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Influence of lactic ferments on Aflatoxin M1 in the industrial manufacture of the Algerian Leben

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ABSTRACT

The physicochemical and microbiological analysis of two types of milk used for the industrial production of fermented milk such Leben give results in accordance with Algerian standards. These same characteristics reveal standards compliance for Leben. The amounts of AFM1 determined by competitive ELISA in both types of milk used for the manufacture of Leben remain higher than those of many countries that have established regulatory limits for this toxin in milk and dairy products, which range from 0, 05 µg/L for European countries to 0.5 µg/L in the USA. The AFM1 levels were comparable ($p < 0.05$) for raw milk and the recombinant, and were respectively $115{,}471.10^{-3}$ ±1,096 and $119{,}057.10^{-3}$ ±0,821µg/L. From the sixth hour incubation, the Leben acidity based raw milk or recombined milk, entered into a free exponential phase from 18 ± 0.87 °D and 20 ± 1.32 ° D to 73 ± 1.5 1 °D and 78± 0.87°D after 18 hours of incubation correspondi ng to a decrease of 93.34% and 96.39% respectively. This reduction was highly significant from 06 hours of incubation for both types of milk ($P < 0.05$). These AFM1 regressions were correlated with the activity of lactic acid bacteria and offer an alternative to direct highly contaminated milk to the manufacture of fermented products.

Keywords: raw milk, recombined milk, Leben, lactic bacteria, aflatoxin M1, ELISA

INTRODUCTION

Aflatoxin B1 (AFB1) is the most worrying and most toxic mycotoxin produced by Aspergillus flavus [1, 2] which contaminates both, animal and human feed, particularly by intermediate cereals. The ingestion of AFB1 by milk producing animals leads to the metabolism of the toxin, which is found in milk in the form of toxic hydroxylated derivative commonly called Aflatoxin M1 (AFM1) [3, 4, 5].

Consumption of food contaminated with mycotoxins is a major public health problem. Even at low doses, AFB1 and AFM1 are considered hepatotoxic, genotoxic, carcinogenic and immunomodulatory [6, 7, 8]. The amount of AFM1 excreted in milk varies from 1 to 6% of AFB1 rate of the ration, and there is a linear relationship between the concentration of AFM1 in milk and the concentration of AFB1 in contaminated food consumed by animals [9, 10, 11]. In Algeria there are no regulations to control the presence of AFM1 in milk products. Regulatory limits for AFM1 exist in more than 60 countries and 34 of these countries define a maximum acceptable level of AFM1 in milk at 0.05 mg / kg [12, 13]. Aflatoxin M1 has been classified in group 2B and considered by IARC as possibly carcinogenic to humans [14, 15]. While it is impossible to completely eliminate mycotoxins from food, it is possible to set maximum values tolerable, does not induce or little damage to health [16]. To allow effective monitoring of possible contamination of milk and milk products by this toxin, more sensitive analytical methods, reliable and

simple were developed [4, 17, 18, 19]. Rosi et al., 2007 [20] showed that for the determination of AFM1 in milk, the ELISA method gives good results in comparison with the high performance liquid chromatography (HPLC). Many studies have shown reduction of AFM1 during fermentation contaminated milk [21, 22, 23, 24]. In our case and for the anxiety that surrounds the possible presence of these toxins in milk and dairy products we characterized the physicochemical and microbiological partly skimmed raw milk and recombined milk, for the industrial manufacture of milk Leben fermented kind. The AFM1 was then sought by competitive ELISA in these products and particularly in the Leben coveted by consumers in our country. Also, the effect of a consortium of mesophilic lactic ferments on this toxin was prospected.

MATERIAL AND METHODS

Our study was conducted at the cheese dairy TESSALA in Sidi Bel-Abbes, a subsidiary of the industrial group of dairy productions (GIPLAIT) in Algeria.

