Technological characterization and sensory evaluation of a traditional Algerian fresh cheese clotted with *Cynara cardunculus* L. flowers and lactic acid bacteria

Wafaa Benheddi¹ · Amina Hellal²

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Abstract Jben is an Algerian traditional fresh cheese produced on a small scale in a limited area of east and west Algeria, in Maghnia and Nadrouma. It is manufactured from raw sheep, cow or goat milks, coagulated with dried flowers of wild thistle *Cynara cardunculus* L. without starter culture addition. In this work, Jben was made using cow and goat pasteurized milks to improve the microbiological quality. Milk was clotted with crude enzymatic extract of dried flowers of wild thistle *C. cardunculus* L. In order to obtain a better organoleptic characteristic, the assays of cheese making were realized with adding strains of lactic acid bacteria isolated from traditional cheeses. Physico-chemical and microbiological analyzes were carried out on the manufactured cheeses. Milk was clotted with 0.5% v/v by aqueous extract (40 g/L) of dried flowers of *C. cardunculus* L. Better organoleptic quality and higher cheese yield were obtained with goat milk. Cheese made from pasteurized goat's milk added with *Lactococcus lactis* subsp. *lactis*, and that added with the mixture of lactic acid bacteria strains *L. lactis* subsp. *lactis*, *Lactococcus raffinolactis*, *Leuconostoc mesenteroides* ssp. *mesenteroides*, were the most appreciated. It is believed that the activity of *L. raffinolactis* in dairy products may contribute to their final sensory characteristics.

Keywords Jben · Traditional cheese · *Cynara cardunculus* L. · Lactic acid bacteria

Introduction

Milk clotting enzymes are the primary active agents in the manufacture of cheeses. Calf rennet was the first and still is the most widely used. The worldwide increase in cheese production along with the reduced supply of calf rennet, has led to an increase in the demand for alternatives sources of milk coagulants (Cavalcanti et al. 2004).

Clotting enzymes extracted from plants have increased the attention for dairy technology (Tavaria et al. 2001). Among the vegetable enzymes that have been reported to produce satisfactory final products cardosins and cyprosins (the proteases present in flowers of *Cynara cardunculus* L.) deserve special mention (Llorente et al. 2014). The use of these coagulants has been shown to preserve the original and distinctive flavors of traditional cheeses (Irigoyen et al. 2002; Fox et al. 2017).

Cynara cardunculus L. is widely distributed in Mediterranean countries. The flowers of cardoon are typically used in Algeria for cheese making of a traditional cheese called Jben, manufactured with raw ewe's milk. It grows wild and abundantly in dry uncultivated places producing flowers all summer. Extraction carried out from various parts of the plant indicated a coagulating activity only with flower collected at an advanced stage of development (Mouzali et al. 2004).

The vegetable rennet *Cynara cardunculus* L. contains two enzymes cardosin A and cardosin B, cardosin A is similar to chymosin where as cardosin B is a pepsine like enzyme (Pereira et al. 2008). Other cardosins (E, F, G, H) have been purified from thistle flowers; they have high similarities to the well-known cardosin A with diverse activity and selectivity (Sarmiento et al. 2009).

Lactic acid is responsible for the fresh acidic flavor of cheese and is important in coagulation of milk casein, which is

✉ Amina Hellal
amina.hellal@g.enp.edu.dz

¹ Ecole Nationale Supérieure Agronomique, Hassen Badi, El Harrach, 16004 Algiers, Algeria

² Laboratoire des Sciences et Techniques de l'Environnement, Ecole Nationale Polytechnique, 10 Avenue Hacen Badi BP 182, El Harrach, 16200 Algiers, Algeria

accomplished by the combined action of rennet (an enzyme) and lactic acid bacteria. As the rennet is an acidic enzyme, its optimum activity occurs in a slightly acidic environment. Therefore, the action of LAB in this phase is crucial as they are required to rapidly release enough lactic acid, to lower the milk pH, and thus creating an appropriate environment for optimum activity of rennet (Carminati et al. 2010).

