

### MOROCCAN TRADITIONAL FERMENTED DAIRY PRODUCTS: CURRENT PROCESSING PRACTICES AND PHYSICOCHEMICAL AND MICROBIOLOGICAL PROPERTIES - A REVIEW

Ghita Benkirane<sup>1,2</sup>, Samir Ananou<sup>\*1</sup>, Emilie Dumas<sup>2</sup>, Sami Ghnimi<sup>2</sup>, Adem Gharsallaoui<sup>2</sup>

**Address(es):**

<sup>1</sup>Laboratoire de Biotechnologie Microbienne et Molécules Bioactives, Faculté des Sciences et Techniques, Université Sidi Mohamed Ben Abdellah, Route Immouzer BP 2202, Fez, Morocco.

<sup>2</sup>Laboratoire d'Automatique, de Génie des Procédés et de Génie Pharmaceutique. Université Claude Bernard Lyon 1, CNRS 5007, 69622 Villeurbanne, France.

\*Corresponding author: [samir.ananou@usmba.ac.ma](mailto:samir.ananou@usmba.ac.ma)

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#### Review



#### ABSTRACT

Milk is an important source of energy and a wide range of vital nutrients, but can also harbor a variety of microorganisms that can be important sources of foodborne diseases. Storage conditions and poor processing of raw milk can lead to spoilage and reduced food safety. Indeed, the role of milk fermentation is to mitigate the impact of these conditions by enhancing shelf life and food safety. Traditional fermented dairy products are widely distributed in Morocco and rely on product specific microbiota responsible for texture, aroma and flavor. Therefore, the knowledge of indigenous microbiota of Moroccan raw milk and fermented dairy products can be of great interest in preservation of Moroccan traditional dairy products. The traditional dairy products included in this review are *Lben* (a fermented milk), *Rayeb* (a coagulated fermented milk), *Zebda beldiya* (a raw fresh butter), *Smen* (a fermented butter), and *Jben* (a fresh cheese). Other products were referred as *Lfrik* (a fermented camel milk) and *Klila* (a hard cheese). Little information is available on the general characteristics and processing practices of these traditional fermented dairy products. Therefore, this review will provide a brief overview of available data about artisanal dairy products preparation, and physicochemical and microbiological characteristics.

**Keywords:** Moroccan fermented dairy foods, *Lben*, *Rayeb*, *Zebda beldiya*, *Smen*, *Jben*, *Lfrik*

#### INTRODUCTION

Milk is an important source of nutrients that can be obtained from a wide variety of animals like cows, sheep, goats and buffalo, as well as humans. Milk delivers high nutrient contents including proteins, vitamins, carbohydrates, minerals, fats and essential amino acids. Milk is often consumed as raw as well as in the form of fermented dairy products for an extended shelf life (Jans *et al.*, 2017).

Fermentation process is one of the oldest and most economical technology for producing and preserving foods as well as improving their nutritional values and sensory properties (García-Burgos *et al.*, 2020; Marco *et al.*, 2017; Rasane *et al.*, 2017). It has been practiced since olden times as a low cost effective method to ensure the longevity of products (beverages and foods) (Gadaga *et al.*, 1999). Traditional fermented foods are defined as foods prepared by indigenous people from animal or plant materials, using their skillful technology and inherited knowledge (Rawat *et al.*, 2018). They can be manufactured by the action of native microorganisms or by the addition of starter cultures containing microorganisms able to convert the substrates into socially and ethically edible products suitable for the local population (Koutinas, 2017).

Fermented dairy products are consumed worldwide, and are a vital components of human diets in many parts of the world (Rasane *et al.*, 2017). Their manufacturing process consists on following the fermentation of milk by a particular group of microorganisms, which results in a decrease of pH and a subsequent coagulation of milk proteins (Hallén, 2008). Most commonly found microorganisms in fermented dairy products are Lactic acid bacteria (LAB). The fermentation action of LAB is widely known to have a crucial role in the preservation and the production of nutritious fermented foods (Satish Kumar *et al.*, 2013).

In recent years, fermented dairy products have experienced a substantial increase due to their high nutritional and health related benefits including the prevention of lactose intolerance and accumulation of galactose (Shiby & Mishra, 2013), due to the action of LAB, which lead to the removal of lactose and galactose. They are also supportive in preventing the gastrointestinal infections as well as reducing serum cholesterol levels (Rasane *et al.*, 2017). In addition, fermented dairy products are beneficial for maintaining a healthy configuration of the celiac microbiota (Kok & Hutkins, 2018). The latter has a crucial protective effect against many diseases and can support physiological homeostasis (Rawat *et al.*, 2018). Fermented dairy products play also a vital role in enhancement of whole quality, aroma and taste of the milk (Sanlier *et al.*, 2019) as well as improvement

of the bioavailability of essential nutrients such as vitamins, amino acids and minerals.

Located in northern Africa, Morocco has a long and ancient tradition in dairy products consumption. Its eating habits, based on vegetables, cereals and animal products, contribute to low prevalence of cardiovascular diseases and obesity (Mehio Sibai *et al.*, 2011; Srairi *et al.*, 2013). Since the independence era (early 1960s), the country has experienced a rapid demographic growth coupled with diet changes. As a result, a sharp increase in food demand has occurred, especially in animal products like meat and dairy products (Lampietti *et al.*, 2011). The latter have an important place in the traditional diet of Moroccan people and are part of the main staple foods (Srairi *et al.*, 2013).

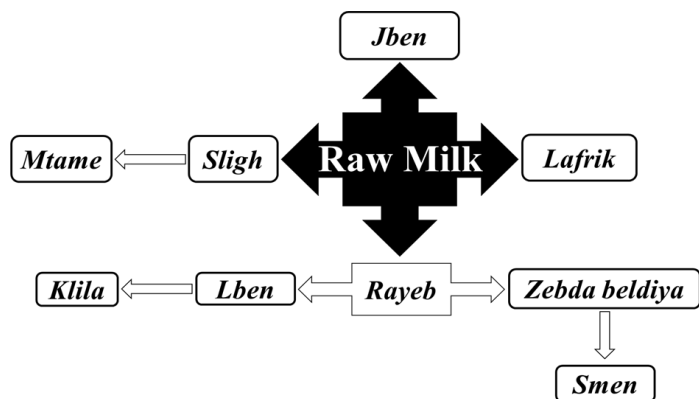
Fermented dairy products in Morocco have an important place in the traditional diet, not only because they serve as a milk preservation form, but also meet the need for diversifying milk products, while contributing to enhance the digestibility, functionality as well as the nutritional profile of milk.

Milk in Morocco comes from four different sources including cows, sheep, goats and camels. According to the Food and Agriculture Organization corporate statistical Database (FAOSTAT, 2021), Morocco was home to an estimated 3.31 million cattle, 5.93 million goats, 21.59 million sheep and 60 thousand camels. Total national milk production has more than doubled in 25 years, going from 513 thousand tons in 1975 to 1.25 million tons in 2000. This production continued to grow very rapidly to reach 2.64 million tons in 2019. The average milk yield in the same year was estimated at 1.46, 0.03, 0.028 and 0.27 tons.year<sup>-1</sup> for cows, sheep, goats and camels, respectively (FAOSTAT, 2021).

The milk in Morocco is processed both by approved industries and by a multitude of small traditional traders working in small dairy shops locally known as "Mahlabas". They are mainly located in popular suburbs in large cities where around 10 to 15% of Moroccan raw milk are processed (Srairi *et al.*, 2013). In these traditional urban dairies, raw milk can be sold out fresh or converted into traditional fermented dairy products, which are an integral part of the Moroccan heritage.

Because of their refreshing qualities, these traditional fermented dairy products are very popular in the country and are highly appreciated and consumed. The most popular Moroccan fermented milk products manufactured using traditional methods are *Lben* (a fermented milk), *Rayeb* (a coagulated milk), *Zebda beldiya* (a raw fresh butter), *Smen* (a fermented butter) and *Jben* (a fresh cheese). Other products were referred as *Lfrik* (a fermented camel milk) and *Klila* (a hardened

cheese obtained by dehydrating the curd from *Lben*). Their physicochemical properties are similar to those commercially produced yogurt, fresh cheese and butter. They are basically manufactured by allowing the raw milk to ferment naturally at ambient temperature for 1 to 3 days depending on the season. The coagulated milk *Rayeb* is thus obtained. It can be consumed as is or churned in an earthen jar to separate *Lben* from *Zebda beldiya*. The latter is transformed into salted butter *Smen* and consumed as a food additive. *Jben* is produced by placing *Rayeb* in a cloth at ambient temperature and draining the whey. With regard to *Lfrik*, this one is obtained by the spontaneous fermentation of camel milk at ambient temperature. *Klila* is produced by heating *Lben* until curdling. The overview of these indigenous fermented dairy products is represented in Figure 1.



**Figure 1** Schematic illustration of production of principal Moroccan traditional fermented dairy products.

Consumers in all over the world demand for dairy products of high quality, hygiene, standards and proper presentation (Zalalem & Faye, 2013). A good hygienic quality of milk is necessary for producing fermented dairy products of good quality and adequate shelf life (Pathot, 2019). The quality control of milk and fermented milk products mainly addresses chemical and microbiological composition. The chemical composition, in particular fat content is usually used as quality test (Zalalem & Faye, 2013). The density of milk is also used to test the addition of water to milk or removal of fats (Ebner et al., 2017). Free fatty acids (FFAs) and peroxide values (PV) serve as an indicators of rancidity of butter (Esfarjani et al., 2019).

The microbial content of milk and fermented dairy products is a major feature in determining their quality. On the one hand, milk and traditionally fermented dairy products are generally a major source of microorganisms that are important in the dairy industry due to the desirable flavors and physical characteristics they produce during their manufacture (Zalalem & Faye, 2013). On the other hand, dairy products may become contaminated with spoilage and pathogen germs or microbial toxins and thereby serve as vehicles for several diseases to consumers (Cancino-Padilla et al., 2017). They are also able to cause physical defects and off flavors in dairy products (Marchand et al., 2017).

Several factors may have a significant influence on the microbial composition of milk and traditionally made dairy products including health status of the animal, origin and quality of milk, environmental conditions such as temperature, manufacturing and sanitary conditions as well as geographic location (Oudghiri, 2009; Owusu-Kwarteng et al., 2020).