Strains and raw materials

In this study, we used a consortium of lactic ferments consisting of *Lactococcus lactis* subsp. *lactis*, *Lactococcus cremoris* subsp. *cremoris*, *Streptococcus thermophilus*, *Leuconostoc cremoris*, *Lactococcus lactis* subsp. *lactis* biovar. *Diacetylactis*, lyophilized and marketed by Vivolac Cultures Corporation (Indiana, USA). These ferments were provided by GIPLAIT unit of Sidi Bel-Abbes. They are typically marketed in aluminum bags impermeable to water and air and may keep twelve months at -30°C.

The milk used was that of raw mixture produced and collected locally undergoing partial skimming. The skimmed milk powder extra grade (less than 1.25% fat) was reconstituted by mixing 94g in one liter of treated water (CaCO3 acceptable hardness <100mg/L) at a temperature between 35 and 45°C. It was then recombined by adding anhydrous liquid fat after degassing for removing the particles of oxygen may oxidize the latter [25].

Manufacture of fermented milk type Leben

Pasteurization and milk inoculation

The milk, recombined and raw partially skimmed, were pasteurized at 72 ° C for 20 min in a pasteurizer plates then cooled to a temperature of 22°C considered optimal for the development of lactic ferments. The milk was directly inoculated with lactic acid bacteria (starter culture) lyophilized, in this case, *Lactococcus lactis subsp lactis, Lactococcus cremoris subsp cremoris, Streptococcus thermophilus, Leuconostoc cremoris et Lactococcus lactis subsp lactis biovar. Diacetylactis.*

Maturing, cooling and conditioning

The maturation time was 16 to 18 hours at 22°C until acidity of between 65-75°D. Once reaching acidity, the milk was rapidly cooled to a temperature of 6 to 10°C to stop fermentation. The fermented milk (Lben) was conditioned at a temperature below 10°C in polyethylene bags.

Sample Collection

Representative samples were concerned partly skimmed raw milk and recombined milk, pasteurized and used in the manufacture of Leben. The sample preparation and removal of the portion used for physical and chemical analysis respect the principle that the aliquot taken for analysis must be as representative as possible of the lot [26]. Sampling was performed during the two Leben manufacturing processes, as well as finished products after manufacture and after 48 hours of storage.

physicochemical analysis

- The milk temperature was measured using a digital thermometer HANNA Instruments, Italy.
- Density was the ratio between the mass of a given volume of milk at 20°C and the mass of same volume of water. It was determined at 20°C using a lactodensimeter (Funke Gerber-Germany) [27].
- The acidity was determined by titrimetric determination of lactic acid using sodium hydroxide in the presence of colored indicator (phenolphthalein). This determination was made on a test sample of 10 ml. The results were expressed in Dornic or percentage degree of lactic acid, 1 Dornic degree (°D) corresponding to 0.1g of lactic acid per liter of milk [28].
- The pH was measured directly using a pH meter (Hanna Instruments, Italy) previously calibrated and by immersing the electrode in the product [29].
- The dry matter (total solids content) was obtained by evaporation and drying 10 ml of milk in an oven (Memmert) at 102 ± 2°C for 05 hours with weighing of the residue [30].

-The fat

The determination of fat, utilizes the acidobutyrique method, which comprises dissolving 11 ml of milk in 10 ml of sulfuric acid. Under the action of a centrifugal force and following the addition of 01 ml of isoamyl alcohol, fat separates in transparent coat. For direct reading of the result, the Gerber butyrometer was used for milk [31].

-Defatted dry matter

Defatted dry matter or fat free dry matter, expressed the milk content of dry elements stripped of total fat. This content was approximately 90 grams per liter. For normal milk, the value was between 90 and 102 grams per liter. A value below 87 indicate a milk adulteration by wetting [32].

Microbiological analysis

10 ml of sample was added to 90 ml of sterile physiological saline. The mother dilution 10^{-1} obtained was used to make decimal dilutions to 10^{-7}. Methods used were in conformity with the Algerian standards according to the Decree of 24/01/1998 relating to the microbiological specifications of certain foodstuffs [33].

