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Effect of milk pre-treatmentS on chemical composition, and sensory quality of traditional fermented milk, raYeb

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Abstract – 'Rayeb' is a Tunisian traditional dairy product which, for centuries, has played a major role in the diet of rural region communities. It is mainly made by rural women according to traditional fermentation procedures. The objective of this study was to investigate the effect of heat pre-treatments of milk on the quality characteristics of Rayeb. The heat treatment methods of cow milk used included heating of cow milk at 65°C for 15 min and pasteurisation at 91°C for 40 s. The results showed that fat and proteins contents and pH values were lower in raw milk Rayebs than thermised and pasteurised milk products ($p<0.05$). Milk heat treatment also induced a reduced lipolysis in Rayeb and impaired essential fatty acid (C18:2, C18:3) contents. The sensory evaluation revealed a decrease in Rayeb taste and texture intensity by heat treatments, leading to a better acceptance of raw milk Rayeb than the heat-treated milk Rayebs.

Keywords: Heat treatment, fatty acids, fermented milk, sensory analysis.

1. Introduction

Rayeb is a popular fermented Tunisian dairy product prepared by spontaneous milk fermentation. This fermented drink has been consumed for centuries, mainly as a fresh beverage, or as a desert after typically traditional Tunisian 'couscous' dishes. Rayeb is a natural probiotic. Probiotics are foods that contain live bacteria, which are beneficial to health (Sharma and Devi 2014). Lactic acid bacteria (LB) are the dominant microflora, which have a significant effect on the overall quality of Rayeb milk (Hamama 1992; Abdelbasset and Djamila 2008; Abd el Gawad et al. 2010; Bendimerad et al. 2012; Bali et al. 2013; Samet-Bali and Attia 2014), and some showed antibacterial activity against pathogenic bacteria such *Listeria monocytogenes*, *Escherichia coli* and *Staphylococcus aureus* (Abdelbasset and Djamila 2008; Abd El Gawad et al. 2010; Arqués et al. 2015).

In traditional production, unpasteurised cow milk is spontaneously fermented at ambient temperature, until it coagulates. The churning of the fermented milk yields fermented milk Rayeb. Microbiological studies have revealed that many species of mesophilic LB, mostly of the genus *Lactobacillus*, *Lactococcus* and *Leuconostoc*, and several yeasts species, especially species of the genera *Saccharomyces* and *Candida*, contribute to the spontaneous fermentation process (Bendimerad et al. 2012; Samet-Bali and Attia 2014). Recently, Rayeb has been produced in pasteurised form by most dairy factories in Tunisia. In dairy processes, heat treatment of milk is performed to reduce microbial loads and eliminate pathogens and most of the spoilage micro-organisms that may be present in milk. The heat treatment can be also applied to improve the keeping qualities by inactivating the enzymes. In Tunisia, dairy product manufacturers pasteurise milk at temperatures higher than 72°C, so as to reduce further the risk of survival of *Mycobacterium avium* ssp. paratuberculosis. In addition to beneficial effects, heat treatment also results in changing the physical as well as chemical properties of milk and dairy products (Ferrer et al. 1999; Singh and Waungana 2001; Benkerroum and Tamime 2004; Banik et al. 2015). In fact, milk pasteurisation is known to adversely affect the development of many quality attributes of dairy products (Imafidon and Farkye 1993; Ferrer et al. 1999; Singh and Waungana 2001; Miloradovic et al. 2016). Thermisation at a sub-pasteurisation temperature (57–68 °C for at least 15 s) can be an alternative to pasteurisation (Codex CX/MMP00/15, 1999). In fact, thermisation can lead to reduce the microflora of raw milk, minimize changes in milk quality and processability prior to conversion into product.

