority at the beginning of processing and then regressed as the cheeses mature to allow the fungal and natural flora signalled by the milk and the protective plant El halfa "Stipa tenacissima" to take over; only Lactobacilli and Leuconostocs remain at a higher level. The Enterobacteriaceae, although they have not completely disappeared at the end of the maturing process, but their

decrease has been visible. These results are in line with those of Benlahcen *et al.* (2017) and Benamara *et al.* (2016).

The continuous modification of the environment of the cheese ecosystem with different physiological characteristics from one stage to another of the j'ben *Elgafs* whose temperature, water activity and pH without forgetting the nutritive conditions lead to this difference in the quantitative appreciation of the microbial populations characterized.

The composition of the milk microbiota depends directly on the composition of the microbiota in the tanks that are in contact with the milk "raw material" teats and milking equipment (Ferdous *et al.,* 2017, Pankaj *et al.,* 2020). Good milking practices, mainly careful teat care and teat washing, as well as disinfection of milking utensils, had a significant impact on the development of the undesirable spoilage flora (0%), represented by bacteria of faecal and non-faecal origin.

The majority of useful microflora, with respective rates of 31% on MRS, 30% on M17 and 22% on MSE, comes from animal and feed from the surrounding meadows of the extensive breeding of local cows as highlighted by Madani *et al.* (2004). For the j'ben native cheese technology, lactic acid bacteria have played a crucial role in the selection of the microorganisms initially present in the milk and which, at the end of the ripening process, are self-sufficient to act as a barrier against spoilage.

Temperature control with curdling temperature maintained between 25 and 28°C allowed the optimal growth of lactic acid bacteria isolated on the specific culture media, MRS, M17 and MSE and promoted rapid acidification limiting the proliferation of spoilage flora. This result is in line with that obtained by Caridi *et al.* (2003) on a similar cheese.

The maturation stage carried out in a controlled environment at maturation temperatures of 12 to 15°C, favoured, on the one hand, the development of acidophilic lactic flora with presumed species (Fig 4), 26% attributed to lactobacilli on the MRS culture medium, 25% to leuconostocs on the MSE medium, 24% to lactococci on M17 and on the other hand to psychrtrophical flora represented mainly on the OGA culture, 20% by yeasts and moulds with lipolytic and proteolytic technological properties giving the organoleptic characteristics typical of cheese j'ben *Elgafs*. The rest, 5% represented by enterobacteriaceae at the end of the refining process can be attributed to contamination by the production environment and the tools used that come into contact with the j'ben cheese.

The control of the relative humidity of the ripening room at 90-95% with the preservation of the protective envelope of the cheeses by a material plant *"Stipa tenacissima"* typical to the region is a selective form to the growth of a fungal flora of interest that gradually evolves at the end of maturation.

Table 1: Culture media used and incubation conditions for the isolation of microbial flora.

Microbial flora	Isolated environments	Temperature °C	Duration	Incubation
Total flora	PCA pH = 6.5	37 and 45	72 hours	Aerobiosis
Lactic flora on M17	M17 pH = 6.5	30 and 37	72 hours	Aerobiosis
Lactic flora on MSE	MSE Hypersalted 6.5% pH = 9.6	25 and 30	5 days	Aerobiosis
Lactic flora on MRS	MRS pH = 6 and pH = 5.5	37 and 45	72 hours	Anaerobiosis
Alteration flora	VRBG pH =7.4	37	18 and 24 hours	Aerobiosis
Fungal flora	OGA pH = 5.6±0.2	15 and 25	5 days	Aerobiosis

Table 2: Average incidence of native microflora during the manufacture of j'ben Elgafs over 03 lactation periods.

Type of analysis	Milk cow Day D	Cheese with demolding Day D+1	Cheese in maturation Day D+9	Cheese to end maturation Day D+14
Physico-chemical				
pH	6.64	4.63	4.99	5.38
Aw	0.916	0.705	0.685	0.672
Temperature in °C	28±2	25±3	15±1	15±3
Microbiological (average log count cfu/ml or g)				
Total flora on PCA	3.95	5.16	5.11	5.08
Lactic flora on MRS	7.60	5.40	6.34	6.34
Lactic flora on M17	7.29	7.31	6.08	6.05
Lactic flora on MSE	5.36	5.44	6.24	6.32
Alteration flora on VRBG	0	4.13	0	1.3
Fungal flora on OGA	4.23	3.83	I.C	5.07

I.C : Indefinite count.