Total mesophilic flora

Enumeration of total viable flora was made on the T.G.E.A medium (Tryptone Glucose Extract Agar). Incubation was performed at 30 °C for 72 hours and the colonies of mesophilic aerobic microorganisms were present in mass, as lenticular form.

Total coliforms

Total coliform research was done on medium Lactose agar deoxycholate and incubation was on solid medium at 37 °C for 24 to 48 hours.

Fecal coliforms

Search and enumeration of fecal coliforms were on nutrient agar EMB (eosin Methyl blue) and incubation in an oven at 44°C for 24 to 48 hours. The total and fecal coliforms appear in mass as small colonies of red color in VRBG middle (Violet Red Bile Agar with Glucose), and green in EMB environment.

Staphylococci

Staphylococci were isolated and counted on a liquid medium Chapman with enrichment medium and potassium tellurite. After incubation at 37 °C for 24 to 48 hours staphylococci were in the form of medium-sized colonies smooth and pigmented in yellow.

Sulfite-reducing clostridia

For Sulfite-reducing clostridia spores at 46°C, milk placed in tubes was previously heated 10 minutes at 80°C and then rapidly cooled to activate the spores of clostridia and destroy germs vegetative form. Then they were counted on the middle of tryptose-sulfite culture cycloserine (TSC) (Institut Pasteur, Algeria). After incubation at 46°C for 20 ± 2 h, only the characteristic colonies, surrounded by a black halo were counted.

Salmonella

For salmonella search, we use Hektoen agar or SS (Salmonella, Shigella). Incubation was performed at 37°C for 48 hours. Salmonella appear in Hektoen environment in the form of green or blue colonies with lactose, sucrose and salicin all negative and positive black center with H_2S. On SS medium in the form of colorless colonies with negative lactose and a black center with positive H_2S.

Immunochemical determination of aflatoxin M1

An aliquot of milk or Leben was placed at 4°C for 2 hours. Centrifugation was then carried out at 3700 rpm to remove fat. 100 µl (per well) of this solution were used in this test [34, 35].

Helica Biosystems Mycomonitor Aflatoxin M1 ELISA kit (Helica Biosystems Inc. Fullerton, CA. USA) was used for quantitative detection of aflatoxin M1 in the samples following manufacturer's instructions. The technique was a competitive ELISA assay in solid phase. An antibody with high affinity for aflatoxin M1 was used for coating polystyrene microwells. Aflatoxin M1 of the sample react competitively with aflatoxin-peroxidase conjugate against coating antibody. After incubation and washing with PBS-Tween 20 of the various wells, the addition of a substrate for horseradish peroxidase, tetramethylbenzidine (TMB), develops a blue color that turns yellow upon the addition of acid stop solution which was measured at 450 nm with microplate reader (Tecan Sunrise, Austria GmbH). The color intensity was inversely proportional to the aflatoxin M1 content of the sample [36, 37, 38, 39]. Standard curve of the absorbency against known AFM1 concentrations (0 to 100 ng/L) was plotted for the determination of AFM1 level by ELISA technique in all samples in this study.

Statistical analysis
The statistical analysis uses the StatView and the results were expressed as mean followed by the standard deviation. The ANOVA test was used to compare the results and the probability P <0.05 was considered significant.

RESULTS AND DISCUSSION

Physico-chemical quality of milk for the manufacture of Leben
Partly skimmed milk and recombined one, used for the manufacture of Leben were subjected to heat treatment (pasteurization at 72°C for 15 to 20 s) to eliminate the common flora and destroy pathogens. The skimmed milk was a local production of milk which undergoes a skimming, whereas the recombinant, it was obtained from the milk powder.

The physicochemical analysis results of the two types of milk used, in this case, the raw skimmed milk and the recombined one, was in accordance with the Algerian standards as stipulated by the Interministerial Order of 24/01/1998 [33] (Table 1).

