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Characterization of Jben: Microbiological and Physicochemical Analysis of Traditional Raw Cow's Milk Cheese with Dried Lamb Rennet from Ain Sefra, Algeria

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ABSTRACT

Background: Jben is a traditional cheese produced in the northwest of Algeria's Ain Sefra province. It is made from cow raw milk, coagulated using "el Hakka" animal rennet.

Methods: Sixteen cheeses prepared during winter and summer were collected, to analyse seasonal effects on their physicochemical characteristics.

Result: The analysis revealed a higher prevalence of lactic acid bacteria, molds, Bacillus, and total coliforms in the winter season. Conversely, mesophilic aerobic bacteria, *Micrococcaceae*, *Enterobacteriaceae*, yeasts, and faecal coliforms were most abundant in the summer. No anaerobic spores or *Salmonella* spp. were detected in any samples. The cheeses had an average pH of 5.76 and a dry matter content of 45.20% (w/w). The average values for fat, protein, ash content, acidity and fat/dry matter were 20.02%, 18.64%, 3.2%, 33.93°D and 44.23%, respectively. Statistical analyses revealed a highly significant difference between the two seasons at $p<0.05$. Traditional Jben is classified as a semi-soft, quarter-fat, and non-ripened cheese.

Key words: Ain sefra, Cow milk, El Hakka, Jben.

INTRODUCTION

In Algeria, the consumption of traditional dairy products is deeply rooted in the culture and linked to livestock farming. Around ten traditional cheeses are produced in different regions of Algeria (Bendimerad, 2013). The most well-known is Bouhezza cheese (Boudalia *et al*., 2020). Other traditional cheeses include Mechouna in eastern Algeria (Derouiche *et al*., 2015), as well as Takammèrite, Kemariya, Aghoughlou, and Ighounane (Leksir *et al*., 2019). In southern Algeria, we find Aoules (Benkerroum, 2013), the Klila (Benamara *et al*., 2022) and Jben (Tadjine *et al*., 2020). They differ in taste, consistency, names and production methods by region.

Jben is a fresh traditional Algerian cheese produced and consumed in the Ain Sefra region, located in the 'Hauts Plateaux' highlands of western Algeria (Naima).These highlands, or steppe, lie between the Tellian Atlas in the north and the Saharan Atlas in the south, extending from Morocco to northwest Tunisia at an average altitude of 1,000 meters. Ecologically, the plateaus are covered by steppe vegetation, dominated by *Stipa tenacissima*, *Artemisia herba-alba Asso*, and *Lygeum spartum*. This vegetation is used to feed livestock, supporting two-thirds of Algeria's sheep and goats (Djebaili *et al*.,1989). The sagebrush and esparto steppes provide grazing, necessitating the movement of herds from south to north in summer and *vice versa* in winter. Pastoralists have adapted their production systems, almost systematically combining cereal crops with livestock (Bedrani, 2001).

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J'ben is the most popular traditional cheese and its manufacturing method is still in use today, with an increase in its consumption in steppe areas, due to its pleasant organoleptic and nutritional properties produced by an indigenous microflora (Dahou *et al.,* 2021). Their is still no scientific information on Jben made from cow raw milk and el Hakka. The present study is planned and executed to characterize this new variant of cheese from various attributes, microbiological view points and to determine the seasonal influence on the quality of Jben.

MATERIALS AND METHODS

Cheese making and sampling

The experiment was conducted rabi session of 01-2016 and 06-2021 at the Laboratory of Applied Microbiology to Agri-Food and Environment "LAMAABE", Tlemcen, Algeria. Sixteen samples of Jben were produced, with equal numbers made in winter and summer. The samples were collected immediately after production, transported in a freezer to the laboratory, and analyzed. Each sample was divided into two aliquots for microbiological and physicochemical analyses.

Microbial analysis

Fifty grams of each sample were homogenized with 200 mL of sterile 2% (w/v) sodium citrate solution at 40-45°C for 3 minutes in a masticator to produce a 1/5 dilution. Decimal dilutions were prepared by mixing 10 mL with 90 mL of sterile 0.1% (w/v) peptone water Arenas *et al*. (2004). The microbial analyses of Aerobic mesophilic bacteria, lactic acid bacteria, *Micrococcaceae, Enterobacteriaceae,* Moulds and yeasts, Spores of anaerobic sulfur-reducing bacteria, Coliforms and *Salmonella* spp were effected as per the standard methods prescribed by Mathot *et al.* (1994); Mennane *et al.* (2007); Medjoudj *et al*. (2017); NF ISO 4831. 2006, ISO 6785/IDF 93. 2001.