Taste and aroma are very important features of fermented dairy products (Grappin and Beuvier 1997; Awad 2006). Consumers make their choice of these products primarily on the basis of flavour characteristics. The typical flavour of fermented dairy products results from lipolysis, proteolysis and further degradation of amino acids by starter cultures and non-starter lactic acid bacteria (Grappin and Beuvier 1997; Smid and Kleerebezem 2014). Free fatty acids (FFA) are released by the actions of lipases from different sources, milk, starter and non-starter bacteria, moulds included as secondary starters, and other exogenous lipases, during lipolysis (Poveda and Cabezas 2006). FFAs contribute positively to the flavour of fermented milk products, particularly when properly balanced by the products of proteolysis and other enzyme-catalysed reactions, and they are precursors of more complex aroma compounds. The pleasant flavour of Rayeb can be attributed to its ethanol, acetaldehyde, diacetyle et acetoïne contents produced by lactic acid bacteria (Samet-Bali and Attia 2014). However, heat treatment of milk inactivates some indigenous milk pro-enzymes and enzymes that could play an important role in flavour development (Awad 2006; Samet-Bali et al. 2010; Velez et al. 2010). Next to flavour, the acceptability is determined by the texture of the curd, which is governed by many factors including type of milk, composition of milk etc. Heat treatment of milk also plays an important role in determining the textural quality of fermented dairy products by way of denaturation of milk proteins (Grappin and Beuvier 1997; Awad, 2006; Samet-Bali et al. 2010).

The objective of this work was to evaluate the effect of milk heat treatments on the chemical composition and sensory quality of Rayeb that may influence the consumers' acceptance.

2. Materials and Methods

2.1. Materials

2.1.1. Milk Sampling

Fresh cow milk (Holstein breed) was collected in April 2016 from a local dairy farm (Cap Bon, Tunisia, Latitude: 36.75 N, Longitude: 10.75 E).

2.1.2. Rayeb Manufacturing

Raw milk from cow was left spontaneously at 25 ± 2°C for coagulation, requiring up to 18 h. After gelation, the product was called "Rayeb". For the Rayeb samples from treated milk, the milk was filtered and thermised either to (i) 65°C for 15 min in water bath or (ii) pasteurised at 91°C for 40 s. The milk was then cooled to about 30°C and added with starter culture at 1% under sanitary conditions. The starter culture (Délice-Danone, Tunis, Tunisia) consisted of *Lactococcus lactis subsp. lactis*, *L. lactis subsp. cremosis*, *L. delbrueckii* and mesophilic *Streptococcus* organisms. After stirring, inoculated milks were incubated at 32°C for about 10 h, and then cooled to about 4°C for 24h, as determined by preliminary tests (data not shown).

2.2. Methods

2.2.1. Milk chemical analysis

Milk total solids, total protein, fat contents, and titratable acidity were determined with MilkoScan Minor (Foss, Danemark) and were carried out in triplicates. All milk samples were tested for antibiotic residues prior to Rayeb making.

2.2.2. Proximate chemical composition of Rayeb samples

The fat content of Rayeb samples was determined by Gerber method and the protein content was determined by Kjeldahl method (AOAC 2012). Similarly, the total solids and ash contents, pH and titrable acidity were done according to the AOAC (2012).

2.2.3. Fatty-Acid Extraction and Preparation of Fatty Acid Methyl Esters (FAMEs).

For each individual, the fatty acids were extracted from Rayeb samples using the continuous Soxhlet extraction technique with petroleum ether (PE) for 3 h. The extracts were filtered and concentrated under reduced pressure at 40°C. FAMEs were prepared according to Lechevallier (1966). In a methylation tube, 0.2 ml of the concentrated extract were saponified with 4 ml of a 0.5m NaOH solution in methanol for 15 min in a boiling H_2O bath at 65°C. As for transmethylations, the mixture was homogenized with

3 ml of a Boron trifluoride (BF3) solution. (14%) in methanol, and the reaction was allowed to proceed for 5 min. Subsequently, 10 ml of H_2O were added to the mixture, and the FAMEs were extracted twice with 10 ml of PE.

2.2.4. Identification of FAMEs by GC

Gas-chromatography (GC) analysis was carried out with an AGILENT 6980 Series II system equipped with a flame-ionisation detector, a split/splitless injector, and a HP-INNOWAX cap. column (30 m x 0.25 mm i.d., film thickness 0.25 m). The oven temperature was programmed isothermal at 150°C for 1 min, then rising from 150 to 200°C at 15°C/min and from 200 to 250°C at 2°C/min, and finally held isothermal at 250°C for 10 min; carrier gas, He (1 ml/min); injection volume, 2 ml. The FAME peaks were identified by comparing their retention time values with those of authentic standards injected under the same chromatographic conditions. The quantification of the FAMEs, expressed as percentages, was done by direct integration of the GC peak areas.

