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Microbiological Control of UHT Sterilized Milk and Milk Products and Effects on Consumers' Health in Western Algeria.

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ABSTRACT

The present study focused on the analysis of microbiological quality of various milk samples such as ultra-high temperature sterilized milk (UHT), melted cheese and yoghurt, obtained from dairy product factories in western Algeria. Hazards identification and development of a quality management standard for these subsidiaries evidenced that the sanitary quality was satisfactory. The result revealed detection of faecal origin contamination 'total and fecal coliforms' in both the yoghurt product and sample of melted cheese where the average content was(1.29±6.72-0.14±0.56 x 10^3cfu/g) and (0.14±0.6-0.022±0.275 x 10^3cfu/g) respectively. It is indicative that all samples were free from *Staphylococcus aureus* and *Salmonella* sp. The study demonstrated that prerequisite programs (PRP) will prevent the occurrence of microbiological hazards origin, which confirms the fact that hygiene measures are the best safety assurance. The hazard analysis of the production process of yoghurt and cheese allowed us to find out their causes, adoption of preventive measures for certain sensitive stages where hazards can be eliminated or reduced at acceptable levels, the determination of critical control points (CCP)and operational prerequisite programs 'PRPo', implementing critical limits, and monitoring plans.

Key Words: Milk and dairy products, HACCP, PRP, CCP, Microbiological quality, Algeria.

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Introduction

A progressive development of the dairy sector has put forward the safety of the food product that is leaving a food chain translating public health risk into a definable goal reflecting in the microbiological hazard at the time of consumption of food. Throughout the world, there are numerous kinds of milk products (FAO, 1995). Milk borne pathogens are well documented. Serious infections in humans have been linked to consumption of dairy products. Many diseases are transmitted through milk and its derivatives (Gran *et al.*, 2002). The World Health Organization confirms that food-borne diseases are a widespread and growing public health problem in developing countries. The principal thrust was reflected on the form associated pathogens entering the commercial chain and consumers rightfully deserve and expect a safe product each time. Consumers should give more importance to the quality of dairy products.

On this view point, we were particularly interested in assessing the hygienic quality of UHT sterilized milk and dairy products namely' yoghurt and melted cheese', and there by identifying and analyzing the hazards that may be encountered at the production chain of these products in dairy subsidiaries of western Algeria.

Materials and Methods

The samples were obtained from retail sale points of dairy products elaborated in governmental factories in western Algeria from January 2013 to August 2013 in accordance with the Algerian standardized sampling methods (ISO 707, 1994). The microbiological analyses were performed in duplicate test on the final products which constituted a total of 86 samples of milk and dairy products shared by 61 samples of yoghurt 'flavored and fruity, dessert cream', 18 samples of soft cheese 'melted cheese portion', and 7 samples of UHT sterilized milk. Samples analysis was carried out in SUDLAIT laboratory 'Igli-Bechar' (Algeria). The main activities of this laboratory are initially to assess the hygienic quality of the analyzed products and identify critical control points 'CCP' in order to check the origins of possible contamination, and finally, establish a plan at the operational level embedded in the food safety management system, such as HACCP, GMP etc. For this purpose we conducted according to a standardized methodology, the analysis of microbiological parameters cited below.

Microbiological Analysis:

Sampling was performed according to Algerian Standard (NA) *e.g.*, NA 676 (1994) standard. Microbiological analysis included after preparing decimal dilutions according to (ISO 8261, 2001), the detection and enumeration of bacteria indicators of fecal contamination or sanitary quality defects, and the search for suspected pathogenic bacteria, including;