Physico-chemical analysis

The pH and titratable acidity of the cheeses were measured by methods prescribed by Garcia Fontán et al. (2001). Dry matter, fat, protein and ash content were determined following the methods outlined in ISO 5534-TDF 004 (2004), NF V04-287 (1972), ISO 8968-1IDF 20-1 (2014), and NA N° 10.96.03. All analysis were carried out in triplicate. For the final Jben, and according to the Codex Alimentarius classification (FAO, 2007), the moisture of defatted cheese (MDC g/100g, H.R.E.S) and the fat in dry matter (FDM g/100g) were calculated.

The energy value of Jben is the sum of the energy values of its basic nutrients per 100 g of fresh Jben (mean ± standard deviation of the 16 samples analyzed) according to Quero *et al*. (2014).

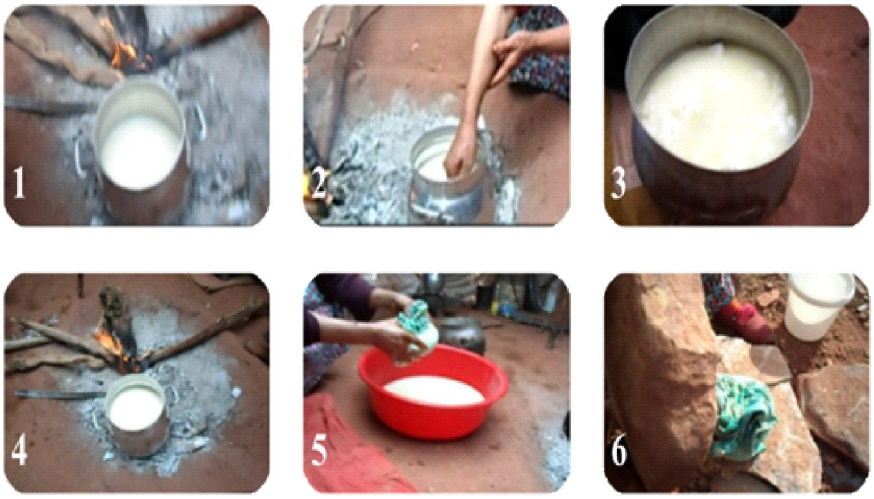
Statistical analysis

For microbiological analysis, results were expressed as log colony-forming units per gram (mean±SD), and comparisons between the two seasons were made using Student's t-test. The significance level was set at $p<0.05$. For statistical analysis of physicochemical results, assays were done in triplicate. ANOVA was used with STATISTICA software and significance was set at $p<0.05$.

RESULTS AND DISCUSSION

In situ study results

The production of Jben requires the preparation of "el Hakka," which is the curd obtained from the proteolytic enzymes obtained from abomasum of a young lamb or kid before it is weaned (still breast feeding). A week after birth, the lamb or kid is slaughtered if it is sick or if its mother dies. The abomasum is neither washed nor emptied of its contents. In some cases, plants such as artemisia, salt are added, and occasionally, sheep's milk is poured into the empty abomasum. It is then sealed and left to dry in the open air, with the drying process occurring more quickly in summer than in winter. This is the traditional method of preparing el Hakka for Jben production.



1. Filtered cow milk, 2. Heating milk up to 32-35°C and rubbing el hakka, 3. Milk curdling after 5-30 min, 4. Reheating the curd milk to separate the lactoserum and then let the milk cool down, 5. Draining with a porous tissue (Hawak), 6. Moulding and pressing between two stones Madoune.

Fig 1: Production protocol of the traditional Algerian Jben.

Jben is produced following a traditional protocol. only old women are allowed to make it, especially in spring when milk production intensifies. Fig 1 illustrates the Jben production process.