Table 1: Physicochemical analysis of partly skimmed milk and recombined milk for the Leben manufacture

Physicochemical parameters	Raw milk, partly skimmed	* Standards	Recombined milk	* Standards
Density (°B)	1032±2	1030-1034	1031±1,73	1028-1032
Acidity (°D)	16,33±0,58	16-18	16,7±058	16-18
Fat (%)	1,80±0,145	0-1,5	1±0,09	<1,25
Total dry extract (g/l)	86±0,99	90-96	98±0,90	95-128
Defatted dry matter (g/l)	84±1,09	/	/	/

** JORA, 1993. Values are presented as mean ±SD (n = 3)*

The average values of titratable acidity studied milks were however lower than those reported by Mathieu, 1998 [40] for ten cow milk samples and in traditional milking conditions. The milk acidity increases with time. Indeed, lactose degradation by microorganisms milk causes acidification, lactose was broken down into lactic acid, indicator of preserving milk and expressed in Dornic degree (°D) [41]. Variabilities are climate-related, stage of lactation, food availability, water intake, health status and conditions of milking cows. In our case the acidity was in accordance with those reported by Aboutayeb, 2011 [42] and the FAO, 2010 [43] (15 to 18°D and 15 to 17°D). Our values also corroborate those of Labioui et al., 2009 [44], which report a titratable acidity of raw milk of 16.75%. Acidity depends on the casein content, minerals and ions salts [45], hygienic conditions during milking, total microbial flora and its metabolic activity [40], the handling of milk.

The average value of the density of our raw milk, partly skimmed and pasteurized was 1032 ± 2. That of recombined milk was 1031 ± 1.73. These values were in conformity with the Algerian standards. They were also corroborated by those reported by FAO, 2010 [43] (1028-1033) and Aboutayeb, 2011 [42] (1028-1035). The density depends on the dry matter content, fat content, increase of the temperature and food availability [46].

The fat content in raw milk partially skimmed pasteurized, and the recombined one, were 1.8 ± 0.145 and 1 ± 0.09% respectively and conform to standards of JORA, 1993 [25]. According to Roudj, 2005 [47], the milk fat content was 16g/l. The average fat content of our two milks remains below the range of 28.5 to 32.5 g/l recommended by ISO 1211: 2010 [48]. The fat content was among the milk solids, the element that was most strongly and quickly modifiable through diet [49]. The variability of fat depends on factors such as weather conditions, stage of lactation and feeding.

The fat free dry matter value of partly skimmed milk (86 ± 0.99) was lower than the Algerian standards (98 ± 0.90) while that of recombined milk was conform [25]. However, our values remain lower than those reported by other authors as Labioui et al, 2009 [44] which show that the dry extract of ten samples of raw milk of cows from two farms was between 113.1 and 121.7 g/l with an average of 117.5 g and depends on climatic and dietary factors. According to Seme, 2015 [50] the dry extract of raw milk from 12 farms in the Maritime region in southern Togo was averaging 133.07 ± 31.12 g/l. In our case the two types of milk have a similar density and acidity (P <0.05) but were different from the fat and total dry extract (p <0.05).

Microbiological quality of milk for the manufacture of Leben
The results of the counts were summarized in Table 2. The enumeration of total viable flora of partly skimmed milk and the recombined one gives results in accordance with regulation [33]. The search for microorganism's indicators of fecal contamination can judge the hygienic condition of product. Even at low levels, they show hygienic conditions deteriorated during milking.