Otherwise, during the ripening process these bacteria play essential roles by producing flavor compounds (e.g. diacetyl, aldehydes) and by producing natural antibiotic substances called bacteriocins that inhibit growth of pathogens and other spoilage microorganisms (Ayad et al. 2002; Bernardeau et al. 2008).

Generally traditional cheeses are manufactured in small farms with raw milk from animals of indigenous breeds. These productions are carried out without the addition of commercial or natural starter cultures. The earliest productions of cheeses were based on the spontaneous fermentation, resulting from the development of the microflora naturally present in the raw milk. The quality of the end product was a reflex of the microbial load and the hygienic quality of the raw material.

Lactococci are generally used as starter cultures in the production of industrial cheeses and cultured milk products (Ayad et al. 2002). For example Cheddar and Colby cheese production, starter cultures include strains of *Lactococcus lactis* ssp. *cremoris* and/or *lactis*. Likewise, blue cheeses require *Lactococcus lactis* ssp. *cremoris* or *lactis*, but the mold *Penicillium roqueforti* is also added as a secondary culture for flavor and blue appearance (Carminati et al. 2010).

The aim of this work was to make Algerian traditional Jben cheese with pasteurized milk for satisfactory microbiological quality, and to improve organoleptic quality by adding isolated lactic acid bacteria strains.

Materials and methods

Vegetable sample of *Cynara cardunculus* L.

The study was carried out on vegetable samples taken in the area of Maghnia, in the west of Algeria. The flowers were dried in a traditional way at ambient temperature and protected of the solar rays for a better conservation.

Determination of the adequate concentration of the crude enzyme solution

Crude enzyme solutions were obtained by maceration of 2 g of dried flowers of *Cynara cardunculus* L. in 10, 20, 40, 50, 80 mL distilled water overnight. There after cheeses are made with the obtained aqueous solutions

added at 1% in order to determine the best concentration ensuring a good and rapid clotting with the least bitterness.

Determination of the rate of curdling by the crude enzyme solution

Once the concentration of the appropriate enzymatic crude extract determined, it was necessary to determine the rate of inoculation of the milk with this crude enzymatic extract. Cheeses manufacturing was tested with 0.2%; 0.5%; 1% and 1.5% (v/v) milk inoculation and submitted to tasting and appreciation tests.

Milk analysis

Goat and cow milks have undergone physicochemical analysis through Eko milk ultrasonic analyzer which measures the contents of dry matter, fat, proteins, water content and the density in the fresh milk.

Cheese making

For the manufacture of the fresh cheese (Fig. 1), cow and goat milks were used. The milk was pasteurized at 63 °C during 30 min and maintained at 30 °C before inoculation with mesophilic lactic acid bacteria strains and addition of the vegetable crude enzymatic extract. After the coagulation (10 h), cheeses were draining in perforated molds. Six assays were conducted, three with cow's milk and three others with goat's milk. The lactic acid bacteria strains used in this work were previously isolated from commercial traditional Jben and identified: *Lactococcus lactis* subsp. *lactis* S7, *Lactococcus lactis* subsp. *lactis* S20, *Lactococcus raffinolactis* S19, *Leuconostoc mesenteroides* ssp. *mesenteroides* S8. Strains were preserved by freezing in skim milk. Before using a subculture of strains during 24 h was performed in skim milk.

Physical and chemical analysis of cheeses

The total solids content was determined by over drying at 105 °C. The fat content was determined by the Van Gulik

Fig. 1 Steps of Jben cheese making process

method (Desfleurs and Bailleul 1975). Kjeldahl method was carried out to the proteins determination using a conversion factor of 6.38 for calculating protein in dairy product (International Dairy Federation 1993). The pH was measured using a pH meter (Inolab, Germany).