2.2.5. Enumeration of mesophilic lactic acid bacteria

Lactic acid bacterial count was estimated using De Man, Rogosa and Sharpe (MRS, Merck Chemicals, Darmstadt, Germany) medium (NF ISO 15214 1998). Plates were incubated at 30° C for 48 h.

2.2.6. Sensory analysis

Sensory analysis of Rayeb was carried out as per standard methods for dairy products (ISO 22935 2009). Quantitative descriptive sensory evaluations of Rayebs were carried out with three replications by 12 trained panellists who were engineer students in INAT. The attributes of Rayeb were organized into odour, taste, texture, and colour categories. Sensory attributes were scored between 1 (the least intense/absent) and 9 (the most intense).

For hedonic evaluation, a total of 120 panellists were recruited from students and staff of the INAT via internal advertisements. The panellists were asked to visually examine, taste the Rayeb samples and express their opinion by writing down the code of their most preferred sample.

2.2.7. Statistical analysis

Values were expressed as mean ± standard deviation of three replicates and statistically analyzed by an one-way analysis of variance (ANOVA) at p<0.05, using GraphPad Prism v. 4.00 software (2003).

3. Results and Discussion

3.1. Physicochemical and microbiological characteristics in raw and heat treated milk Rayebs

The physicochemical characteristics and mesophilic LB counts of raw milk and Rayebs made from raw, thermised and pasteurised milks are presented in Table 1.

Table 1. Physicochemical and microbiological characteristics of Rayebs made from raw, thermised and pasteurised milks (n=3)

		pH	Titratable Acidity (°Dornic)	Total solids (g/L)	Protein (g/L)	Fat (g/L)	Ash (g/L)	FFA (mg/g)	LB (CFU/g)
Milk		6.74 ± 0.02^a	15.1 ± 0.8^a	107.6 ± 0.8^a	31.4 ± 0.6^a	27.9 ± 0.5^a	7.2± 0.1^a	-	-
Rayeb from milk	Raw	4.63 ± 0.03^b	69.3 ± 0.1^b	94.4 ± 0.6^b	21.5 ± 0.4^b	20.5 ± 0.3^b	7.1 ± 0.1^a	3.24 ± 0.10^a	52.1 x 10^6 ± 312^a
	Thermised	4.85 ± 0.04^c	54.5 ± 0.1^c	97.5 ± 0.9^c	24.9 ± 0.4^c	23.0 ± 0.3^c	6.8 ± 0.8^a	0.89 ± 0.02^b	2.2 x 10^6 ± 307^b
	Pasteurised	4.87 ± 0.10^c	53.6 ± 0.5^c	98.9 ±0.6^c	25.4 ± 0.3^c	25.4 ± 0.5^d	6.2 ± 0.1^a	0.88 ± 0.03^b	1.1 x 10^6 ± 278^c

FFA: Free Fatty Acids; LB: Lactic Bacteria.
Results are expressed as means ± standard deviation.
Different letter superscripts a,b,c,d in the same column indicate significant difference (P<0.05).

Chemical composition of raw milk indicated suitable technological properties (NT 14.141 2004). Significant differences in physicochemical composition were observed between Rayebs made from raw and heat treated milk (p<0.05). In fact, heat treated milk Rayebs were characterised by higher total solids, protein and fat contents (P <0.05), in agreement with results obtained with cheeses and fermented milks (Singh and Waungana 2001; Samet-Bali et al. 2010; Miloradovic et al. 2016). These differences could be due, not only to the milk heat treatment but also to the nature of starter bacteria. No significant differences in the ash contents were noticed between raw and heat treated milk Rayeb samples.
Rayeb was characterized by a higher titrable acidity and a lower pH when compared to raw milk. In raw milk, the natural microflora, composed in part of lactic acid bacteria, would ferment lactose to lactic acid. Thus, the action of the natural milk microflora, could be probably responsible for a high level of acidity observed in Rayeb made from raw milk. This result was in agreement with those reported by Samet-Bali and Attia (2014) in South of Tunisia, Hamama (1992) in Morroco, Abdelbasset and Djamila (2008) in Algeria, and Abd El Gawad et al. (2010) in Egypt. Rayebs made from thermised and pasteurised milk were characterised by higher pH and lower titrable acidity values than raw milk Rayeb, and no significance difference was found between thermised and pasteurised milk products. During the curd formation, the pH fell faster in raw milk than in heat treated milks (pH<0.05 ; data not shown). This difference in acidity could be due to a high LB content of Rayebs from raw milk than thermised and pasteurised milk, as shown in Table 1. This richness on LB could lead to the biosynthesis of more nutritional and aroma compounds (Samet-Bali and Attia 2014). The distinction in acidity could also be presumably explained by the non starter microflora initially present in the raw milk and destroyed by heat treatment (Samet-Bali et al. 2010; Bendimerad et al. 2012).