Table 2: Microbiological analysis of partly skimmed milk and recombined milk for the Leben manufacture

Microbiological criteria	partly skimmed raw milk	*Standards	Recombined milk	*Standards
Aerobic mesophilic flora	22.10^3±180	30 10^3/ml	20 10^3±132	30 10^3/ml
Total coliform	20±1	10/ml	30±1	10
Fecal coliform	Abs	Abs	Abs	Abs
Staphylococcus	Abs	Abs	Abs	Abs
Fecal Streptococcus	Abs	Abs	/	/
Sulfite-reducing *clostridia*	Abs	Abs	/	/
Salmonella	Abs	Abs	/	/
Shigella	Abs	Abs	/	/

**JORA, 1998. Values are presented as mean ±SD (n = 3)*

The partly skimmed milk and recombined were free of fecal coliforms in accordance with Algerian legislation. However, the total coliform levels were slightly above the recommended threshold. Other germs sought in this case the Staphylococci, Streptococci Fecal, sulfite-reducing Clostridium, Salmonella and Shigella were absent from both studied milks. Pasteurization allows the destruction of a large part of the bacteria. These results show the importance of observing good hygiene breeding, milking and handling conditions of these raw materials. Logically, a low bacterial load will always be easier to remove than a heavy load. The nutritional quality of dairy products still depends on raw milk [51].

Physicochemical and microbiological quality of Leben

The Leben obtained by the method of manufacturing of the Giplait unit in the western region of Algeria has physicochemical characteristics in conformity with the Algerian standards [25] (Table 3). PHs of our milk, raw and recombined, were respectively 4.67 ± 0.072 and 4.74 ± 0.101 with a similar acidity of 73 ± 1 ° D.

Tableau 3: Physicochemical characterization of industrial Leben

Physicochemical parameters	Leben from partly skimmed milk	Leben from recombined milk	*Standards
pH	4,67±0,072	4,74±0,101	/
Acidity (°D)	73±1	73±1	65-75
Fat (%)	1,85±0,132	1±0,22	0 - 5
Total dry extract (g/l)	90,8±2,03	90,8±1,59	90 - 96
Defatted dry matter (g/l)	88,8±2,43	89,8±3,56	–
Temperature (°C)	20±1	20±1	/

**JORA, 1993. Values are presented as mean ±SD (n = 3)*

According Ouadghiri et al., 2009 [52], fermented and skimmed milk samples Leben, showed a pH ranging from 4.25 to 4.57 with an average of 4.38 and an acidity ranging from 73.12 to 112.5 °D with an average of 83.05 °D. Acidic pH of Leben, were related to the technology of these products and were explained by the acidifying activity of lactic acid bacteria. Our results were in agreement with many works particularly in Morocco [53, 54, 44]. Milks used in our case for the industrial manufacture of Leben were standardized with respect to the fat. Unlike traditional Leben manufacturing process there was no churning that causing a phase inversion and a collection of butter. The milk was then pasteurized and directly inoculated with mesophilic ferments.

The fat present almost similar rate of 1.85 ± 0.132% and 1% ± 0.22 respectively for raw and recombined milk ($p < 0.05$). The work of Boubekri et al., 1984 [53] report a fat content of Leben ranging from 0.2 to 1.8% with an average of 0.893 almost similar to that of our product. The total dry extract for both types of Leben were similar ($p < 0.05$) respectively 90.8 ± 2.03% and 90.8 ± 1.59%. The defatted extracts were also similar ($p < 0.05$) with respectively 88.8 ± 2.43% and 89.8 ± 3.56% for both types of Leben. These results were in accordance with Algerian regulations. According Boubekri et al., 1984 [53] total dry extract and defatted of Leben represent on average 88.96 and 80.04 g/l respectively. The work of Samet-Bali et al., 2012 [55] show that industrial Leben product with commercial ferments has a total dry extract of 82.19 ± 1.40 g/kg. The chemical composition of Leben depends on the quality of raw milk used and varies among localities, regions and farms [54]. The microbiological quality of Lebens, produced from the two types of milk was satisfactory and in conformity to the Algerian regulations (Table 4). Despite the presence of Total coliforms rate of 25.10^3 ± 226 and $23,10^3$ ± 217 respectively for Leben produced from skimmed raw milk and from recombined milk, the absence of fecal coliforms, *Staphylococcus*, *Salmonella*, *Shigella* and Sulfite-reducing *clostridia* was noted.