While the knowledge on the hygienic aspects of the most popular Moroccan fermented dairy products and raw milk has been widely discussed (El Marmissi et al., 2013; Filali, 2018; Hnini et al., 2018; Mennane et al., 2007a), few studies have addressed the characterization and identification of their microbiota. In fact, a thorough identification of the microbiota of these products can be useful in order to establish and preserve the microbial species diversity of Moroccan dairy products, as well as the selection of appropriate starter cultures for dairy fermentation (Oudghiri et al., 2005). Thus, this review is intended to provide the current status of knowledge from available data of the main microbiota identified in Moroccan raw milk and traditional fermented dairy products. The review also summarizes research results on the physicochemical properties of these products, as well as their indigenous manufacturing process.

## Moroccan traditional fermented dairy products

### Raw milk

The Codex Alimentarius defines milk as normal mammary secretion from milking animals obtained from one or more milkings, without any addition or subtraction, intended for consumption as milk liquid or further processing (CODEX STAN 206-1999, 1999). Milk is considered as a nutritious food for humans and also constitutes a favorable environment for microbial growth, including spoilage and food-borne pathogens (Chye et al., 2004).

Over the past decade, the traditional Moroccan diet -which was previously characterized by high consumption of cereals and vegetables and a low consumption of meat and dairy products- is rapidly transforming into a westernized diet (El Kinany et al., 2020). One of the most nutritional changes was the increasing consumption of dairy products (103 g/person/day) (FAO, 2011).

In Morocco, several animal species like cow, goat, sheep and camel provide milk for human consumption. The national milk production in 2019 was estimated in 2550000, 45935, 35512 and 8860 tones for cows, goats, sheep and camels, respectively (FAOSTAT, 2021). Cow's milk is considered as the most common in terms of availability and quantity produced.

Cow's milk is considered as a staple in many diets, healthy drink and its consumption is associated with a quality diet. It provides an easily accessible matrix containing a wide variety of essential nutrients such as vitamins, minerals and easily digestible proteins, which are essential for all body functions (Steijns, 2008). It is sold at 6 MAD (Moroccan Dirham).L<sup>-1</sup>.

Along with meats, grains, fruits and vegetables foods, dairy products are nutrient dense foods, providing many nutrients that are relatively low in energy and are considered as essential for health throughout the life cycle (Drewnowski, 2005). Furthermore, their consumption is also associated with beneficial effects on human health (Mitsui et al., 2007).

Also, milk and dairy products have served as functional food ingredients vectors including fatty acids, phytosterols and various probiotics. In addition, these products are a rich source for the development of large variety of innovative health promoting ingredients, which find their way onto market like "dietary supplements" (Michaelidou & Steijns, 2006). Furthermore, milk proteins are preferentially introduced into special formula intended to reconstruction of muscle mass and tissues for infantile, aged, athletes, and hospital patients (Steijns, 2008).

### Moroccan traditional fermented dairy products

Morocco has a wide range of traditional fermented dairy products included in consumers habits and highly appreciated (Benkerroum & Tamime, 2004). Their nature depends on type of milk used, pretreatments, fermentation conditions and subsequent treatments. The dairy production enjoys a very special status in agricultural sector and development plans. In addition to income generation opportunities, it contributes to supply of food for Moroccan population, in full demographic development, and whose consumers habits are evolving towards more quality products in dietary protein (Sraïri et al., 2005).

#### *Lben*

The traditional Moroccan *Lben* is a fermented milk obtained by spontaneous fermentation mainly of cow's milk, or in smaller quantities of goat's, camel's or sheep's milk. Therefore, these minority milks are used alone or in combination with cow's milk (Benkerroum & Tamime, 2004; Tantaoui-Elaraki & El Marrakchi, 1987). It is sold at 5 MAD.L<sup>-1</sup>.

In order to prepare *Lben*, raw milk is placed in an earthenware jar or in a goat leather bag called "*Checoua*" and kept at room temperature for 24-72h - depending on the temperature during the summer and winter, respectively- until souring and coagulation occurs (Samet-Bali et al., 2012). After coagulation, the resulting product is called *Rayeb*, which is separated after vigorously shaking -during 30 to 40 min- into *Lben* and *Zebda beldiya* (raw butter) (Benkerroum & Tamime, 2004; Tantaoui-Elaraki & El Marrakchi, 1987). Finally, *Lben* is added with 10% of water to facilitate butter recovery (water is added cold or hot depending on ambient temperature to bring the temperature of the whole to a level suitable for collecting the grains of butter).

Traditional Moroccan *Lben* usually lasts for 2 or 3 days, since afterwards it becomes more acidic over time and very sour, acquiring a bitter taste and a strong yeast flavor (Mangia et al., 2014). This can be attributed to the growth of undesirable microorganisms present in raw milk (such as coliforms, enterococci and *Bacillus* species), equipments and personal (Benkerroum & Tamime, 2004; Erkmen & Bozoglu, 2016; Mangia et al., 2014).

#### *Rayeb*

*Rayeb* is a traditional Moroccan coagulated milk, obtained by spontaneous fermentation, which has some resemblance to yoghurt. It is semi-solid product and has a pleasant taste and odor. It is also smooth, thick and of uniform appearance and sold at 25 MAD.L<sup>-1</sup>.

*Rayeb*, a very popular traditional fermented product, is consumed in Morocco by weaning age children and elderly. It constitutes a primary fermented whole milk product, from which other products can be processed (*Lben* and *Zebda beldiya*). Several milk types are used (cow's, goat's, ewe's or camel's milks) in *Rayeb* process. The fermentation takes place normally at ambient temperature (approx. 15-30°C) for 24-72 h (Bendimerad et al., 2012).

### Zebda beldiya

*Zebda beldiya*, product with great commercial value (60 MAD.kg<sup>-1</sup>), can be obtained by churning fresh milk, fermented milk, or cream (Mourad & Bettache, 2020). Indeed, the use of fermented milk has several advantages:

- The product is homogenous and stable.
- The production is easy.
- It allows the accumulation of milk for several days.
- Its process is faster than that based on fresh milk.

Moreover, with fermented milk, a higher percentage of milk fats may be extracted. It gives a more stable product, probably due to the presence of low pH which can eliminate some pathogens (Mourad & Bettache, 2020; O'Connor & Tripathi, 1992).

Sometimes, a limited amount of water (about 10%) is added to improve the cohesion of fat globules and to increase yields of *Zebda beldiya* recovered manually (Benkerroum & Tamime, 2004). In addition, some manufactures filtered *Lben* on a canvas in order to collect the maximum amount of butter (Tantaoui-Elaraki & El Marrakchi, 1987).

The use of mechanical churning machine contributes to flotation of fat globules on *Lben* surface. Subsequently, it will be removed using a perforated spoon. Due to its higher moisture content, this butter has a strong diacetyl flavor and a smoother body compared to industrially produced butter (Benkerroum & Tamime, 2004).

### Smen

*Zebda beldiya* can be transformed into another homemade traditional product called *Smen* (Benkerroum & Tamime, 2004). Indeed, *Smen* is a fermented and salted Moroccan butter (contain approx. 8-10% of salt), mainly consumed as a food additive or as an ingredient in processed foods (Sarhir et al., 2021). It is also designed to conserve and protect butter for a long time (Benkerroum & Tamime, 2004). Also, its pleasant and unique aroma can enhance and improve the taste of many Moroccan plates (Sarhir et al., 2021; Tantaoui-Elaraki & El Marrakchi, 1987).

This Moroccan traditional fermented butter has been sold at more than 120 MAD.kg<sup>-1</sup>. The preparation of *Smen* mainly brings out the following characteristics: a) absence of any heat treatment; b) salting constitutes element of preservation.

*Smen* manufacture is carried out in several steps:

- First, the curdled milk is prepared through spontaneous fermentation of cows', ewes 'or goats' raw milk,
- The raw butter is separated from the fermented milk by the churning step (Sarhir et al., 2021).
- The butter obtained is washed with warm water, decanted and then replaced by fresh salted water. This step is repeated so many times until the rinsing water is becoming clear. This indicates that the product is free from any residual *Lben* that may cause spoilage/putrefaction during the maturation step (Benkerroum & Tamime, 2004).
- *Smen* is salted (at approx. 8-10% of salt) and packaged in an earthenware pot, completely compacted to avoid trapped air. The pot is completely filled to minimize free space and promote anaerobiosis.
- *Smen* is stored in dark and cool place for about 3 to 6 months for maturation. In some Moroccan regions, this container is buried underground to ensure anaerobiosis, darkness and to minimize temperature variations (Benkerroum & Tamime, 2004).

Moreover, storage conditions are also standardized (anaerobic conditions and ambient temperature) (Marrakchi et al., 1986). This artisanal technology, practiced throughout Morocco, is subject to some modifications (according to provinces and regions). Indeed, raw butter can be made from a mixture of cow's and goat's milk. This method of preparation is carried out in the southern and northern regions of Morocco, where goat farming predominates (Marrakchi et al., 1986). Besides, before salting, raw butter can be exceptionally submitted to heat during 1 hour to promote residual water evaporation. This type of *Smen* is only made in Marrakech (Marrakchi et al., 1986). Finally, *Smen* can be flavored with coarsely ground leaves of aromatic plants, like thyme or rosemary, to prevent molds growth and to enhance the flavor of end product. They are either placed on *Smen* surface just before packaging step or incorporated in raw butter during manufacturing steps (Benkerroum & Tamime, 2004).

### Jben

*Jben* is an artisanal fresh cheese, manufactured with traditional techniques, without intentional addition of starter cultures. It is the most famous fresh cheese in Morocco and has been widely manufactured and consumed for a very long time

(Ouadghiri, 2009). The consumption of *Jben* has increased lately, due to the installation of a large number of traditional dairies in urban areas. This traditional product is prepared from raw milk using often artisanal procedures. Indeed, the artisanal methods are essentially based on the knowledge acquired from a long experience (Salmerón et al., 2002). Besides this traditional sector, some semi-industrial dairy units are also interested in manufacture of *Jben*, using both raw or pasteurized milk, and more or less improved preparation processes (Benkerroum & Tamime, 2004). Consequently, as result of these different methods used for preparation of *Jben*, different varieties of this white fresh cheese (as an artisanal cheese of special characteristics) are marketed under popular name of *Jben* (Benkerroum & Tamime, 2004). Indeed, the technological parameters have a great influence on final characteristic of cheese and can play an important role in its microbial composition, which is considered by manufactures and consumers as a special characteristic of artisanal cheese (Randazzo et al., 2009).