Microbiological analysis

Cheese samples (10 g) were homogenized with 90 mL of a sterile peptone solution in a stomacher; sequential decimal dilutions were inoculated, on to the plates in duplicate. Total viable counts were determined on plat count agar (PCA) incubated at 30 °C for 3 days. Yeasts and fungi were cultivated on agar glucose oxytetracyclin at 25 °C for 5 days. Total coliform bacteria were determined on desoxycholate agar at 37 °C for 24–48 h. Faecal coliforms were determined using green bile lactose broth at 44 °C for 48 h. For the research of Staphylococci, Chapman media was used with incubation conditions at 37 °C for 48 h. *Clostridium* sp., was tested on meat liver media with adding iron alum and sodium sulfate with incubation at 45 °C for 72 h. Test tubes were previously heated in water bath 80 °C for 10 min in order to destroy vegetative forms. Presumptive media of Roth and confirmation media of Litsky were used for faecal Streptococci incubation 37 °C for 24–48 h. The search for *Salmonella* occurs through 3 stages, pre-enrichment, enrichment broth with sodium selenite, isolation on Hoektoen agar with incubation at 37 °C for 24 h. Researches of the different floras were carried out according to the protocols reported by Bourgeois and Leveau (1991) and Gonzalez-Fandos et al. (2000).

Sensory analysis

The texture, flavor and appearance were evaluated according to the French notation norm (AFNOR 1991) on the cheese obtained with the goat's and cow's milks. The scale used for the appreciation of the characteristics varies from 0 to 4:

- 0 Missing character
- 1 Recognized low level character
- 2 Medium intensity recognized character
- 3 Recognized intensive nature
- 4 Very intensive character

Sensory analysis was conducted by a taste panel consisting of twenty panelists. The samples were equilibrated at 20–25 °C for an hour before they were scored. The samples were served in plates with a consumer sensory evaluation questionnaire on an anonymous labeled basis. Principal component analysis was used to study the correlations of sensory attributes. Sensory data input was achieved with XLSTAT Software to categorize the Jben cheese samples.

Results and discussion

The adequate concentration of the crude enzyme solution

Cheeses were made from pasteurized milk 63 °C 30 min. Once cooled milk is renneted by 1% v/v of crude enzyme solutions obtained by maceration of 2 g dried flowers of *Cynara cardunculus* L. in various volumes of distilled water. The influence of this concentration on the technological properties of cheeses is summarized in the Table 1.

Based on the obtained results, the concentration of 2 g dried flowers of *Cynara cardunculus* L./50 mL distilled water was retained for this study.

The curdling rate of the milk by the aqueous enzymatic solution

When the crude enzymatic extract (2 g dried flowers/50 mL distilled water) was added to milk at less than 0.5% (v/v), it induced slow coagulation and when added at greater than 1% (v/v) it induced a high proteolysis which is responsible of the bitterness of the cheeses (Table 2). As the purpose of this study is to improve the organoleptic characters without affecting the proper course of coagulation, the rennet rate of 0.5% was chosen for cheese making.

Milk composition

The results related to the physico-chemical analyses on cow and goat milks (Table 3) showed that goat's milk presented higher degreased dry matter and proteins content than cow's milk. On the contrary, cow's milk contains more fat than goat's milk. These differences in composition

Table 1 Influence of the enzymatic solutions concentration on the technological properties of cheeses

Concentration of crude enzyme solution (g dried flowers/mL distilled water)	Observations
2/10	Extensive proteolysis resulting weight loss and pronounced bitterness. Impossible to carry out the dripping of the cheese due to formation of very fine particles of casein
2/20	Weight loss and pronounced bitterness
2/40	Fairly good coagulation; a slight taste of bitterness
2/50	Good coagulation getting a firm cheese; absence of bitterness
2/80	Slow coagulation, absence of bitterness

Table 2 Effect of rennet rate on bitterness of cheese

Rate of crude enzymatic extract (%v/v)	Observations
0.2	Absence of bitterness, very slow coagulation
0.5	Absence of bitterness, good coagulation
1	Perceptible bitterness, good coagulation
1.5	Pronounced bitterness, proteolysis formation fine particles of casein

Table 3 Composition of the cow and goat milks

	Degreased dry matter/%	Fat %	Proteins %
Goat milk	7.54 ± 0.32	3.56 ± 0.23	2.86 ± 0.13
Cow milk	6.01 ± 0.05	4.44 ± 0.06	2.31 ± 0.02

are related to the animal species, feeding and period of lactation.