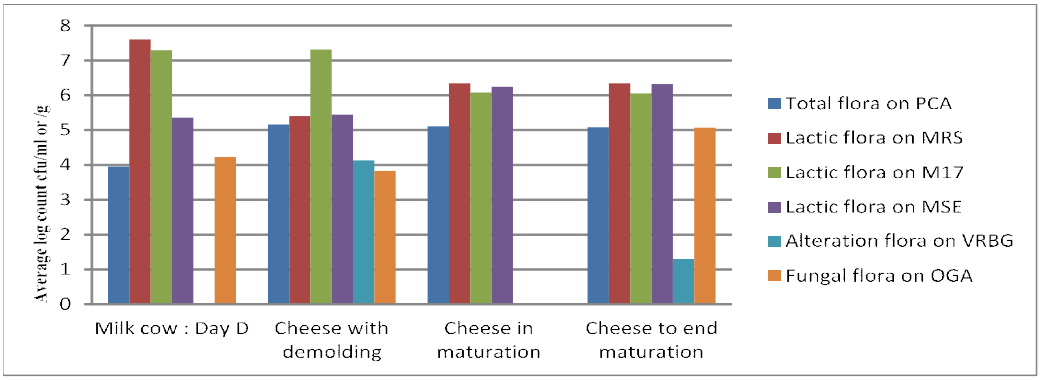

Fig 3: Evolution of microbial flora isolated during the manufacture of the j'ben *Elgafs.*

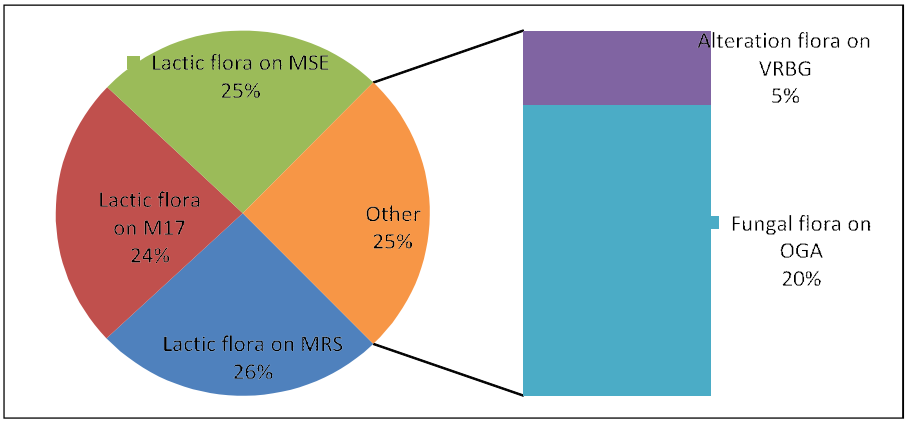

Fig 4: Availability of microbial flora listed on finished cheese product j'ben Elgafs.

The evolution of pH during the cheese-making process is also responsible for the sequential growth of the various listed microbial groups that colonize cheese over time (Benlahcen *et al.,* 2017; Benyagoub *et al.,* 2016; Meribai *et al.,* 2017). At the beginning of maturation (when the cheese is removed from the mould) the pH value is quite low (around 4.63), due to the acidification of cheese curd by lactic acid bacteria, the main agents of lactic fermentation. This pH value evolves towards 5.38 because of the gradual proliferation of yeasts which consume lactic acid on the surface essential for the proliferation of useful moulds of the the penicillium genus brought back by the plant material *"Stipa tenacissima*".

Controlling Aw water activity around 0.9 is a parameter that defines the availability of water in the curd by controlling the surrounding atmosphere (Caridi *et al.,* 2003). Indeed, cheeses are complex in their chemical composition; they usually contain a solid phase consisting of a protein gel containing a fat emulsion and a liquid phase consisting of an aqueous solution of lactose, lactic acid, mineral salts, soluble proteins. In such an environment, water is not completely free, but bound to the substrate by multiple interactions: solvent water of small dissolved molecules, water hydration of sugars, salts, proteins, water physically blocked by capillarity, by absorption; the remaining water is considered free; it can intervene in microbial, enzymatic and chemical reactions that can transform the cheese substrate and thus obtain the typical organoleptic qualities. The measurement of relative equilibrium humidity (R.H.E between 90 and 95%) directly reflects this availability of water to maintain stable water activity and trigger the metabolic activities necessary for the maturation of the cheese j'ben *Elgafs.*

The statistical analysis of the results, carried out by software R, version 4.0.3, 2020, gave significant values ($P<0.05$) with a direct impact on the development of the lactic flora and the controlled physico-chemical parameters of Aw and pH having a direct effect on the different metabolisms that are established for the enzymatic maturation of the curd (by glycolysis, lipolysis and proteolysis) that give j'ben Elgafs its texture, its organoleptic qualities and its typical nutritional value.