In general, *Jben* is made from either cow's or goat's milk. The manufacturing process requires three major steps: coagulation of milk, drainage of whey and molding (Benkerroum et al., 2007a).

- The coagulation consists in incubating raw milk at room temperature for a variable period to promote the multiplication of lactic acid bacteria (that will play an important role in acidification of milk). This coagulation can be spontaneous or induced by rennet or any other coagulating enzyme. The coagulant activity (consistency) is determined by milk temperature, acidity and strength of rennet (Randazzo et al., 2002).
- After renneting, milk is left to stand at room temperature for 6 to 10 hours. Then, the curd (coagulum) is recuperated by filtration using canvas. The curd obtained, characterized with high moisture content, is salted at 1 g.100g<sup>-1</sup> (Hamama et al., 2002) to improve draining of residual whey and to extend product shelf life.
- The drained *Jben* is processed in molds that give cheese its shape and can be submitted optionally to mechanical pressure actions.

### Other traditional Moroccan products

#### Lfrik

In Morocco, precisely in southern area, the traditionally fermented raw camel milk is called *Lfrik*. The preparation of this product is carried out in goat skin bags (named "*Tassoufra*") by spontaneous fermentation at ambient temperature during about 12h (Alaoui Ismaili et al., 2017,2018). Generally, *Lfrik* is prepared from unpasteurized camel milk, however, some preparations are based on pasteurized milk. As result, different sensory characteristics of *Lfrik* are obtained (Alaoui Ismaili et al., 2017).

In addition to *Lfrik*, other locally fermented camel milk products (such as *Sligh* and *Mtame*) are processed using similar equipment and techniques and with different incubation periods. Indeed, *Sligh* and *Mtame* are fermented during 3h and 24h respectively, which conferred to product different sensory properties (Alaoui Ismaili et al., 2017).

#### Klila

*Klila* is a traditional fresh cheese manufactured from raw cow's milk with spontaneous fermentation (Guetouache & Guessas, 2015). *Klila* is prepared by heating *Lben* to obtain curd. The curd cheese is drained and dried (Leksir et al., 2019; Mennane et al., 2007a,b). Cheese (*Klila*) can be consumed fresh or after drying step. This product is mainly prepared in Taza, Tetouan, Tangier and Oujda (Morocco).

### Physicochemical characteristics of raw milk and traditional fermented dairy products of Morocco

#### Raw milk

Milk is a white, matte and slightly viscous liquid whose composition and physicochemical characteristics vary significantly depending on the animal species, and even between races (Soryal et al., 2004). These variations are primarily due to the state of animal such as age, health status, heredity, diet, lactation period, milking speed and conditioning at milking time (Ercolini et al., 2009).

Milk constitutes an emulsion of fat in a liquid, which has similarities with blood plasma. This liquid is itself a suspension of protein material in a serum. The latter is a neutral solution containing mainly lactose and mineral salts (Musambyemaria, 2011). Also, regarding its physicochemical composition, milk is considered as a solution, an emulsion and a suspension. Indeed, lactose and mineral salts (in ionic state or undissociated salts) are in solution. The nitrogenous

materials (like caseins) are in suspension, and fat being in emulsion (Benacir, 1983).

Milk contains a large amount of water, carbohydrates, lipids, proteins, and mineral salts. It contains other minor constituents such as enzymes (lipase, phosphatase, lactoperoxidase, protease), pigment ( $\beta$  carotene), vitamins (A, D, B) and dissolved gases (carbon dioxide, nitrogen, oxygen) (Amiot et al., 2002).

Recent studies conducted in different Moroccan regions showed that the chemical properties of Moroccan raw milk are usually variable depending on the type of milk and the region. With regard to cow's milk, samples collected from Gharb Chrarda Beni Hssen farms region located in the north-west of Morocco showed a significant variations in terms of chemical composition of fats, proteins and total solids content (Chrif et al., 2019). The average fat content varied from 3.45 to 3.81% with an average value of 3.57%. The protein content varied from 2.92 to 3.17% with an average of 3.05% and the total solids ranged from 8.92 to 9.48 % with an average value of 9.2%.

Another study conducted by Hnini et al. (2018) showed a variability average in the nutritional quality of cow's milk collected from different regions of Morocco. The milk fat content average obtained from the west-North of the country ranged from the highest value of 37.6 g. L<sup>-1</sup> to the lowest value of 33.4 g. L<sup>-1</sup> recorded in the North-west areas. The other graded values were about 34.56 and 34.51 g. L<sup>-1</sup> in the North and West areas, respectively. Moreover, the average values recorded for milk protein content were between 27.8 and 30.6 g. L<sup>-1</sup>, whereas for the total solids, they ranged from 115.9 to 122.9 g.L<sup>-1</sup>.

Regarding Moroccan camel milk, analysis on its physicochemical characteristics revealed an average pH of 6.47, a density of 1.026, a dornic acidity of 0.19%, 0.18% of chlorides, 0.87% of ashes, 2.55% of proteins and 2.72% of fats (Alaoui Ismaili et al., 2016). The same authors revealed a significant effect on the period of the year on fats, proteins and lactose milk contents. In fact, summer camel milk was revealed to have the lowest total solids contents. Another study conducted by Kouniba et al. (2005) on Moroccan camel milk revealed an average pH value of 6.61, a titratable acidity of 0.17% and density of 1.032. The camel milk was also composed of 10.8% of total solids, 2.7% of fats, 3.3% of proteins, 4.1% of lactose, 0.83% of ash and 0.24% of chlorides.

In addition to cow and camel milk, the chemical composition of Moroccan goat's milk was also revealed by Moroccan authors. According to Noutfia & Said (2017), goat's milk was characterized by a pH value of 6.64, a lactic acid content of 1.77 g.L<sup>-1</sup>, 13.3% of dry matters, 0.73% of ash, 4.16% of fat and 3.6 % of total nitrogenous matters. Another study conducted by Zantar et al. (2010) revealed that goat's milk samples collected from two regions of Morocco (the north and oases regions) showed a relatively similar results with a slight superiority concerning dry matters in the milk from oases regions with values of (14.49 vs 13.38%), ashes (0.75 vs 0.67%) and fat content (4.53 vs 3.58%) and from the north region for proteins (3.54 vs 3.98%).

The chemical composition of raw milk varies depending on breed differences, lactation stage, climatic conditions, environment, animal health, diet, feeding practices and animal genetic (Adler et al., 2013; Arnould et al., 2009; Chrif et al., 2019; Hnini et al., 2018; Moran et al., 2018; Parmar et al., 2020). This variation affected directly yield and quality of processed dairy products (Parmar et al., 2020; O'Callaghan et al., 2017). Indeed, in addition to physicochemical properties, the processed dairy products quality can be linked principally to fat, fatty acid and protein (casein) contents of raw milk (Murphy et al., 2016; Parmar et al., 2020).

### Moroccan traditional fermented dairy products

#### Lben

In addition to type of milk used, the physicochemical composition of Lben varies considerably between different farms, regions and localities (Ouahghiri et al., 2009). This variation also depends on chemical composition of raw milk and manufacturing procedures (Benkerroum & Tamime, 2004; El-Baradei et al., 2008).

Lben was characterized by a high acidity (0.81%) and a low pH (4.25), which consequently give this product its specific acid taste (Tantaoui-Elaraki & El Marrakchi, 1987). According to Benkerroum & Tamime (2004), fermentation of lactose increases the titratable acidity in Lben to more than 0.6% of lactic acid. Therefore, pH and lactose fall below 4.7 and 3.7 g.100 g<sup>-1</sup>, respectively. In another study carried out by Marnissi et al. (2013), pH of Lben samples analyzed was 4.5 and titratable acidity measured was 80°D. Lben also contained 2.7, 0.9, 2.5, 6.5 and 0.17 % of lactose, fat content, proteins, total solids and chlorides, respectively (Tantaoui-Elaraki & El Marrakchi, 1987).

The fermentation of citrate in milk by microorganisms (especially *Lactococcus lactis* strains, *Enterococcus*, *Leuconostoc* and *Lactobacillus* species) (Beresford, 2011) results in volatile compounds accumulation, including ethanol, acetoin, acetaldehyde and diacetyl (Figure 2). These components play an important role in development of aroma in Lben (Tantaoui-Elaraki & El Marrakchi, 1987). Ethanol was reported to be present at 179.3 mg.L<sup>-1</sup>. It was quantitatively the most important volatile compound that might contribute to a typical flavor and aroma of the product. However, its concentration in Lben was too low to give it a typical alcoholic taste (Benkerroum & Tamime, 2004). The amounts of diacetyl and

acetoin in Moroccan Lben were are also very small (Tantaoui-Elaraki & El Marrakchi, 1987).

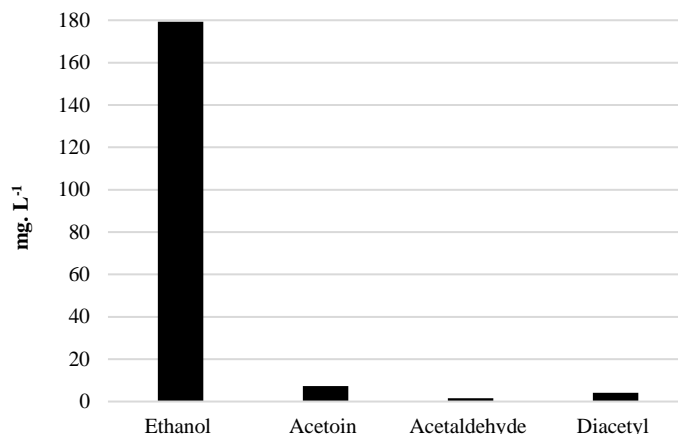


Figure 2 Aroma compounds found in traditional Moroccan Lben (adapted from Benkerroum & Tamime, 2004) .

#### Rayeb

Rayeb is considered as a product with important nutritional values, since it constitutes a high source of major nutrients (carbohydrates, protein, fat and vitamins). When consumed fresh, it constitutes low health risks due to its low pH (ranging from 4.2 to 4.5), at which most spoilage and pathogenic microorganisms are inhibited (Gonfa et al., 2001).