The contents of goat's milk fat and proteins are lower than those obtained by St-Gelais et al. (2005) where the fat content was 4.1% and the content of the proteins 3.5%.

Physical–chemical analysis of manufactured Jben cheese

Cheeses are manufactured using pasteurized milk, crude enzymatic extract of *Cynara cardunculus* L. flowers and mesophilic lactic bacteria strains.

According to the results of the physico-chemical analysis of the cheeses (Table 4), the level of the dry matter varies from 27.98 g/100 g to 34.61 g/100 g for goat's milk cheeses. This result is high compared to that obtained for a homemade fresh goat cheeses from northern Morocco (El Galiou et al. 2015). For cow's milk cheeses dry matter varied from 29.88 g/100 g to 33.05 g/100 g. Another Algerian traditional cheese, Bouhezza, made with cow's milk presented dry matter varied from 20.77 g/100 g at the first week to 35.86 g/100 g at the tenth week (Zitoun et al. 2011).

Table 4 Physico-chemical characteristics of Jben cheeses

Cheese	Dry matter %	Fat %	Proteins %	pH	Yield (g/L)
Goat milk					
Mixed strains S8, S20, S19	27.98 ± 0.68	11 ± 0.70	14.64 ± 0.14	4.76 ± 0.07	185.1 ± 3.57
<i>L. lactis</i> subsp. <i>lactis</i> S7	34.61 ± 0.55	14 ± 0.26	14.54 ± 0.14	4.85 ± 0.07	215.4 ± 1.85
<i>L. lactis</i> subsp. <i>lactis</i> S20	31.25 ± 0.35	10 ± 0.16	14.21 ± 0.14	4.81 ± 0.22	207.33 ± 2.24
Cow milk					
Mixed strains S8, S20, S19	31.53 ± 0.66	10 ± 0.14	18.22 ± 0.07	4.61 ± 0.08	185.66 ± 2.84
<i>L. lactis</i> subsp. <i>lactis</i> S7	33.05 ± 0.49	10 ± 0.07	15.36 ± 0.16	4.90 ± 0.14	138.03 ± 2.69
<i>L. lactis</i> subsp. <i>lactis</i> S20	29.88 ± 0.70	17 ± 0.70	17.14 ± 0.07	4.42 ± 0.11	127.9 ± 3.43

Lactococcus lactis subsp. *lactis* S7, *Lactococcus lactis* subsp. *lactis* S20, *Lactococcus raffinolactis* S19, *Leuconostoc mesenteroides* ssp. *mesenteroides* S8

In this study, Jben cheese was manufactured in the same way but the results of the physico-chemical analyses present differences due firstly to the origin and the composition of the milk and other differences related to the nature of the strains. During manufacture cheese, coagulation occurs under the combined effect of the coagulating enzyme and lactic acid bacteria.

The milk, whether of bovine or caprine origin, is clotting by the same quantity of crud enzymatic extract. The observed differences in the quality could be related to the lactic acid bacteria strains because the enzymatic environment differs. It has been shown that the enzyme acts on k-casein cleaving the Phe105-Met106 peptide bond on bovine and ovine k-casein, whereas caprine k-casein is preferentially cleaved at Lys116-Thr117 (Silva and Malcata 2000). Each strain is characterized by its own proteolytic activity. Its own enzymatic arsenal depends on the goat or cow milks substrate casein (Llorente et al. 2014).

The weight of cheeses for cow's milk varies from 127.9 to 185.66 g/L (Table 4). It corresponds to the result obtained by Mouzali et al. (2004) who found 177.4 g/L. The cheese yield is higher with goat's milk. The weight of cheeses from goat's milk varies from 185.1 to 215.4 g/L.

The pH for the majority of cheeses varies between 4.42 and 4.90. In Bouhezza cheese the pH remained 4 (Zitoun et al. 2011), in Kulek cheese, the heated one with adding starter the pH varied between 4.79 and 4.88 (Dervisoglu and Aydemir 2007).

Microbiological analyses

Microbiological analysis were performed on pasteurized milk, crude enzymatic extract and manufactured fresh cheeses, dating from 1 day to 1 week stored at 4 °C. The results obtained are shown in Table 5.