3.2. FFA contents in raw and heat treated milk Rayebs

Milk heat pre-treatment significantly (P<0.05) induced a decrease in total FFA content in Rayebs (Table 1); that could be due to the inactivation of indigenous milk lipoprotein lipase (Ray et al. 2013). It is well known that lipoprotein lipase is a relatively heat-labile enzyme, which may be completely inactivated by heating at 78°C for 10 s (Ray et al. 2013). This finding was in agreement with earlier results from McSweeney et al. (1993) who reported higher levels of free fatty acids in cheese made from raw milk, and from Atasoy and Türkoğlu (2009) and Velez et al. (2010) who showed that heat treatment had a significant effect on cheese lipolysis and volatile compounds production.
The profiles of individual saturated free fatty acids in the Rayebs are shown in Fig. 1.

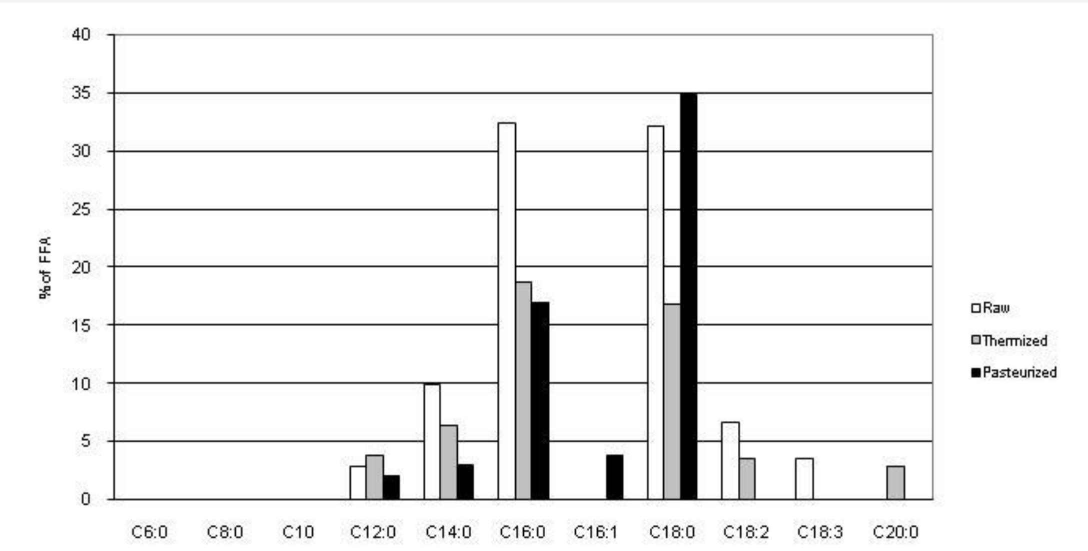

Figure 1. Free fatty acid profile of Rayeb made drom RAW, Heat-treated or pasteurised cows' milk (N=2).
Values represent the average of 2 replicates