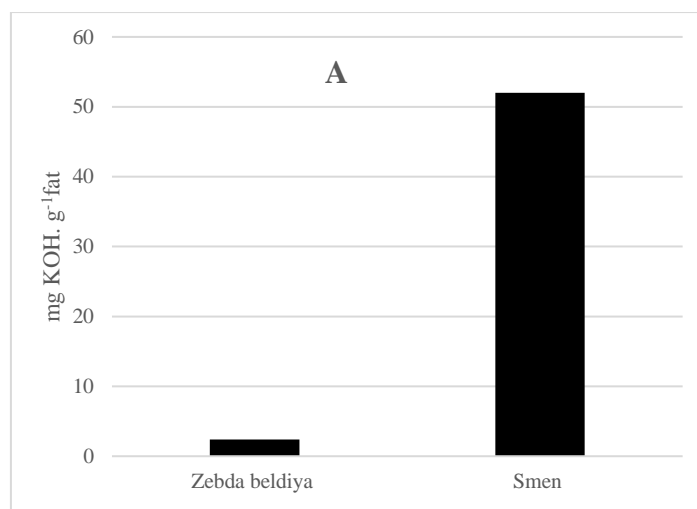
The chemical composition of Moroccan Rayeb, was determined by Hamama & Bayi (1991). The values of chemical composition were 10.7%, 2.22%, 3.1%, 4.2%, 0.17%, 0.54% for solids, fat, protein, lactose, chloride and ash, respectively. As stated above, Rayeb was also characterized by a low pH (approx. 4.2), a lactic acid content of 0.67% and a titratable acidity of 70.4°D (Hamama & Bayi, 1991; Marrakchi et al. 1993).

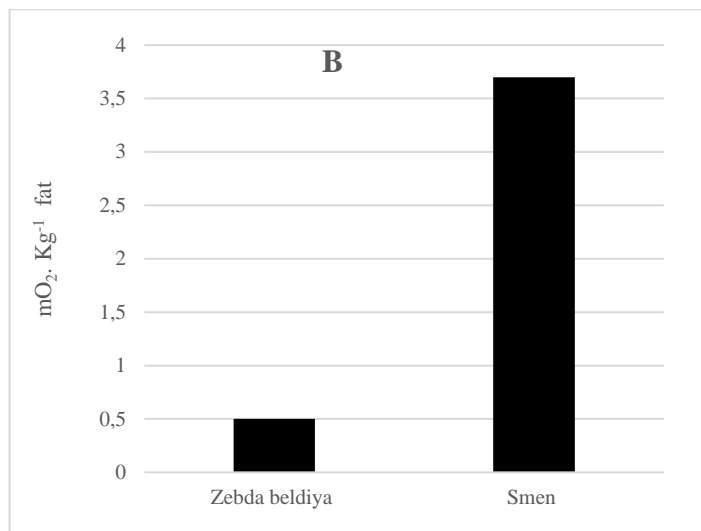
#### Zebda beldiya

The physicochemical composition of Zebda beldiya is depending on several factors such as the animal, its breed, age, health, status and stage of lactation. Environmental conditions as well as herd management also affect the product composition (Alganesh & Yetenayet, 2017).

According to Marrakchi et al. (1986), traditional Zebda beldiya was characterized by pH of 4.5 and lactic acid content of 0.77%. These values resulted from fermentation activity of indigenous microbiota adapted to product. The moisture content value of 23% was referred by Hamama (1997).

Moroccan raw butter contained approx. 73.7%, 1.8%, 1.2% and 76.7% of fats, proteins, lactose and total solids respectively (Marrakchi et al., 1986). In addition, Zebda beldiya had an average acid index of 2.42 mg KOH.g<sup>-1</sup> of butter as well as peroxide value of 0.5 mO<sub>2</sub>.kg<sup>-1</sup> of fat (Figure 3 A,B).





**Figure 3** Zebda Beldiya's and Smen's acid index (A) and peroxide index (B) (adapted from Tantaoui-Elaraki & El Marrakchi, 1987).

#### Smen

The traditional Moroccan *Smen*, is produced through abiotics and biotics (enzymatic) reactions (Benkerroum & Tamime, 2004; Tantaoui-Elaraki & El Marrakchi, 1987). During the ripening period, lipolysis of butter is considered as the main biochemical mechanism, which determines the typical flavor of *Smen* (Benkerroum & Tamime, 2004). Lipolysis pathway could originate principally from microbial cells and/or microbial free lipases (Benkerroum & Tamime, 2004). The release of free fatty acids (FFAs) from fat hydrolysis witnesses the successful ripening of *Smen* (Iradukunda et al., 2018). During this process, the short chains fatty acids (C4-C10) are the first to be released, followed by the unsaturated chains (mainly oleic acid "C18:1"), and finally, the saturated fatty acids (C12-C20) are the last to be released (Benkerroum & Tamime, 2004). The FFAs inhibit progressively *Lactococcus* and *Leuconostoc*, whereas *Lactobacillus* takes over due to its capacity to metabolize FFAs (Tantaoui-Elaraki & El Marrakchi, 1987). Then, both reactions have a major effect on *Smen*'s aroma, taste and rheological characteristics (Benkerroum & Tamime, 2004).

In recent study, Sarhir et al. (2021) studied aroma-active compounds and odor activity in Moroccan *Smen* using gas chromatography-mass spectrometry-olfactometry (GC-MS-olfactometry). Indeed, thirty-four volatile compounds were identified in *Smen*, including alcohols, acids, esters and ketons. Twenty-two and nineteen aroma active compounds were revealed, with flavor dilution factors ranging from 4 to 409, and odor activity values (OAVs) between 1 and 3359. Ethyl lactate, ethyl butanoate, ethyl hexanoate, hexanoic acid and butanoic acid were the main potent odorants in Moroccan *Smen* producing creamy-whey, fruity rose, overripe fruit, rancid buttery/soapy and ripened cheese odors, respectively (Sarhir et al., 2021).

Moreover, the chemical study of Moroccan *Smen* showed a pH value of 4.2, 81.34% of fat content and 1.5% of chlorides (Tantaoui-Elaraki & El Marrakchi, 1987). Salting and storage conditions (room temperature for at least 6 months) lead to significant chemical modifications characterized essentially by lipolysis process. During Moroccan *Smen* maturation undergoes two important transformations. The first one is related to hydrolysis of fat, and the second is linked to decrease of aqueous phase. These reactions are attributed particularly to microbial enzymes. *Smen* was also characterized by acid index of 52 mg KOH.g<sup>-1</sup> of butter and peroxide index of 3.7 mO<sub>2</sub>.kg<sup>-1</sup> of fat (Figure 3A,B).

#### Jben

As a result of different methods used for preparation of traditional Moroccan *Jben*, the physicochemical characteristics are no well-defined. The inconsistency in manufacture of *Jben* leads to large variation in chemical composition (Benkerroum & Tamime, 2004). The physicochemical characteristics, aroma and organoleptic properties of *Jben* depend mainly on those of raw milk, which depends on animals breed and their type of diet (Poznanski et al., 2004).

The composition of Moroccan *Jben* showed high levels of fat (16.4%) and proteins (15.8%). It was also characterized by low pH values of 4.1 and higher acidity (1.04% of lactic acid) (Hamama & Bayi, 1991). Also, pH and titratable acidity, which are least variable parameters in *Jben*, were reported to be less than 4.2 and higher than 0.99% respectively (Benkerroum & Tamime, 2004). In addition, pH value of 4.3 and titratable acidity of 95°D were referred in *Jben* samples (Marnissi et al., 2013).

The most variable parameter in Moroccan *Jben* was reported to be dry matter and total fat content (Benkerroum & Tamime, 2004). This variability can be due to different manufacturing procedures used in *Jben* preparation and composition of raw milk. Indeed, some *Jben* samples, especially from north of Morocco, need

more draining time (up to 10 days), whereas samples from other regions -with a temperate climate- require shorter draining period (2-3 days) (Hamama & Bayi, 1991).

#### Other products

##### Lfrik

*Lfrik* was characterized by pH ranging between 4.7 and 5.9. Similar values were reported for other traditional African fermented camel's milk such as "Suusac" in Somalia and "Gariss" in Sudan (Alaoui Ismaili et al., 2017; Jans et al., 2012; Suliman & El Zubeir, 2014).

The presence of inhibitor agents and protective proteins in raw camel milk like lactoferin, lysozyme, immunoglobulin and lactoperoxidase (Kavas, 2015) decreases the rate of lactic acid content in *Lfrik*. According to Alaoui Ismaili et al. (2017), lactic acid values ranged from 0.32 to 0.5% which was very much lower than lactic acid content obtained from the traditional fermented cow milk *Lben* (Boubekri et al., 1984).

Other components such as fat, proteins, ash, total solids, density and chlorides showed average values of 3.41, 2.46, 1.027, 0.84, 9.55 and 0.21%, respectively (Alaoui Ismaili et al., 2017). However, due to fermentation process of *Lfrik*, lactose content was very much lower than that of raw camel milk with an average value of 3.8%.

##### Klila

Due to its high protein content and very low moisture content, *Klila* can be considered as hard cheese (Mennane et al., 2007a). *Klila* samples analysed by Mennane et al. (2007a) showed an average pH of 4.28, the free acidity value of 75°D, whereas the fat and proteins contents of 12.68% and 10.38 %, respectively.

#### Microbiological overview of Moroccan raw milk and traditional fermented dairy products

##### Raw milk

Raw milk is a food with a very limited shelf life. The knowledge of its microbial composition is of particular interest to milk processors and farmers. In udder cells, milk is sterile (Taponen et al., 2019), but udder skin, mammary gland, bedding, milking equipment, breeder's practices and air quality are sources of contamination (Addis et al., 2016; Quigley et al., 2013). Due to its high nutrients, fragility and pH close to neutrality, milk is easily altered by a heterogeneous microbial population (bacteria, yeasts and molds), including spoilage and food-borne pathogens (Fusco et al., 2020).

Prior to pasteurization, LAB is a dominant population in raw milk. The most common include *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Enterococcus* and *Streptococcus* (Issa & Tahergorabi, 2019). Many other Gram positive bacteria can be found in milk like *Bacillus*, *Staphylococcus*, *Microbacterium*, *Micrococcus*, and *Propionibacterium* (von Neubeck et al., 2015). Also, Gram negative bacteria were associated with milk such as *Pseudomonas*, *Ralstonia*, *Acinetobacter*, *Psychrobacter*, *Faecalibacterium*, *Stenotrophomonas*, *Sphingomonas*, *Chryseobacterium*, *Aeromonas*, *Bacteroides*, *Porphyromonas*, *Comamonas*, *Fusobacterium*, *Enterobacter*, *Klebsiella* and *Hafnia* (Addis et al., 2016; Quigley et al., 2013; von Neubeck et al., 2015) as well as various yeasts and molds.