The absence of pathogenic germs, as well as the absence of the total mesophilic flora and faecal coliforms reflects the good pasteurization of milk. It would be necessary to filter the crude enzymatic extract in order to decrease its

Table 5 Microbiological quality of pasteurized milk, crude enzyme extract, fresh cheese dating of 1 day and 1 week

CFU/g	Pasteurized milk	Crude enzymatic extract	Fresh cheese 24 h	Fresh cheese 1 week
Total aerobic mesophilic flora	Abs	350	< 30	189
Total coliforms	Abs	100	Abs	< 30
Faecal coliforms	Abs	40	Abs	Abs
Staphylococci	Abs	Abs	Abs	Abs
Yeasts and molds	Abs	Abs	Abs	Abs
Faecal streptococci	Abs	Abs	Abs	Abs
Sulphito reducer clostridia	Abs	Abs	Abs	Abs
Salmonella	Abs	Abs	Abs	Abs

Abs absence of germs, CFU colony forming unit

microbial load because it contains faecal coliforms and yeasts and molds which may compromise the hygienic quality of the product. In fact Chazarra et al. (2007) obtained better microbiological quality when crude enzymatic extract was filtered and treated with additive benzoic acid. The absence of pathogenic germs in all analyzed products is remarkable. These results show the absence in the manufactured cheeses of yeasts, molds and faecal coliforms. Cheeses could be contaminated by the raw enzymatic extract but the action of the mesophilic lactic acid bacteria strains are responsible for the good microbial quality of the cheeses due to their antagonistic effect. These results can be explained by the fact that lactic acid bacteria possess bactericidal effect by a production of bacteriocins. In the production of Kulek cheese, used starter microorganisms had significantly decreased the undesirable germs (Dervisoglu and Aydemir 2007).

The fresh cheese kept in the refrigerator after 1 week has a good microbiological quality, compared to another Algerian traditional cheese Bouhezza where count of total mesophilic flora ranged from 7 to 8 log CFU/g of cheese. It may be attributed to unpasteurised milk (Zitoun et al. 2011). The cheeses produced in the present study using *Cynara cardunculus* L. and lactic acid bacteria are of excellent microbiological quality.

Sensory analyses

The jury of tasting, questioned on the characters and flavor of goat and cow milk cheeses of Jben (Fig. 2) presented in an anonymous way, was able to appreciate the aspect, the texture, the color and the flavor of the manufactured cheeses with different mesophilic lactic strains.

The sensorial parameters were evaluated using principal component (PCA) analyses. PCA was conducted based on the correlation parameters which might explain most of the variability. PCA was applied to classify the Jben cheese samples using texture, flavor and appearance data listed in Table 6. As can be seen (Fig. 3) PCA proved to be very

successful to determine the most effective variables and to reveal relationships among the variables. Indeed, it indicates type of milk (goat vs. cow) accounted for 30% of the variance: the cheeses made from goat milk are in the upper quadrants; in the lower quadrants are cheeses made using cow milk. Starter of lactic acid bacteria accounted for 39.31% of the variance: the cheese made with *Lactobacillus lactis* strains alone are on the left of the plot and the cheeses made with the mixture strains on the right side.

The PCA analysis reflects the attributes that characterize or correlate with each cheese. Goat's cheese has a slightly more accentuated color with a slight taste of milk and a rather compact appearance with less whey residue.

Goat's cheeses were more appreciated than cow's cheeses. Cheese made from pasteurized goat's milk added with the mixture of lactic acid bacteria strains *Lactococcus lactis* subsp. *lactis* S20, *Lactococcus raffinolactis* S19, *Leuconostoc mesenteroides* ssp. *mesenteroides* S8, was the most appreciated. It is believed that the activity of *Lactococcus raffinolactis* in dairy products may contribute to their final sensory characteristics, this species might be associated with the authenticity and originality of the cheese (Fernández et al. 2010). Cheeses are creamy and without bitterness.

The cheeses made with pasteurized cow's milk added with the mixture of strains (*Lactococcus lactis* ssp. *lactis* S20, *Lactococcus raffinolactis* S19, *Leuconostoc mesenteroides* ssp. *mesenteroides* S8) have been less appreciated because of the strong presence of the serum.