The amounts of free fatty acids were more important in the traditional Rayeb that the heat treated milk rayebs, confirming a higher degree of lipolysis in the artisanal product.
Higher values of FFA are desirable in cheese and could be attributed to the specificity of the strains of culture micro organisms used (Awad 2006). They derived from two majors sources: breakdown of fat by lipolysis and metabolism of carbohydrates and amino acids by bacteria. Total FFA concentration and short/long-chain FFA ratio have been related to the type and the amount of lipase used during cheese ripening, and to the sensory characteristics of the cheese (Poveda and Cabezas 2006; Atasoy and Türkoğlu 2009; Giaccone et al. 2016).
In raw milk Rayeb, the major free fatty acids are myristic (C 14: 0), palmitic (C16:0) and stearic (C18:0) acids. In the pasteurised milk Rayeb, stearic acid (C18: 0) was predominant, and to a lesser extend, palmitic acid (C16:0), but no essential fatty acids (C18: 2, C18:3) were detected, suggesting that the industrial pasteurisation process could generate oxidation side reactions leading to destruction of these unsaturated fatty acids. However, linoleic acid (C18:2) was detected in thermized milk Rayeb. The relative amounts of short chain fatty acids (C4 - C10) were low in all Rayebs (about 2%), a display of the low lipolytic activity from the starter and non starter micro-organisms. The contents of fatty acids medium-chain (C14) were higher in the raw milk Rayeb than in the heat treated milk Rayebs. These volatile fatty acids (C6, C8, C10 and C12) give the cheese its odour (McSweeney and Sousa 2000). Giaccone et al. (2016) have established significant correlations between the concentration of several FA in cheese and the intensity of sensory descriptors. Furthermore, the free fatty acids can affect the specific flavour of each fermented dairy product and be the precursors of aromatic compounds such as lactones, secondary alcohols and organic acids (Smid and Kleerebezem 2014).

3.3. Sensory evaluation

Table 2 showed the effect of milk heat treatment on Rayeb sensory quality.

Table 2. Quantitative descriptive analysis of Rayebs made from raw, thermized and pasteurised milks (n= 12 trained panellists)

Rayeb from milk	**Colour**	**Odour**	**Texture**	**Taste**
Raw	4.8^a	3.9^a	6.6^a	6.0^a
Thermized	4.2^a	2.9^a	5.3^b	4.9^b
Pasteurised	4.1^a	2.4^b	4.9^b	4.5^b

[a,b] Different letter superscripts in the same column indicate significant difference (P<0.05).

Pasteurised and thermized milk Rayebs were not perceived as significantly different in colour, when compared with raw milk Rayeb. However, the intensity scores for odour, texture and taste decreased significantly as a result of heat pre-treatment (p<0.05). According to Samet-Bali and Attia (2014), the microstructure of Rayeb consisted of individualized particles that were coalesced in chains leading to relatively homogeneous sieve. LAB are involved in protein matrix since bacterial membranes could interact milk proteins (Samet-Bali and Attia 2014). Milk heat pre-treatments induced changes in protein matrix, and therefore in Rayeb texture. The difference could be attributed to the types of micro-organisms involved in the fermentation, which produce different flavour compounds (Buchin et al. 1998; Pogačić et al. 2016). This suggests that the non-starter microflora could have a significant effect on sensory quality of Rayeb made from raw milk. This was in agreement with others studies indicating that traditional fermented milks can be considered to be more aromatic than similar industrial products (Buchin et al. 1998; Benkerroum and Tamime 2004: Samet-Bali et al. 2010). Bendimerad et al. (2012) have shown that Rayeb was flavoured by strains of *Lactococci* and *Leuconostocs*. Thus, heat treatment and pasteurisation, which both reduced the number of nonstarter microflora, could lead to a large reduction in aromatic components in Rayeb, particularly in the organic acid amounts (Samet-Bali et al. 2010). Moreover, the lower pH (about 4.5) could contribute to aroma development in fermented milks (Murti et al. 1992).
A consumers' acceptance test on 120 naive panellists showed that 52% preferred raw milk Rayeb, while Rayebs made from thermized and pasteurised milks were preferred by 24% and 24% panellists, respectively. Although industrial processes are used to produce safe fermented milk and to provide the

product with standard characteristics, consumers prefer traditional Rayeb made from raw milk, due to its organoleptic quality (fresh and sour taste and characteristic aroma).

4. Conclusion

Heat pre-treatments induced significant changes in acidity and chemical composition of Rayeb. In addition, our results showed that these treatments were able to produce a Rayeb with a lower level of lipolysis than raw milk Rayeb, but pasteurisation led to the destruction of essential FFA. Furthermore, descriptive sensory data indicated a decrease in odour, taste and texture compared to the raw milk Rayeb. Several changes could be attributed to the biochemical changes of milk components due to the heat and to the quality of starter cells. Spontaneous starter cells present in raw milk are responsible of a typical taste appreciated by consumers. Future studies are needed to define the conditions (time, temperature) required to achieve the correct balance of positive effects of heat treatment.

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