In Morocco, raw milk was characterized by a rich biodiversity in LAB. However, their prevalence depends on regions, farms, common practices of producers and mode of production (Hamama et al., 1992). Insufficient control of the milking operation (the first squirts of milk not eliminated, unwashed udders) promotes increased levels of LAB in raw milk (Bacic et al., 1968). Many LAB genus were reported to predominate in Moroccan raw milk -collected from different regions such as El Jadida/Mohamedia, Kenitra, Rabat and Tetouan, including *Lactococcus*, *Lactobacillus* and *Leuconostoc* (Ouadghiri et al., 2009). According to Khedid et al. (2009), *Lactococcus* species isolated from Moroccan raw milk include *Lc. lactis* subsp. *cremoris*, *Lc. lactis* biovar *diacetylactis*, *Lc. garvieae*, *Lc. raffinolactis* and *Lc. lactis* subsp. *lactis*. Among them, *Lc. lactis* is a typical technologically very important LAB in milk products (Jans et al., 2017). It was isolated from dairy products from Morocco (Ouadghiri et al., 2009) and other African countries such as Algeria (Hassaine et al., 2007), Egypt (El-Baradei et al., 2008), Mali (Wullschleger et al., 2013), and Kenya (Mathara et al., 2004). Moreover, *Lc. lactis* is a mesophilic LAB, which has a highly adapted lactose metabolism, limited proteolysis and diacetyl formation for flavor development and food preservation. All this had led to its widespread application in industrial fermentation process (Jans et al., 2017).

In regards to *Lactobacillus*, several species were isolated from Moroccan raw milk including *Lb. paracasei*, *Lb. brevis*, *Lb. plantarum*, *Lb. delbrueckii* subsp. *lactis*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. delbrueckii* subsp. *delbrueckii*, *Lb. casei* subsp. *casei*, *Lb. helveticus* and *Lb. amylophilus* (Bennani et al., 2017; Khedid et al., 2009; Ouadghiri et al., 2009). They are widely known for their excellent fermentation and probiotic properties.

*Leuconostoc* species are generally native to plants. However, *Leuc. mesenteroides* and *Leuc. pseudomesenteroides* besides other *Leuconostoc* species, such as *Leuc. mesenteroides* subsp. *cremoris*, *Leuc. lactis*, *Leuc. kimchi* and *Leuc. citreum* were identified in Moroccan raw milk (Bennani et al., 2017; Khedid et al., 2009; Ouadghiri et al., 2009). In milk with high proteolytic activity, *Leuconostoc* species can produce many metabolites such as lactate, acetaldehyde and acetate (Issa & Tahergorabi, 2019).

Different *Enterococcus* species were also isolated from Moroccan raw milk such as *E. faecium*, *E. gilvus*, *E. durans*, *E. casseliflavus* and *E. hirae* (Ananou et al., 2020; Khedid et al., 2009; Ouadghiri et al., 2009). Enterococci are widely known to be producers of bacteriocins (a ribosomally synthesized antimicrobial peptides called also enterocins) (Ananou et al., 2007, 2020; Foulquié Moreno et al., 2006). *Pediococcus* and *Streptococcus* were also detected in Moroccan raw milk. These genera include *P. acidilactici*, *P. damnosus*, *P. pentosaceus*, *Strep. cameli*, *Strep. salivarius* subsp. *thermophilus*, *Strep. equinus* and *Strep. tangierensis* (Bennani et al., 2017; Kadri et al., 2015; Khedid et al., 2009). Moreover, another LAB genus, which is *Weissella* (*W. cibaria*, *W. confuse*, *W. paramesenteroides* and *W. viridescens*) was isolated from Moroccan raw milk (Ouadghiri et al., 2009). *Weissella* spp. are frequently characterized by a large set of technological and functional properties (enhance nutritional, sensory and safety characteristics). In addition, *W. cibaria* and *W. confuse* have been widely described as high producers of exopolysaccharides (Fessard & Remize, 2017). Table 1 summarizes the identified lactic acid bacteria of Moroccan raw milk.

Molds and yeasts were also present in raw milk at moderate level (Ouadghiri et al., 2009). Their prevalence in milk is dependent on animals feeding, health and weather. Due to their broad range of physiological and biological properties, these microorganisms are important in dairy products. Yeasts including *Saccharomyces*, *Candida*, *Kluyveromyces*, *Cryptococcus* and *Trichosporon*, and molds including *Aspergillus*, *Penicillium*, *Geotrichum*, *Mucor* and *Fusarium* were isolated from several raw milk samples (Issa & Tahergorabi, 2019). In this context, it is of great

relevance that a very large number of molds can produce mycotoxins (Creppy, 2002).

On another side, microbial analyses carried out on Moroccan raw milk have revealed the occurrence of some pathogenic microorganisms including *Staphylococcus aureus*, *Yersinia enterocolitica* and *Listeria monocytogenes* (El Marnissi et al., 2013; Hamama et al., 1992; Marnissi et al., 2013).

Bendahou et al. (2008) reported the *Staphylococci* incidence in Moroccan raw milk and milk products. Results showed presence of coagulase positive staphylococci (*St. aureus* (40%), *St. intermedius* (2%) and *St. hyicus* (4%)) with a predominance (54%) of coagulase negative staphylococci (*St. arlettae*, *St. cohnii*, *St. epidermidis*, *St. gallinarum*, *St. hominis*, *St. lentus*, *St. simulans* and *St. xylosus*) in milk samples. Different sources of contamination such as milking machines, manipulators not respecting hygienic conditions or from cows with mastitis are responsible of *Staphylococcus* presence (Marnissi et al., 2013). Also, *Li. monocytogenes* with a prevalence of 8.33% (El Marnissi et al., 2013) and *Y. enterocolitica* were detected in Moroccan raw milk (Hamama et al., 1992; Issa & Tahergorabi, 2019). Factors such as environment, poor silage fermentation, manipulator's hygiene, milk equipment and water used can lead to contamination of milk by these pathogens (Raghianti, 2018).

Several studies have reported the isolation of bacteriocin-producing LAB strains with anti-pathogens activities. Such are the cases of enterocin OS1 with anti-listerial activity produced by *E. hirae*, isolated from Moroccan raw cow's milk, enterocin RM6 produced by *E. faecalis*, enterocin F420 produced by *E. hirae* isolated from Moroccan raw goat milk as well as diverse bacteriocins isolated from camel milk, produced by *E. durans* (E204 and E214), *E. faecium* (R11 and R122) *Lc. lactis* (R75 and R76), *E. faecalis* and *Strep. salivarius* subsp. *thermophilus* (Achemchem et al., 2012; Ananou et al., 2020; Benkerroum et al., 2007b; Choho et al., 2008; Huang et al., 2013; Khay et al., 2011).

Table 1 Identified microbiota in Moroccan raw milk

| Moroccan dairy products | Identified microbiota   | References  |
|-------------------------|---|---|
| Raw milk                | <p><b>Predominant :</b> <i>Lc. lactis</i>, <i>Leuc. pseudomesenteroides</i>, <i>Leuc. mesenteroides</i>, <i>Lb. plantarum</i>.</p> <p><b>Others:</b> <i>Lc. lactis</i> subsp. <i>cremoris</i>, <i>Lc. garviae</i>, <i>Lb. paracasei</i>, <i>Lb. brevis</i>, <i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i>, <i>Lb. delbrueckii</i> subsp. <i>lactis</i>, <i>Lb. delbrueckii</i> subsp. <i>delbrueckii</i>, <i>Leuc. kimchi</i>, <i>Leuc. citreum</i>, <i>Leuc. mesenteroides</i> subsp. <i>cremoris</i>, <i>P. damnosus</i>, <i>P. pentosaceus</i>, <i>W. cibaria</i>, <i>W. confusa</i>, <i>W. paramesenteroides</i>, <i>W. viridescens</i>, <i>E. durans</i>, <i>E. faecium</i>, <i>E. gilvus</i>, <i>E. hirae</i>, <i>E. casseliflavus</i>. <i>Strep. salivarius</i> subsp. <i>thermophilus</i>, <i>Strep. equinus</i>.</p> <p><i>St. arlettae</i>, <i>St. cohnii</i>, <i>St. epidermidis</i>, <i>St. gallinarum</i>, <i>St. hyicus</i>, <i>St. hominis</i>, <i>St. lentus</i>, <i>St. simulans</i>, <i>St. xylosus</i>.</p> <p><b>Associated pathogens :</b> <i>St. aureus</i>, <i>Yersinia</i>, <i>Li. monocytogenes</i>.</p> | <p>Ananou et al., 2020; Bendahou et al., 2008; Bennani et al., 2017; Ferdous et al., 2017; Hamama et al., 1992; Marnissi et al., 2013; Ouadghiri, 2009</p>  |
|                         | Camel milk  | <p><b>Predominant :</b> <i>Lc. lactis</i> subsp. <i>lactis</i>, <i>Lb. helveticus</i>, <i>Lb. casei</i>, <i>Lb. plantarum</i>, <i>Strep. salivarius</i> subsp. <i>thermophilus</i>.</p> <p><b>Others :</b> <i>Lb. delbrueckii</i> subsp. <i>lactis</i>, <i>Lb. delbrueckii</i> subsp. <i>delbrueckii</i>, <i>Lb. brevis</i>, <i>Lb. paracasei</i> subsp. <i>tolerans</i>, <i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i>, <i>Lb. amylophilus</i>, <i>Lb. casei</i> subsp. <i>rhamnosus</i>, <i>Lc. garviae</i>, <i>Lc. lactis</i> subsp. <i>cremoris</i>, <i>Lc. lactis</i> biovar <i>diacetylactis</i>, <i>Lc. raffinolactis</i>, <i>Leuc. mesenteroides</i> subsp. <i>mesenteroides</i>, <i>Leuc. lactis</i>, <i>Leuc. mesenteroides</i> subsp. <i>cremoris</i>, <i>Leuc. mesenteroides</i> subsp. <i>dextranicum</i>, <i>E. casseliflavus</i>, <i>E. faecalis</i>, <i>Strep. salivarius</i> subsp. <i>thermophilus</i>, <i>P. acidilactici</i>, <i>P. damnosus</i>, <i>P. pentosarus</i>.</p> |

Moroccan traditional fermented dairy products

The microbiota of fermented dairy products is clearly variable (Figure 4). The fermentation of milk depends principally on microbial activities to produce various metabolites. These metabolites have antagonistic and preservative effects to prevent the pathogenic and spoilage food microorganisms. The microbiota of raw milk significantly influences safety, quality and general characteristics of fermented dairy product (Agyei et al., 2020; Ananou et al., 2007).