It is important to note that traditional raw milk cheeses are highly valued for their natural flavors, while large-scale products are often perceived by the consumer as savorless (Law 2001), a consequence of the elimination by pasteurization, of the flora that has a key role in flavor development; and this puts the food industry under pressure to look for alternative lactic acid bacteria cultures capable of improving products flavor (Leroy and De Vuyst 2004). In our case, the addition of the lactic acid bacteria to the

Fig. 2 Pictures of some elaborated Jben cheeses

Different cheeses made with cow and goat pasteurized milks clotted with crude enzymatic solution of *Cynara cardunculus* L

Goat cheese curdled with crude enzymatic solution of *Cynara cardunculus* L and lactic strain *Lactococcus lactis* subsp. *lactis* S7

Cheeses made from pasteurized goat and cow milks clotted with crude enzymatic solution of *Cynara cardunculus* L and lactic bacteria strains

Goat cheese obtained by addition of the mixture of strains: *Lactococcus lactis* subsp. *lactis* S20, *Lactococcus raffinolactis* S19, *Leuconostoc mesenteroides* ssp. *mesenteroides* S8

Table 6 Mean values of Jben cheese sensory attributes (n = 20 judges with 3 replicates)

Cheese	Color	Presence of lactoserum	Smell of milk	Fluidity	Roughness	Acidity	Bitterness	Oiliness	Homogeneity	Preference
Goat M	1.42	1.6	2	1.4	0	1.4	0	2.4	3	3.62
Goat 7	2.6	0.75	1.33	0	2	2.5	0.5	1.5	1.5	3
Goat 20	2.66	0.66	2.33	1.33	2	2.33	0.66	2.66	2.66	2.75
Cow M	1.22	2.3	1	2	1	1.66	0	3	3.33	2.16
Cow 7	1.5	1	0.83	1.8	2.6	1.8	0	1.6	1.6	2.16
Cow 20	1.6	1.2	0.66	0.33	0.25	1	0.66	1.8	2.4	2.2

Goat M Cheese prepared with goat milk and mixed strains of LAB S8, S20, S19

Goat 7 Cheese prepared with goat milk and *L. lactis* subsp. *lactis* S7

Goat 20 Cheese prepared with goat milk and *L. lactis* subsp. *lactis* S20

Cow M Cheese prepared with cow milk and mixed strains of LAB S8, S20, S19

Cow 7 Cheese prepared with cow milk and *L. lactis* subsp. *lactis* S7

Cow 20 Cheese prepared with cow milk and *L. lactis* subsp. *lactis* S20

pasteurized milk has considerably improved the Jben cheese sensorial quality.

Conclusion

In the present study the interest of using traditional vegetable enzyme of *Cynara cardunculus* L. has been demonstrated. Hence, use of the plant enzyme extract with

goat's milk gave better products than cow's milk; with higher cheese yield. The use of traditional coagulants has been shown to preserve the original and distinctive flavors of traditional cheeses.

Milk pasteurization has contributed to the good microbiological quality of cheese and the addition of lactic acid bacteria strains improved organoleptic quality cheese and inhibited the effect of germs present in the enzymatic crude extract. Lactic acid bacteria are important in cheese

Fig. 3 Principal component analysis of 6 Jben cheeses, showing the sensory attributes and the mean values from Table 2

Goat M = Cheese prepared with goat milk and mixed strains of LAB S8, S20, S19

Goat 7 = Cheese prepared with goat milk and *L. lactis* subsp *lactis* S7

Goat 20 = Cheese prepared with goat milk and *L. lactis* subsp *lactis* S20

Cow M = Cheese prepared with cow milk and mixed strains of LAB S8, S20, S19

Cow 7 = Cheese prepared with cow milk and *L. lactis* subsp *lactis* S7

Cow 20 = Cheese prepared with cow milk and *L. lactis* subsp *lactis* S20

processing because they increase food safety through the release of lactic acid and bacteriocins. They produce aromas and flavor and accelerate the maturation process of cheese.

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