Table 4: Microbiological characterization of industrial Leben

Microbiological criteria	Leben from partly skimmed milk	Leben from recombined milk	*Standards
Total coliform	25.10^3±226 germs/ml	23.10^3±217 germs/ml	30.10^3 germs/ml
Fecal coliform	Abs	Abs	30/ml
Staphylococcus	Abs	Abs	300/ml
Salmonella	Abs	Abs	Abs
Shigella	Abs	Abs	Abs
Sulfite-reducing *clostridia*	Abs	Abs	Abs

**JORA, 1998, Abs : Absent*

The work of El Marnissi et al., 2013 [56] showed that three milk products marketed in the city of Fez in Morocco have unsatisfactory hygienic quality due to non-compliance with good hygiene practices both during milking, the collection or transport of raw milk, or during its transformation into Lben and Jben in traditional dairies.

Determination of aflatoxin M1 in Leben

Quantitative determination of AFM1 by competitive ELISA during production of Leben both from, partly skimmed milk, and recombined milk, uses a calibration curve (Figure 1) and the results showed a decrease of this toxin throughout the process of Leben manufacture (Table 5).

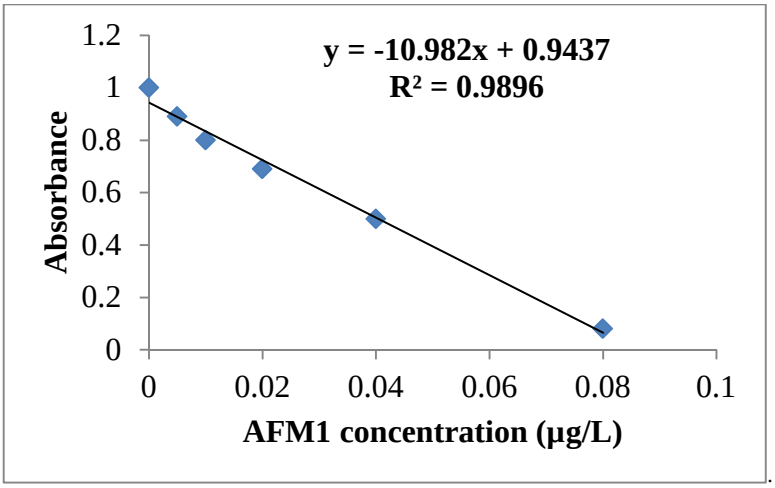

Figure 1: Enzyme-linked immunosorbent assay calibration curve for AFM1

Indeed, for Leben made from partially skimmed raw milk, there was a decrease of AFM1 passing of $115,471.10^{-3}$ ± 1.096 µg/L in early to $7,889.10^{-3}$ ± 0,321µg/L after 02 days of Leben storage of a decrease of 93.34%. This reduction was highly significant from 06 hours of incubation ($P < 0.05$). Similarly, the decrease in this AFM1 in Leben made from pasteurized recombined milk was more pronounced and represents 96.39%. It was also significant from the 6th hour of incubation ($P < 0.05$). Initially the amounts of AFM1 in both types of milk used for the manufacture of Leben remain higher than those of many countries that have established regulatory limits for AFM1 in milk and dairy products, which range from 0.05 µg/L for European countries to 0.5 µg/L in the USA [57, 58]. The AFM1 in milk was not significantly affected by thermal processes used in the dairy industry, namely pasteurization and UHT treatment [59, 60].

Tableau 5: Concentration in AFM1 during Leben production from partly skimmed raw milk and recombined milk

Samples	*AFM1 µg/L	**AFM1 µg/L
Starting milk	$115,471.10^{-3}$±1,096	$119,057.10^{-3}$ ±0,821
After 6 hours of incubation	$76,024.10^{-3}$±1,035	$108.29.10^{-3}$ ±1,054
After 12 hours of incubation	$70,645.10^{-3}$±1,176	$70,645.10^{-3}$ ±0,731
After 18 hours of incubation (Leben)	$32,991.10^{-3}$±0,600	$29,405.10^{-3}$ ±0,559
Leben after 2 days of storage	$7,889.10^{-3}$±0,321	$4,303.10^{-3}$ ±0,303