Enumeration of total aerobic mesophyll flora incubated at 30°C for 72h according to (NF V 04-016, 1985) on Plate Count Agar (PCA) (Fluka, Spain); Detection and enumeration of coliform organisms and thermo-tolerant coliform incubated at 37 and 44°C respectively for 24 to 48h according to (ISO 4832, 1978) on the middle Violet Red Bile Lactose Agar (VRBL) (Biochem, Canada). All red colonies (lactose +) with a minimum diameter of 0.5 mm appeared in 24h are regarded as coliforms; Detection and enumeration of *Staphylococcus aureus* according to (ISO 5944, 2001) on the middle of Giolitti Cantoni (Institut Pasteur, Algeria) and Baird Parker Agar (Fluka, Switzerland); Detection of *Salmonella spp* according to (ISO 6785, 2001) after pre-enrichment in non-selective liquid medium and enrichment in selective media (Rappaport-Vassiliadis and selenite/cystine middle) in a deep tube; Search and enumeration in a liquid medium Rothe (Scharlau, Spain) for faecal Streptococci incubated at 37°C for 48h following the method described by (Afif*et al.*, 2008). The contents of positive tubes with turbid appearance, were then subcultured on Litsky medium (Institut Pasteur, Algeria) with a platinum loop and subjected to incubation at 37°C for 48h; and finally, enumeration of fungal flora on agar Sabouraud 4% glucose (Fluka, India) according to (ISO 6611, 1996). Enumeration of Petri dishes having microorganisms is based on the standard set by legislation (AFNOR, 1980).

Interpretation of results

The criteria for microbiological quality employed were in accordance with the publication on dairy products (NA 2692, 1992; NA 69, 1993 and NA 35, 1998), which is based on a 3-class plan following the specifications; n, c, m and M.

The interpretation of the standards is based on the analysis of five units component the sample (n=5) and measure the frequency of values between m to M range. Values at m above M in any sample are unacceptable relative to either health hazard, sanitary indicators a

spoilage potential.

Identification and hazard analysis 'approach HACCP'

The implementation of HACCP "Hazard analysis and critical control points" system is performed in several successive stages, at first, it is to apply the prerequisite programs (PRP) to reduce the level of certain contaminants, identify hazards at each stage of production, processing or preparation of the product, evaluate the corresponding risks and to determine the stage where it is possible to act effectively (Faye and Loiseau, 2000).

The quality management by the analysis of risks or potential hazards associated with a product or process (HACCP) must be applied to the entire chain from the cow to the consumer (Leyral and Vierling, 2007). Hazard identification was performed using the Ishikawa diagram 'diagram cause-effect' detailing 5 causes to consider " 5MMethod " (Medium, Workforce, materials, methods and equipment) to maintain a hygienic environment throughout the production, whereas the risk by approaching Zurich Hazard Analysis which involves assessing qualitatively and semi-quantitatively the risk, severity and frequency.

Results

Microbiological Analysis

All samples free from fecal Streptococci, presumed pathogenic Staphylococci and *Salmonella sp*. The average load fungal flora were 0.86±2.49 x 10^4cfu/g.Microbiological analysis of UHT sterilized milk showed a total germ load exceed the limits set by the Algerian regulations (NA 35, 1998), whose average was revealed 1.38±1.48 x 10^3cfu/ml. The presence of fecal contamination (coliform organism, thermo-tolerant coliform and fecal streptococci) was absent, and the fungal flora had an average of 0.2±0.29 x 10^3fu/ml (Table 1).

Table 1: Microbiological characterization of 'flavored and fruity' yoghurt, dessert cream, melted cheese and UHT sterilized milk

Product analyzed	Microbiological parameters: Average value ± standard deviation						
	GAMT (10^4cfu/g)	Coliforms (10^3cfu/g)		*St. aureus*	Sal.	LM (10^4fu/g)	Str. F
		CT	CF				
YA	4.6±7.7	Abs	Abs	<1	Abs	0.71±0.96	<1
YF	4.84±6.94	1.29±6.72	0.14±0.6	<1	Abs	1.3±2.19	<1
CD	0.138±0.148	Abs	Abs	<1	Abs	0.038±0.073	<1
FP	3.05±8.49	0.141±0.556	0.022±0.275	<1	Abs	0.86±2.49	<1
UHT SM	0.138±0.148	<1	<1	--	--	0.02±0.029	<1