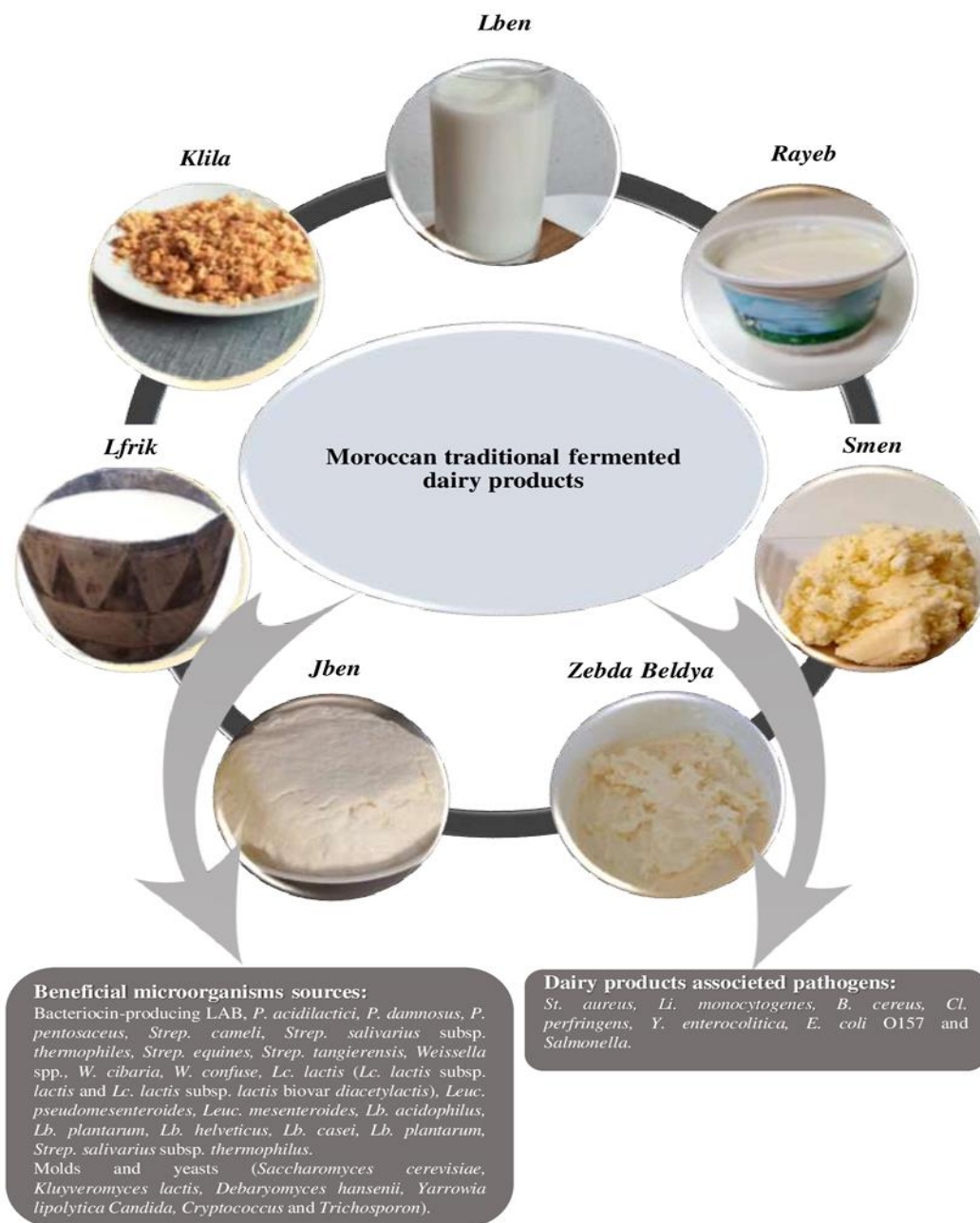
Lben

*Lben* is manufactured by a spontaneous fermentation of raw milk. The main microbiota of Moroccan *Lben* is represented by microorganisms responsible for acidification, aroma and flavor development (Tantaoui-Elaraki & El Marrakchi, 1987). *Lactococcus* and *Leuconostoc* were main LAB involved in fermentation of Moroccan *Lben* (Tantaoui-Elaraki & El Marrakchi, 1987) (Table 2). *Lactococcus* was also reported as abundant microorganism in *Lben*, which increases from 2.9x10<sup>5</sup> CFU.mL<sup>-1</sup> to reach 7.6x10<sup>8</sup> CFU.mL<sup>-1</sup> at end of *Lben*

elaboration (Berrada et al., 1983). In addition, *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *lactis* biovar *diacetylactis* are the main *Lactococcus* species predominating -with more than 10<sup>9</sup> CFU.mL<sup>-1</sup>- in Moroccan *Lben* (Mangia et al., 2014). However, *Lc. lactis* subsp. *cremoris* was also detected at low levels.

With regard to *Leuconostoc*, the main species found in *Lben* were *Leuc. lactis* and *Leuc. mesenteroides* subsp. *dextranicum* (Mangia et al., 2014). These species certainly play a major role in acidification and flavor elaboration of *Lben* (Berrada et al., 1983). These species can be distributed between homofermentative acidifying species (such as *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *lactis* biovar *diacetylactis*) and heterofermentative species (*Leuc. lactis*) or homofermentative flavoring species (*Lc. lactis* subsp. *lactis* biovar *diacetylactis*) (Berrada et al., 1983).

Lactobacilli were isolated from several traditional fermented dairy products. This genus is extremely diverse and widely used in dairy industry as starter culture to milk (in order to facilitate the fermentation process) and/or flavoring culture (Issa & Tahergorabi, 2019). *Lactobacillus*, such as *Lb. brevis*, was isolated from Moroccan *Lben* at low levels (1.8x10<sup>2</sup> CFU.g<sup>-1</sup>) (Mangia et al., 2014; Tantaoui-Elaraki & El Marrakchi, 1987).



**Figure 4** Moroccan traditional fermented dairy products and principal microbial groups associated

*Enterococcus* was also isolated from Moroccan *Lben* with more than  $10^5$  CFU.g<sup>-1</sup> (Mangia et al., 2014). *Enterococcus* constitutes the most debatable group of LAB, arising from the fact that different strains of *Enterococcus* exhibit different properties. On the one hand, some strains are considered (and consequently used) as starter, protective cultures or even, probiotics; on the other hand, some strains are considered spoilage and pathogens microorganisms (Issa & Tahergorabi, 2019). Enterococci have also several enzymatic activities (proteolysis) leading to the formation of flavors, which make them essential in fermentation process (Bhardwaj et al., 2008). Furthermore, Enterococci are widely known to be producers of bacteriocins (Ananou et al., 2007, 2020).

In addition to bacteria, molds and yeasts were reported in finished product (Berrada et al., 1983; Tantaoui-Elaraki & El Marrakchi, 1987). This can be due to favorable acidity developed and shaking (aeration) applied during *Lben* preparation (Berrada et al., 1983). Moreover, the poor hygienic conditions during handling and milk storage could also be an important source of molds and yeasts in Moroccan *Lben* (Tantaoui-Elaraki & El Marrakchi, 1987). The most dominant yeasts presented in *Lben* -with more than  $10^5$  CFU.g<sup>-1</sup>- were *Saccharomyces cerevisiae* and *Kluyveromyces lactis* (Benkerroum & Tamime, 2004; Mangia et al., 2014).

Despite their relatively low counts, compared to other microorganisms (LAB), the role of molds and yeasts cannot be neglected. On the one hand, they can activate LAB growth and, on the other hand, they can contribute to make the specific organoleptic properties to *Lben* (Berrada et al., 1983). Indeed, the physiological properties of certain molds and yeasts -like *Debaryomyces hansenii* and *Yarrowia lipolytica*-, especially their proteolytic activity, make them essential to elaborate

the flavor and aroma of fermented dairy products (Büchl & Seiler, 2011; Van Den Tempel & Jakobsen, 2000).

However, *Lben* contains undesirable microorganisms in variable levels, depending on several factors such as raw milk quality and hygiene processing. Indeed, the use of initially contaminated raw milk and water -which is generally added during the churning step in order to favor aggregation of butter grains- are sources of contamination. Several studies reported the unsatisfactory hygienic quality and poor sanitary conditions adopted during manufacture and storage of Moroccan *Lben*. Filali (2018) showed that mesophilic counts and coliforms counts -which must not exceed  $3 \times 10^6$  and 5 CFU.g<sup>-1</sup>- (BO: 5214, 2004) achieved  $1.7 \times 10^9$  CFU.mL<sup>-1</sup> and  $1.6 \times 10^7$  CFU.mL<sup>-1</sup> respectively. Another study reported counts of  $7.8 \times 10^6$ ,  $1.8 \times 10^4$  and  $8.4 \times 10^4$  CFU.mL<sup>-1</sup> for mesophilic, coliforms and enterococci, respectively (Marnissi et al., 2013). In addition, Hadrya et al. (2012) reported  $9 \times 10^6$  CFU.mL<sup>-1</sup> of mesophilic counts,  $2 \times 10^7$  CFU.mL<sup>-1</sup> of total coliforms, and  $4.5 \times 10^5$  CFU.mL<sup>-1</sup> of fecal coliforms.

The occurrence of pathogens in Moroccan *Lben* has been also investigated. The prevalence of pathogens has been referred as 75% and 55.55% for *E. coli* and *Clostridium perfringens*, respectively (Filali, 2018). *Li. monocytogenes* prevalence was between 5.7% and 19.88% (El Marnissi et al., 2013; Filali, 2018). Also, prevalence of 23.8% for *Yersinia* spp., and between 18.2% and 75% for *St. aureus* were referred in *Lben* samples (Bouymajane et al., 2021; El Marnissi et al., 2013; Filali, 2018; Hadrya et al., 2012; Hamama et al., 1992).