*Aflatoxin M1 of *partly skimmed raw milk , **recombined milk.*
Values are presented as mean ± SD (n = 3)

During the Leben manufacturing process from the two types of milk, rates of AFM1 and acidity were inversely proportional (Figure 2). The higher the activity of lactic acid bacteria becomes, the greater the acidity increases, the more the rate of AFM1 decreases. From the sixth hour the Leben acidity, made from raw milk or recombined milk

enters frank exponential phase respectively from 18±0.87°D and 20±1.32°D to 56±1.73°D and 60±1.32°D at the 12th hour and continues to rise up to 73±1.51°D and 78±0.87°D after 18 hours 'incubation. Storage of two types of Leben during 02 days does not cause a significant variation in acidity compared with that of 18 hours.

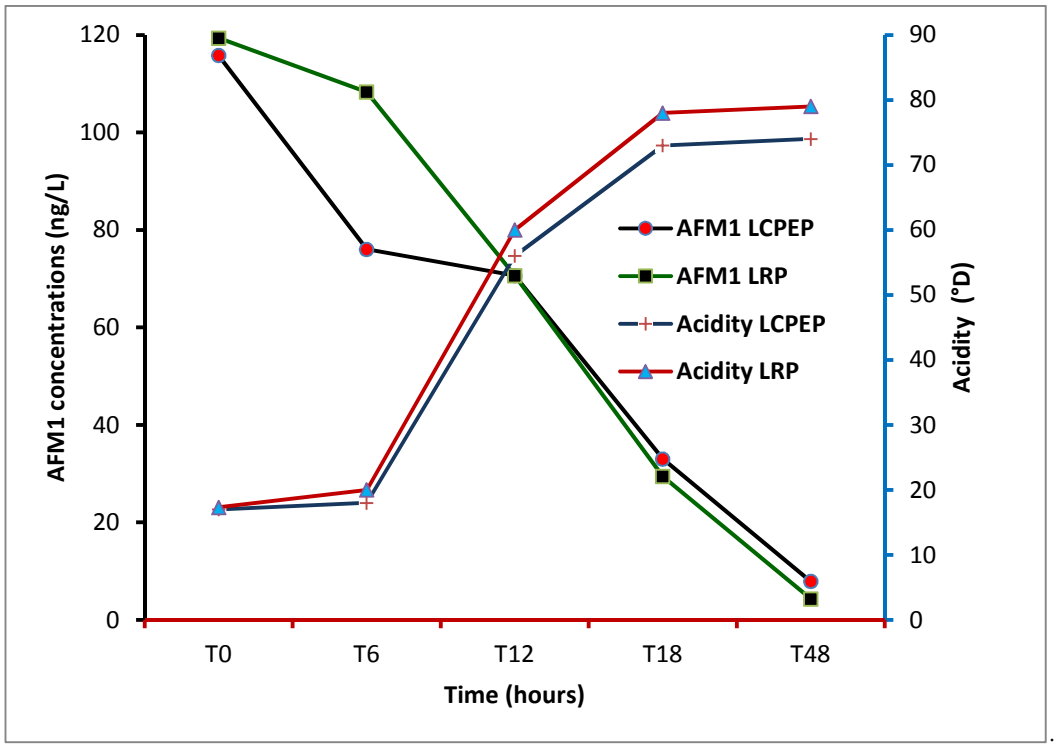

Figure 2: Correlation curve of AFM1 and acidity levels of Leben during manufacture

AFM1 concentrations and acidities are presented as mean ±SD (n = 3)
LCPEP : Partly skimmed raw milk
LRP : Recombined milk