Microbiol and physicochical

The different numbers of microbial groups in Jben produced with cow's milk and El Hakka in the two seasons are shown in Table (1). The mean values of the total mesophilic aerobic flora of Jben is 7.60±1.19 log cfug^{-1} in winter and 7.86±0.52 log cfug^{-1} in summer. *Lactococcus* exhibits a mean of 6.93±1.17 log cfug^{-1} in winter and 6.84±1.02 log cfug^{-1} in summer. *Leuconostoc* shows a mean of 6.27±1.43 log cfug-1 in winter and 5.19± 1.33 log cfug^{-1} in summer. *Lactobacillus* has a mean of 6.47±1.13 log cfug^{-1} in winter and of 5.90±1.60 log cfug^{-1} in summer. Mean Micrococcaceae counts were 2.86±1.85 log cfug^{-1} in winter and of 3.47±1.50 log cfug^{-1} in summer. *Enterobacteriaceae* counts averaged 3.31±1.54 log cfug^{-1} in winter and 4.59±1.05 log cfug^{-1} in summer. Yeast and mould populations ranged from 3.43±0.48 log cfug^{-1} in winter and 3.71±0.89 log cfug^{-1} in summer. Total coliforms were present at 4.17±1.19 log cfug^{-1} in winter and 3.87±1.36 log cfug^{-1} in summer. Fecal coliform counts averaged 2.62±1.55 log cfug^{-1} in winter and 2.78±1.50 log cfug^{-1} in summer.

Anaerobic spores and S*almonella* were not detected in any of the cheese samples. Statistical comparison between the two seasons indicated no significant difference at $p<0.05$ among the microbial groups . *Lactobacillus, Lactococcus, Leuconostoc* and total coliforms were highest in winter. Mesophilic aerobic bacteria, *Micrococacceae, Enterobacteriaceae*, yeasts, moulds, and fecal coliforms were highest in summer.

The physico-chemical parameters of Jben elaborated in the two seasons are shown in Table 2. The different seasons had significant differences at $p<0.05$ in pH, acidity, dry matter, moisture, fat, protein and ash.

The presence of various microbial groups in cheeses affects the microbiological quality of milk. The lack of adherence to hygienic conditions during milking and the addition of el Hakka, prepared under similar unhygienic conditions, may impact the microbiological quality of cheese.

The counts of total mesophilic aerobic bacteria were slightly lower than those reported in Bouhezza Algerian and Moroccan, cheeses by previous authors (Aissaoui *et al*., 2011; El Galio *et al*., 2015). However, they were comparable to those found in other fresh Algerian cheeses as reported by (Dahou *et al.* 2020; Tadjine *et al*. 2020).

Table 1: Results of microbiological analysis of traditional Jben cheese produced in North-West Algeria in winter and summer.

Seasons	Microflora counts (logcfu/g) n=16					
	Winter			Summer		
Microflora	Min	Mean±sd	Max	Min	Mean±sd	Max
Mesophilic aerobic bacteria	5.12	7.6±1.19	8.51	7.32	7.86±0.52	8.86
Lactobacilli	3.93	6.47±1.13	7.36	2.4	5.90±1.60	7.28
Lactococci	4.07	6.93±1.17	7.53	4.34	6.84±1.02	7.32
Leuconostoc	3.39	6.27±1.43	7.37	2.74	5.19±1.33	6.79
Micrococcaceae	0	2.86±1.85	4.71	0	3.47±1.50	4.70
Enterobactériaceae	0	3.31±1.54	4.45	3.04	4.59±1.05	6.57
Yeasts and molds	2.69	3.43±0.48	4.05	3.32	3.71±0.89	4.21
Anaerobic spores	0	0.00	0	0	0.00	0.00
Total coliforms	2.6	4.17±1.19	6.05	2.6	3.87±1.36	6.05
Faecal coliforms	0	2.62±1.55	4.95	0	2.78±1.50	5.30
Salmonella spp	0	0.00	0	0	0.00	0.00

Table 2: Results of physicochemical analysis of traditional Jben produced in northwestern Algeria in winter and summer.

Variable	Winter			Summer		
	Mean±S.D	Min	Max	Mean	Min	Max
pH	5.61±0.45^{a}	5.00	6.20	5.92±0.42^{a}	5.12	6.40
Acidity (°d)	36.60±9.38^{a}	24.50	50.00	31.26±8.99^{a}	21.00	49.30
Dry matter (%)	45.77±3.52^{a}	39.44	51.36	44,74±2.58^{a}	39.50	48.45
Fat(%)	21.02±2.90^{a}	14.00	26.50	19.02±3.78^{a}	13.00	27.00
Fat/dry matter(%)	45.95±5.64^{a}	31.96	55.25	42.52±7.74^{a}	30.44	55.78
Proteins(%)	18.79±3.29^{a}	12.10	22.41	18.49±3.24^{a}	13.25	23,51
Ash(%)	3.00±0.31^{a}	2.34	3.35	3.92±1.23^{a}	2.60	6.00

Values are expressed as the mean±standard deviation (n=3). Values in same line with superscript (a) were significantly different at p<0,05.