(cfu/ml), (fu/ml) for UHT Sterilized Milk

GAMT: total aerobic mesophilic bacteria, CT: total coliforms, CF: fecal coliforms, *St. aureus:Staphylococcusaureus*, Str. F: fecal streptococci, YA: flavored yoghurt, YF: fruity yoghurt, CD: dessert cream, FP: Melted cheese portion, ufc: colony forming unit, uf: fungal unit, LM: yeast and mold, Abs: Absent, UHT SM: Ultra High temperature Sterilized Milk, Sal :*Salmonella sp*.

The average load of different microbiological parameters determined for processed cheese is summarized in Table 2. More than 61% of the samples analyzed evidenced a rather high level in aerobic germs with an average of 3.05±8.49 x 10^4cfu/g. The analysis also revealed a fecal contamination, more than 27 and 11% of samples, respectively, by the total and fecal coliforms (Table 2) which averaged 1.41±5.56 and 0.22±2.75 x 10^2cfu/g, respectively. That is not complying with the standard according to (NA 35, 1998).

Table 2: Percentage of non-compliance of bacteriological results of fruity yoghurt and melted cheese.

Bacteriological Indicators	Percentage of non-compliance (% and IC 95%)	
	Fruity yoghurt	Melted cheese
Total coliforms	40 (37.06 – 42.94)	--
Fecal coliforms	25 (22.38 – 27.62)	--

Flavored and fruity yoghurts samples tested showed a variable load of aerobic germs, between 4.6 and 4.84 x 10^4cfu/g, but the load in the dessert cream was found lower as 0.13±0.14 x 10^3cfu/g (Table 1). The assessment revealed that the flora of fecal contamination was not detected in flavored yoghurt and dessert cream. However it is interesting to note that nearly 40% of fruity yoghurt samples (Table 2) were not in compliance with the particular standard (NA 35, 1998), because they indicated a fecal contamination (total and fecal coliforms) containing average of 1.29±6.72 and 0.14±0.6 x 10^3cfu/g, respectively. It is remarkable that all samples were free from fecal Streptococci and *Staphylococci* pathogens, but the average loads fungal flora of flavored yoghurts, fruity yoghurts, and dessert cream were found 0.71±0.96, 1.3±2.19 and 0.038±0.073 x 10^4cfu/g, respectively, with significant standard deviations, indicating the variability of conditions and parameters of production of yoghurt an production to another.

Discussion

Microbiological analysis

The total bacteria count can be employed as indicator of the sanitary quality of the product which informs us about the quality of production operations, transport and storage (Hassainya *et al.,* 2006).The present study evidenced that the total germs in dairy products remained high. These are thought to be accidentally occurred during subsequent handling of the food or it is voluntarily added in the raw material (ferments).

The results for the enumeration of total bacteria in melted cheese were lower compared to that reported by Hamama (1989) (3.05 x 10^4cfu/g against 2.5 x $l0^8$cfu/g). This may be the results of pH and water content which was correlated with the density rate of total bacteria.

A though the different samples of yoghurts have revealed a lower count of total germs compared to that findings (Bonfoh *et al.,* 2002) in Mali, where they found an average of 1.2 x 10^7cfu/g for yoghurt and 5.5 x 10^8cfu/ml for fermented milk and these were lower than that found in our research about fermented milk. A considerable quantity of fermentation reaction could explain the high rate of aerobic mesophyll bacteria.

The pH is one of the intrinsic factors of the product that allows the growth of these microorganisms. According to (Hamza-Chaffai, 1990), a pH greater than 5.7 not only promotes proliferation of micro-organisms in the cheese during storage, but also favors the development of various alterations: putrefaction, swelling.