CONCLUSION

The influence of physico-chemical parameters on the evolution of the microorganisms studied has been proven. The changes that occurred on the j'ben *Elgafs* during maturation did not in any way create conditions too restrictive for the majority of microbial groups invested since the accounts were consistent during maturation and even less for Enterobacteriaceae. The divergence in traditional practice at different stages of milk production and cheese

maturation, leads to a versatility of environments and microbial consortium. The challenge is to maintain a quality microbial diversity to preserve environmental biodiversity, but also to maintain the typicality and to offer a cheese of origin ever safer and quality elaborated in accordance with tradition.

ACKNOWLEDGEMENT

I would like to thank all the staff of of the Laboratory of Sciences and Technics of Animal Production and the DGRSDT for their contribution to the development of scientific research in Algeria. My thanks also go to the managers of the “Hatabi, Hadj Zoubir and Hadj Kaddour” farms for providing me with the materials necessary to make the cheese and the samples necessary to carry out this study.

REFERENCES

Benamara, R.N., Gemelas, L., Ibri, K., Moussa-Boudjemaa, B. and Demarigny, Y. (2016). Sensory, microbiological and physico-chemical characterization of *klila*, a traditional cheese made in the south-west of algeria. african. Journal of Microbiology Research. 10(41): 1728-1738.

Benlahcen, K., Mahamedi, A.E., Djellid, Y., Sadeki, I.F. and Kihal, M. (2017). Microbiological characterization of algerian traditional cheese “klila”. Journal of Purity, Utility Reaction and Environment. 6(1): 12-18.

Benyagoub, E.H., Guessas, B., Ayat, M. and Sanebaoui, B. (2016). Physicochemical and bacteriological properties of some Algerian traditional dairy products ‘klila and jben’ marketed in southwest of algeria and their impact on consumers health. International Journal of Advanced Research. 4(12): 760-768.

Caridi, A., Micari, P., Foti, F., Ramondino, D. and Sarullo, V. (2003). Ripening and seasonal changes in microbiological and chemicalparameters of the artisanal cheese caprino d’aspromonte produced from raw or thermized goat’s milk. Food Microbiology Journal. 20(2): 201-209.

Dahou, A.A., Bekada, A.A. and Homrani, A. (2021). Identification of a *Lactococcus lactis* isolated from a fresh local cheese of the western Algerian steppe. Asian Journal of Dairy and Food Research. 40(1): 40-44.

Dahou, A.A., Bekada, A.M.A, Medjahed, M., Tahlaiti, H., Rechidi, S.N. and Homrani, A. (2020). Characterization of Natural Lactic Flora in Soft Cheese “Camembert of the Tessala” made from thermised milk of local breed cow “Brown of the Atlas”. Asian Journal of Dairy and Food Research. 39(1): 30-34.

Delfosse, C. (2006). The location of cheese production: changing geographical approaches. Geocarrefour Open Edition Journals. 81(4): 311-318.

Ferdous, N., Hnini, R., Chigr, F. and Najimi, M. (2017). Hygienic quality of raw cow milk produced by smallholder dairy farmers in Benimellel area in Morocco. World Journal of Research and Review (WJRR). 5(2): 09-16.

Guiraud, J.P. (2003). Food microbiology, Dunod edition, first edition, Paris, France.

I.D.F (International Dairy Federation) Reference ISO 707/ F.I.L. (2018). Standards set for microbiological and chemical analysis of milks and dairy products. Rome. pp 358.

Idoui, T. and Karam, N.E. (2008). Lactic acid bacteria from Jijel’s butter: insulation, identification and major technological features. J. Gr. Y. Aceites. 59(4): 361-367.

Licitra, G. (2010). World wide traditional cheeses. Banned for business. Dairy Science and Technology Journal. 90: 357-374.

Madani, T., Mouffof, C. and Frioui, M. (2004). Effect of the level of concentrate in the ration on the profitability of milk production in semi-arid Algerian situations. Mr. Renc. Rech. Ruminant Journal. 11(1): 244-245.

Meribai, A., Jenidi, R., Hammouche, Y. and Bensoltane, A. (2017). Physico-chimical characterization and microbiological quality evaluation of klila, an artisanal hard dried cheese from Algerian’s arid areas: preliminary. Journal of News Sciences, Agriculture and Biotechnology. 40(4): 2169-2174.

Montel, M.C., Buchin, S., Mallet, A., Delbes-Paus, C., Vuitton, D.A., Desmasures, D. and Berthier, F. (2014). Traditional cheeses: rich and diversified microbiota with associated benefits. International Journal of Food Microbiology. 177(2): 136-154.

Pankaj, K.G., Dinesh, C.R., Vinod, K.P., Rajendra, P. and Ashok, K.Y. (2020). Study on physico- chimical and microbial quality of raw milk collected from different places of Assi region in Varanasi city. Asian Journal of Dairy and Food Research. 39(1): 1-9.