Other staphylococci species were also present in Moroccan *Lben*. *St. cohnii*, *St. epidermidis*, *St. hominis*, *St. lentus*, *St. sciuri*, *St. simulans* and *St. xylosus* (as coagulase negative staphylococci), and *St. hyicus* as well as *St. intermedius* (as

coagulase positive staphylococci) were isolated from *Lben* samples (Bendahou et al., 2008).

Thus, the occurrence of pathogens in Moroccan *Lben* can be considered as food safety alert. The health authority must provide consumer protection with

establishment of hygiene standard, a broad microbiological assessment, sensibilization of producers and consumers about the importance of milk safety.

**Table 2** Identified microbiota in Moroccan *Lben*.

| Moroccan dairy product | Identified microbiota  | References   |
|------------------------|--|--|
| <i>Lben</i>            | <p><b>Predominant :</b> <i>Lc. lactis</i> (<i>Lc. lactis</i> subsp. <i>lactis</i> biovar <i>diacetyllactis</i>, <i>Lc. lactis</i> subsp. <i>lactis</i>), <i>Leuc. pseudomesenteroides</i>, <i>Lb. plantarum</i>, <i>Klyveromyces lactis</i>, <i>Saccharomyces cerevisiae</i>.</p> <p><b>Others :</b> <i>Leuc. mesenteroides</i>, <i>Lb. paracasei</i>, <i>E. coli</i>, <i>E. faecalis</i>, <i>Lb. brevis</i>, <i>Lc. lactis</i> subsp. <i>cremoris</i>, <i>Leuc. mesenteroides</i> subsp. <i>dextranicum</i>, <i>Leuc. lactis</i>, <i>St. cohnii</i>, <i>St. epidermidis</i>, <i>St. hyicus</i>, <i>St. intermedius</i>, <i>St. hominis</i>, <i>St. lentus</i>, <i>St. sciuri</i>, <i>St. simulans</i>, <i>St. xylosum</i>.</p> <p><b>Associated pathogens :</b> <i>St. aureus</i>, <i>Li. monocytogenes</i>, <i>Clostridia</i>, <i>Y. enterocolitica</i>.</p> | <p>Bendahou et al., 2008; Hamama et al., 1992; Mangia et al., 2014; Marnissi et al., 2013; Ouadghiri, 2009</p> |

*Rayeb*

Moroccan *Rayeb* is the main product used for preparation of several Moroccan traditional fermented dairy products such as *Lben*, *Zebda beldiya* and *Smen*. Several microbial species were isolated from *Rayeb* (Elotmani et al., 2002; Hamama & Bayi, 1991). *Lactococcus*, *Lactobacillus*, *Leuconostoc* and several yeasts (especially *Saccharomyces* and *Candida*) contribute to the spontaneous fermentation process of Moroccan *Rayeb* (Bendimerad et al., 2012; Hamama & Bayi, 1991). Indeed, *Lactococcus* predominated LAB with  $1.4 \times 10^8$  CFU.mL<sup>-1</sup> versus  $2.6 \times 10^6$  CFU.mL<sup>-1</sup> for *Lactobacillus* and  $2.8 \times 10^6$  CFU.mL<sup>-1</sup> for *Leuconostoc*. Also, yeasts and molds reached  $5.3 \times 10^4$  CFU.mL<sup>-1</sup> in Moroccan *Rayeb* (Hamama & Bayi, 1991). Due to their strong proteolytic and lypolytic activity, yeasts growth can be essential for development of typical texture and aroma profiles of several fermented dairy products (Álvarez-Martín et al., 2008). It was reported that yeasts produce several valuable nutrients including amino-acids and vitamins, as well as various aromatic compounds like ethanol and diacetyl in these products (Samet-Bali et al., 2010). In addition, a symbiosis

between yeasts and LAB was widely suggested in fermented dairy products. In fact, LAB provide acidic conditions which are favorable to yeasts growth, and yeasts provide other growth factors to LAB (Samet-Bali, 2012). Several studies evaluated hygienic quality of Moroccan *Rayeb*. Coliforms counts reached  $1.7 \times 10^5$  CFU.mL<sup>-1</sup> (Hamama & Bayi, 1991), which proved a poor hygienic quality of this product. Moreover, *Rayeb* may also be the carrier of many pathogens responsible for serious food-borne diseases such as *Li. monocytogenes* (at 10% of prevalence) (Marrakchi et al., 1993). In fact, the frequently use of unpasteurized raw milk, unhygienic and unfavorable environmental conditions mean that *Rayeb* has variable characteristics and also higher spoilage risks. However, previous studies reported the presence of potential bacteriocinogenic LAB in Moroccan *Rayeb*. Elotmani et al. (2002) characterized several bacteriocins, with anti-listerial activity, produced by LAB strains (lactococci R10/1 and R9/2 produced by *Lc. lactis*, enterocin R18 produced by *E. faecium* and enterocin R69 produced by *E. faecalis*). Table 3 summarizes the main microbiota identified in Moroccan *Rayeb*.

**Table 3** Identified microbiota in *Rayeb*.

| Moroccan dairy product | Identified microbiota  | References   |
|------------------------|--|--|
| <i>Rayeb</i>           | <p><b>Predominant:</b> <i>Lc. lactis</i> subsp. <i>Lactis</i>, <i>Lc. lactis</i> subsp. <i>lactis</i> biovar <i>diacetyllactis</i>, <i>Leuc. mesenteroides</i>, <i>Leuc. pseudomesenteroides</i>, <i>S. cerevisiae</i>, <i>Candida</i>.</p> <p><b>Others :</b> <i>E. faecium</i>, <i>E. faecalis</i>.</p> <p><b>Associated pathogens:</b> <i>E. coli</i> O157, <i>Li. monocytogenes</i>, <i>St. aureus</i>, <i>Salmonella</i>.</p> | <p>Bendimerad et al., 2012; Benkerroum &amp; Tamime, 2004; Elotmani et al., 2002; Hamama et al., 1992; Hamama &amp; Bayi, 1991</p> |

*Smen*

The chemical study of Moroccan *Smen* revealed the importance of microbial enzymes to product maturation. However, microbiota of Moroccan *Smen* is very low (El Marrakchi et al., 1988a), due to combined effect of free fatty acids (FFAs) and salt (8-10%), as well as nutrients (proteins, lactose and mineral salts) decrease in aqueous phase (Tantaoui-Elaraki & El Marrakchi, 1987). Moroccan *Smen* contains principally salt-tolerant microorganisms, mainly represented by Gram positive bacteria. This microbial population is predominated by *Bacillus* (at 96%), followed by staphylococci (Benkerroum & Tamime, 2004; Tantaoui-Elaraki & El Marrakchi, 1987). The predominance of *Bacillus* species (such as *B. alvei*, *B.*

*cereus*, *B. brevis* and *B. firmus*) (Table 4) is due to lipolytic activity, FFAs resistance and salt tolerance. In fact, several species of *Bacillus* are salt-tolerant bacteria, many of which grow at NaCl concentration of 25% and pH 2 (El Marrakchi et al., 1988a; Tantaoui-Elaraki & El Marrakchi, 1987). Also, *St. cohnii* and *Aeromonas hydrophilic* (these species can grow at salt concentration of 7%) with lipolytic activity are found in Moroccan *Smen*. In addition, some *Lactobacilli* (especially *Lb. plantarum*, *Lb. casei*, *Lb. acidophilus* and *Lb. helveticus*) remained at low levels in *Smen* (Tantaoui-Elaraki & El Marrakchi, 1987).

**Table 4** Identified microbiota in Moroccan *Smen*.

| Moroccan dairy product | Identified microbiota   | References  |
|------------------------|---|---|
| <i>Smen</i>            | <p><b>Predominant:</b> <i>B. alvei</i>, <i>B. brevis</i>, <i>B. firmus</i>.</p> <p><b>Others:</b> <i>St. cohnii</i>, <i>Aeromonas hydrophyla</i>, <i>Lb. plantarum</i>, <i>Lb. casei</i>, <i>Lb. acidophilus</i>, <i>Lb. helveticus</i>.</p> <p><b>Associated pathogens:</b> <i>St. aureus</i>, <i>Cl. perfringens</i>, <i>Cl. botulinum</i>, <i>B. cereus</i>.</p> | <p>Benkerroum &amp; Tamime, 2004; El Marrakchi et al., 1988a,b; Tantaoui-Elaraki &amp; El Marrakchi, 1987</p> |

*Zebda beldiya*

In traditional Moroccan raw butter, mesophilic counts were around  $10^7$  CFU.g<sup>-1</sup> due to absence of salt and unpasteurized milk use (Hamama, 1989; Rady & Badr, 2003). In addition, shaking and separation processes can enhance microbial counts of raw butter (Idoui et al., 2010).

The lipolytic and proteolytic populations are mainly represented by Gram negative bacteria including *Acinetobacter*, *Pseudomonas*, *Flavobacterium*, *Vibrionaceae* and *Enterobacteriaceae* (El Marrakchi et al., 1988b). LAB are only presented in this product (Mourad & Bettache, 2018). Indeed, LAB are predominated by *Streptococci* with  $5.0 \times 10^6$  CFU.g<sup>-1</sup>, followed by *Lactobacilli* with  $2.4 \times 10^5$  CFU.g<sup>-1</sup> and *Leuconostoc* with  $1.8 \times 10^4$  CFU.g<sup>-1</sup> (Hamama, 1989).

The fecal contamination was also referred in butter samples with values of  $6.5 \times 10^4$  CFU.g<sup>-1</sup>,  $2.1 \times 10^4$  CFU.g<sup>-1</sup> and  $8.6 \times 10^4$  CFU.g<sup>-1</sup> for total coliforms, fecal coliforms

and enterococci respectively (Hamama, 1989). Their existence might be attributed to contaminated equipment, producers unhygienic, handling and/or processing methods and milk origin (Alganesh & Yetenayet, 2017; Gökçe et al., 2010).

*Jben*

The microbiota of Moroccan *Jben* was dominated principally by LAB ( $10^8$ - $10^9$  CFU.g<sup>-1</sup>). The majority of strains belonged to *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Enterococcus*. LAB species were mainly represented by *Lc. lactis*, *Leuc. mesenteroides* and *Lb. casei* (Hamama, 1997). Also, *Lc. plantarum*, *Leuc. pseudomesenteroides*, *Lc. lactis* and *Leuc. mesenteroides* were referred as predominated species in Moroccan *Jben* (Ouadghiri et al., 2005). In addition, less dominant species, such as *Lc. paracasei*, *Lc. brevis*, *Lc. garviae*, *Lc. raffinolactis*,



*Lc. rhamnosus* and *Lc. buchneri*, were reported in Moroccan *Jben* (Ouahghiri et al., 2005) (Table 5).