Tantaoui-Elaraki et al., 1983 [61] reported that, lactic streptococci and *Leuconostoc* were the main groups responsible for acidification of milk during its transformation into Lben and that the most important species are *Streptococcus lactis, S. diacetylactis, Leuconostoc lactis* and *L. cremoris*. According Khaddor et al, 2003 [24], alone or combined use of mesophilic bacteria (*Lactococcus diacetylactis, Lactococcus lactis*) or thermophilic (*Lactobacillus bulgaricus, Streptococcus thermophilus*) results in substantial degradation AFM1 during the lactic fermentation. In our case the consortium of bacteria used in the fermentation of two types of milk, assumed that some of them such as *Lactococcus lactis subsp lactis biovar. Diacetylactis, Lactococcus lactis* and *Streptococcus thermophilus* can influence the regression of AFM1. After 18 hours of incubation, respectively AFM1 decreases in Leben product from raw milk and recombined milk from 71.43% to 75.30%. After 48 hours or 2 days of storage, with acidity respectively of 74 ± 0.87 and 79 ± 1.5 ° D, the decreases reach in order, 93.17% and 96.39%. This storage time of the Leben generally allows its maturation that lasts 18 hours and the product should be consumed within a period which may not exceed 48 hours. Beyond, its acidity would alter its organoleptic characteristics. Some researchers agree that it was very unlikely that the only acidity was the cause of the destruction of this toxin. Al-Delaimy and Mahmoud, 2015 [21] reported a reduction of AFM1 following various treatments of milk, such as heat, fermentation and the addition of plant extracts, carrot juice seemed to have a considerable reduction effect of this toxin. The carotenoids compounds in carrots as antioxidants may interact and bind the AFM1 configuration. According Govaris, 2002 [62], the decrease of AFM1 in the production of yogurt can be attributed to factors such as low pH, presence of lactic acid bacteria, organic acids and fermentation by products. PH reduction due to the increase of the acid can affect the structure of milk casein and influence AFM1 [63]. On the contrary, Blanco et al., 1993 [64] reported that the yoghurt production does not affect AFM1 while Munksgaard et al., 1987 [65] showed a slight increase in the concentration of AFM1 in yoghurt compared with that initially present in raw milk. The work of Iha et al., 2013 [22] on yogurt and cheese naturally contaminated, have found no significant influence of the manufacturing process on the AFM1. Moreover, According to the work of El-Nezami et al., 2002 [66], Niderkorn et al., 2009 [67] mycotoxin sequestering efficiency was comparable between viable bacteria and thermolysed bacteria suggesting that the activity was not due to the metabolic activity of living cells. The polarity of the toxins plays an important role in sequestration mechanism. Thus the rate of fixed aflatoxin decreases in order of decreasing polarity

AFB1> AFG1> AFB2> AFG2 [68] and that AFM1 was removed less efficiently than AFB1 [69]. According Niderkorn et al., 2009 [67], interaction involves the peptidoglycan of the cell wall. Yiannikouris et al., 2004 b, c, [70, 71] showed that β-D-glucans, provide most of the adsorption of mycotoxins. They were able to establish a correlation between the amount of glucans present in the wall and its sequestration capacity. According to these authors the mannan content were little involved and the content of chitin has a negative effect.

CONCLUSION

The results of these studies show that milk used as raw material for the industrial production of fermented milk type Leben in GIPLAIT unit in western Algeria exhibit physicochemical and microbiological characteristics in conformity to the Algerian regulations. The analytical techniques used were all in accordance with those of the International Standards Organization (ISO). In this unit little importance was given to the possible presence of toxins such as AFM1 in milk products. The unit has no upstream power to oversee animal feed especially during closed period's barn where cows feed products often stored in humid conditions favoring the appearance of AFB1. The amounts of AFM1 in both types studied milks exceed European standards fixed to 50 ng/L. The orientation of the milk to manufacture Leben proves advantageous in reducing this toxin by lactic acid bacteria. Other alternatives were however possible for the use of contaminated milk, by associating them with extracts from plants (food, condimentary, aromatic and medicinal plants) to sequester the toxin and to design functional foods, products new.

Conflict of interest

No conflict of interest exists in the submission of this manuscript.

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