Lactic acid bacteria, were identified, with *Lactococcus* predominating. These counts were close than observed by Saidane *et al.* (2021) and by Garcia Fontan *et al*. (2000). Among these bacteria, lactococci were most abundant, with a mean count of 6.88±1.06 (Table 1). Vasek *et al*. (2008) have also noted this dominance of lactococci. Lactobacilli exhibit a slower metabolism compared to lactococci and leuconostoc, resulting in slower initial growth. However, they are acid-tolerant microorganisms with an optimal growth pH similar to that of lactococci and *Leuconostoc*.

Micrococcaceae were found in Jben cheese with values as low as 3.16±1.66, likely due to their resilience to salt and dehydration (Ordiales *et al*., 2013). These findings align closely with those reported by Tadjine *et al*. (2020).

Enterobacteriaceae and coliforms are widely used as indicators of food microbiological quality globally (Psoni *et al*., 2003). Enterobacteriaceae are recognized as undesirable flora, indicating poor hygiene practices, potentially causing texture defects, blistering, off-flavors, and linked to raw milk from animals with mastitis (Ordiales et al., 2013). In this study, Enterobacteriaceae were detected at lower levels compared to those reported by Aissaoui *et al*. (2011).

Moulds and yeasts are spoilage organisms indicating environmental contamination. The acidic pH likely favored yeast growth. The observed yeast and mold counts were lower than those reported by Vasek *et al.* (2008), El Galio *et al*. (2015), and Tadjine *et al.* (2020). However, the total coliform counts were similar to those documented by the same authors.

Conversely, the values obtained for fecal coliforms are lower than those reported by Tadjine *et al.* (2020), higher than those found by El Galio *et al*. (2015) and Medjoudj *et al*. (2017), but similar to those reported by Vasek *et al*. (2008).

The pH values are higher than those reported by Benheddi *et al*. (2019) and Tadjine *et al*. (2020) for an Algerian Jben cheese made with vegetable rennet (4.42 and 4.69, respectively). They are also higher than those found in traditional homemade fresh goat cheese from northern Morocco El Galio *et al*. (2015). A pH above 5.0 creates ideal conditions for pathogen growth, leading to a decrease in the cheese's hygienic quality. The titratable acidity values observed in this study are similar to those reported by Tadjine *et al*. (2020) but lower than those found by Benkerroum *et al*. (2004) for Jben cheese made from raw milk in Morocco (104°D).

The dry matter content exceeds that of other Moroccan fresh goat cheeses El Galio *et al*. (2015) but aligns with Serbian Valsena cheese (47.2%) Terzic-Vidojevic *et al*. (2013) and Turkish Aho cheese Temiz *et al.* (2015). It is also similar to Corrientes cheese from Argentina (40-50%) Vasek *et al.* (2008). The lower DM values (40.27 - 49.73%) indicate high moisture content in most Jben cheeses, a key spoilage factor.

The fat content was close to that in Chihuahua cheese made from raw milk in Mexico (22.2 g/100 g) Sanchez *et al.* (2018) and Corrientes cheese from Argentina Vasek *et al.* (2008), but higher than that found by Ordiales *et al*. (2015) and Temiz *et al.* (2015). However, the average fat in dry matter was lower than in Moroccan cheeses noted by Ordiales *et al*. (2015). Protein contents were lower than those reported by Vasek *et al*. (2008) and Temiz *et al*. (2015). The ash values were higher than those reported by Ordiales *et al*. (2015).

According to the Algerian food microbiological criteria (JORADP N° 39 2017), two samples exceeded the "m" criterion for faecal coliforms, while six samples exceeded the "m" criterion for staphylococci.

CONCLUSION

This study provides a comprehensive analysis of the traditional Jben cheese made with El Hakka and raw cow's milk across winter and summer seasons in Ain Safra, Algeria. The results demonstrate that Jben cheese meets the required standards for its physicochemical and bacteriological parameters. According to FAO standards, Jben is classified as a semi-soft cheese with moisture in defatted cheese (MDC) and fat/dry matter ratio of 68.44 g/100 g and 44.23, respectively. The Jben energy value is 252 (167- 314) Kcal/100 g. Lactic acid bacteria were found to dominate the microbial flora, highlighting their role in the cheese's development and flavor profile. Future research should focus on exploring the specific properties of lactic acid bacteria in Jben cheese and investigating the microbial dynamics of El Hakka. Additionally, further investigation into microbial species with potential antimicrobial properties during cheese production would be beneficial.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or

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