The population of yeasts and molds exceeded 10<sup>6</sup> CFU.g<sup>-1</sup> in Moroccan *Jben* (Benkerroum & Tamime, 2004). Yeasts can be considered as cheese spoilage agents. Indeed, at higher levels, they can cause several organoleptic alterations such as viscosity or slimy appearance, discoloration, and strong alcoholic odor. However, at moderate levels, yeasts can contribute to flavor elaboration of cheese (Geronikou et al., 2020; Ouahghiri, 2009; Wyder & Puhane, 1999).

Yeast species identified in traditional Moroccan *Jben*, elaborated with goat's milk, were *Kluyveromyces lactis*, *Saccharomyces cerevisiae*, *Yarrowia lipolytica*, *Candida parapsilosis*, *Kazachstania unispora*, *Kluyveromyces marxianus* and *Pichia fermentans* (Table 5) with percentages of 19.1, 11.7, 10.3, 10.3, 8, 7.4 and 5.9% respectively (Ouahghiri et al., 2014).

Besides, the production of Moroccan *Jben* using unpasteurized milks, equipment handlers and non-standardized conditions are responsible for food spoilage and

food safety reduction. Several authors have evaluated the hygienic quality of Moroccan *Jben* (Benkerroum & Tamime, 2004; Marnissi et al., 2013; Mennane et al., 2007a). In fact, fecal contaminants achieved levels of 10<sup>5</sup> CFU.g<sup>-1</sup> (Benkerroum & Tamime, 2004). In addition, many staphylococci species, including coagulase negative staphylococci (such as *St. auricularis*, *St. cohnii*, *St. epidermidis*, *St. hominis*, *St. lentus*, *St. simulans* and *St. xylosus*) as well as coagulase positive staphylococci (like *St. aureus*, *St. intermedius* and *St. hyicus*), were isolated from Moroccan *Jben* (Bendahou et al., 2008). Even though the limited acidification of *Jben* (a fresh cheese), pathogens of serious public health concern, such as *Y. enterocolitica*, *Salmonella* spp. and *Li. monocytogenes*, were detected at 4.1%, 10% and 18.1% of samples respectively (El Marnissi et al., 2013; Hamama, 1997). Thus, Moroccan *Jben* production must comply with official hygiene -and other regulatory standards- and follows informative marketing routes to be industrialized.

**Table 5** Identified microbiota in Moroccan *Jben*.

| Moroccan dairy product | Identified microbiota   | References   |
|------------------------|---|--|
| <i>Jben</i>            | <p><b>Predominant:</b> <i>Lc. lactis</i>, <i>Leuc. mesenteroides</i>, <i>Lb. casei</i>, <i>Lc. plantarum</i>, <i>Leuc. pseudomesenteroides</i>.</p> <p><b>Others :</b> <i>Lc. paracasei</i>, <i>Lc. brevis</i>, <i>Lc. rhamnosus</i>, <i>Lc. buchneri</i>, <i>Lc. garviae</i>, <i>Lc. raffinolactis</i>, <i>E. saccharominus</i>, <i>St. auricularis</i>, <i>St. cohnii</i>, <i>St. epidermidis</i>, <i>St. hyicus</i>, <i>St. intermedius</i>, <i>St. hominis</i>, <i>St. lentus</i>, <i>St. simulans</i>, <i>St. xylosus</i>. <i>S. cerevisiae</i>, <i>Y. lipolytica</i>, <i>C. parapsilosis</i>, <i>K. unispora</i>, <i>K. marxianus</i>, <i>K. lactis</i>, <i>P. fermentans</i>.</p> <p><b>Associated pathogens :</b> <i>Y. enterocolitica</i>, <i>Salmonella</i>, <i>Li. monocytogenes</i>, <i>St. aureus</i>.</p> | <p>Bendahou et al., 2008; Hamama, 1997; Ouahghiri et al., 2005, 2014</p> |

*Other products*

*Lfrik*

The microbiota of *Lfrik* was mainly dominated by LAB (*Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Streptococcus*) (Table 6). The major proportion was *Lactobacillus* (at 1.2x10<sup>7</sup> CFU.mL<sup>-1</sup>) represented with several species (especially, *Lb. brevis*, *Lb. fermentum*, *Lb. delbrueckii* subsp. *bugaricus*, *Lb. delbrueckii* subsp. *lactis*, *Lb. acidophilus* and *Lb. plantarum*). *Lactococcus* achieved 3.7x10<sup>7</sup> CFU.mL<sup>-1</sup> and was represented by *Lc. lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis* biovar *diacetylactis*, *Lc. lactis* subsp. *hordniae*, *Lc. raffinolactis* and *Lc. plantarum*. Besides, *Leuc. mesenteroides*, *Leuc. dextranicum*, *Leuc. lactis* and

*Strep. salivarius* subsp. *thermophilus* were also isolated from Moroccan *Lfrik* (Alaoui Ismaili et al., 2017).

Molds and yeasts were also detected in *Lfrik* at average values of 1.13x10<sup>4</sup> CFU.mL<sup>-1</sup> and 8.3x10<sup>6</sup> CFU.mL<sup>-1</sup> respectively (Alaoui Ismaili et al., 2017). The positively effect of yeasts -at high levels- on LAB activities was also reported (Gadaga et al., 2001; Pereira-Dias et al., 2000; Suzzi et al., 2003).

In regards to hygienic quality of Moroccan *Lfrik*, high levels of fecal contamination were detected (5.6x10<sup>6</sup> CFU.mL<sup>-1</sup> and 3.57x10<sup>6</sup> CFU.mL<sup>-1</sup> for total coliforms and fecal coliforms respectively), which reflect a poor hygienic quality. However, pathogens such as *Salmonella* and *St. aureus* were undetectable (Alaoui Ismaili et al., 2017). This can be attributed to camel milk use, fermentation duration (about 12h), and *Lfrik* pH (on average 5.2).

**Table 6** Identified microbiota in Moroccan *Lfrik*.

| Moroccan dairy product | Identified microbiota  | References                         |
|------------------------|--|------------------------------------|
| <i>Lfrik</i>           | <p><b>Predominant:</b> <i>Lc. lactis</i> subsp. <i>lactis</i> biovar <i>diacetylactis</i>, <i>Lb. brevis</i>, <i>Strep. salivarius</i> subsp. <i>thermophilus</i>.</p> <p><b>Others :</b> <i>Lc. lactis</i> subsp. <i>cremoris</i>, <i>Lc. lactis</i> subsp. <i>hordniae</i>, <i>Lc. raffinolactis</i>, <i>Lc. plantarum</i>, <i>Lb. fermentum</i>, <i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i>, <i>Lb. delbrueckii</i> subsp. <i>lactis</i>, <i>Lb. acidophilus</i>, <i>Lb. plantarum</i>, <i>Leuc. mesenteroides</i> subsp. <i>dextranicum</i>, <i>Leuc. lactis</i>.</p> | <p>Alaoui Ismaili et al., 2017</p> |

*Klila*

*Klila* is manufactured under traditional conditions. Levels of 5.1x10<sup>6</sup> CFU.g<sup>-1</sup> and 2.2x10<sup>5</sup> CFU.g<sup>-1</sup> of total aerobic mesophilic flora (TAMF), with predominance of LAB, were referred in Moroccan *Klila* (Mennane et al., 2007a,b). These values are accepted by Moroccan standard limits for raw cheeses consumption (Haas, 2018). In addition, fecal contamination as well as pathogens bacteria were not detected in Moroccan *Klila*. However, anaerobic spore-forming bacteria (ASF) was also referred (Mennane et al., 2007a,b). Also, yeasts and molds were detected in Moroccan *Klila* at levels of 2.3x10<sup>3</sup>-4.4x10<sup>6</sup> CFU.g<sup>-1</sup> and 2.1x10<sup>3</sup> CFU.g<sup>-1</sup> respectively (Mennane et al., 2007a,b).

These results can be attributed, probably, to LAB predominance, heat treatment (to obtain curd) and/or low moisture content (due to drainage and drying applied during *Klila* preparation). Nevertheless, ASF was only referred owing to heat and dry resistance of spores. Also, yeasts and molds were described resistant to low a<sub>w</sub>. Moreover, in addition to their flavor and texture development, LAB displays numerous antimicrobial activities against spoilage and pathogenic bacteria (mainly by H<sub>2</sub>O<sub>2</sub>, organic acids and bacteriocins production) (Ananou et al., 2007; O'Sullivan et al., 2002).

**CONCLUSIONS**

In this review, we found that traditional Moroccan dairy products are diverse. The Moroccan dairy processing is characterized by indigenous processing methods, which are sometimes accompanied by the application of other transformation steps and the addition of different spices. However, the manufacturing of these traditional products is unfortunately still not standardized. Due to their particular characteristics (flavor and nutritional value), the preservation of these products can be of great importance, especially due to

increasing of consumer awareness and interest in fermented dairy products and their nutritional and health benefits.

The great microbial diversity of traditional Moroccan dairy products, making them interesting foods, must be preserved due to the importance of their positive aspects, even if measures have to be implemented to guarantee the hygienic safety of these products. The microbiota of Moroccan raw and fermented dairy products is predominated by LAB and their exact role and prevalence can be product-specific. Moreover, the metabolic interactions between LAB and others microorganisms (particularly with yeasts) are responsible for the typical taste appreciated by consumers. However, the study on biodiversity of Moroccan traditional fermented dairy products and microbial interactions is still limited by the small number of studies given the characterization and identification of their microbiota.

For this purpose, further studies are required, on the one hand, in the isolation of the most abundant strains and the investigation of their physiological and biochemical characteristics to approximate to their role in the fermentation process. On the other hand, these products may become interesting source of beneficial microorganisms. Indeed, the new traditional strains, with attractive technological and functional properties, can be developed and used as starter, protective cultures or even, probiotics. These traditional cultures can be also used to ensure the hygienic safety with maintaining the original organoleptic properties of Moroccan traditional fermented dairy products as well as for new products elaboration with new tastes.

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