According to Eck and Gillis (1997), water activity is an important parameter as temperature and pH for the growth and proliferation of micro-organisms and the propagation of certain chemical reactions. Since cheeses have high water activity, therefore all types of germs can grow in them easily. Regulating the water activity of the cheese the relative means of action to control the micro flora in cheese can be implemented. It is well recognized that total number of these in habitant bacteria decrease with the introduction of heat treatment which at post-harvest also depends on storage conditions (Eck and Gillis, 1997). The rate of aerobic germs of UHT sterilized milk analyzed was found relatively high. The study revealed that this might be due to improper transport and storage conditions (Benyagoub and Ayat, 2013).

Coliforms and thermo-tolerant coliform organisms are absent in samples of flavored yoghurts and desserts cream, and are below the specified values for processed cheese according to (NA 35, 1998). However these contaminants are reported in the work of (Mourgues *et al.,* 1977) (1.48 x 10^2 to 2.98 x 10^3cfu/g) (Quinto and Cepeda, 1997; Branger *et al.,* 2007) (1.30 x 10^1 to 6.01 x 10^2cfu/g) for cheese samples, which are absent in our work.

Absence of this flora indicates the practices GMP and GHP. The high load of coliforms may be responsible for incidental swelling, generating undesirable flavors and textures (Amiot-Carlin *et al.,* 2010),

poor condition of manipulating and manufacturing (Hassainya *et al.*, 2006).

Very few coliforms are also related to good sanitary practice and personal hygiene, environmental and animal health implication on the one part, and on the other part to hygiene during introduction of ferments 'Direct inoculation', and cleaning and disinfection operation of tanks, bins and piping systems of the production line of yoghurt before and after each production through the CIP unit 'clean in place'. In addition, the production of acid and nisin that is bactericidal and sporocide by lactic acid bacteria, inhibit the growth of pathogens by lowering the pH of the medium (Morou, 2010).

Total coliforms and thermo-tolerant coliforms were found respectively in 40% and 25% (1.29 x 10^3 and 1.4 x 10^2cfu/g) of fruity yoghurt samples analyzed. These results are similar to Alio Djabril (1996) revealed in Niger. Found that 32.5% and 12.5% respectively, remained below the observations made in the traditional curds in Morocco showed a highe numeration of microorganisms presumably indicators of fecal contamination. Earlier it was found that 57% of curdled milk artisanal in Cameroon is contaminated with coliform. Dieng (2001) found only 19% of samples contaminated with coliforms. Dib *et al.*,(2008) reported in their study in Lebanon on local cheeses, an alarming rise of total coliform ranging from1.33-2 x 10^3cfu/g in 72 to 86% of the samples, while fecal coliforms are present in 23 to 71% of cheese.

Presence of coliform bacteria is generally employed as an indicator of poor hygienic practices in the handling of milk (Labioui *et al.*, 2009), while *E. coli* frequently reflects fecal contamination revealing the presence of other enteric bacteria in the sample (El zyney *et al.*, 2007). This contamination in the present study is probably originated from the contamination either during inoculation (introduction of ferments) or during post-fermentation handling (adding flavor and fruit, packaging, etc). Katinan *et al.*, (2012) and Dib *et al.*, (2008), showed that stressed onpoor environmental conditions during production and bases in refrigeration are factors of high yield of coliform.

As regards the cheese samples, the level of hygiene, handling conditions, production, cleaning utensils and equipment, lack of mastery on the one hand, the temperature during transport and storage, and secondly, the inherent dangers to manipulation and inefficient pasteurization or post-pasteurization contamination, could be the source of bacterial contaminants and/or pathogens in dairy products (Morou, 2010).

However, all samples in this study were free of contaminating microorganisms and pathogens namely 'fecal streptococci and *Staphylococcus aureus*'. The results satisfy the local regulations. Reports from other researches (Hamama, 1989) that artisanal cheese, demonstrated5.3 x 10^5cfu/g and 7 x 10^4cfu/g respectively and 4.9-4.03 x 10^2 cfu/g for *S. aureus* reported by (Cosentino and Palmas, 1997).

Absence of pathogenic microorganisms like *Salmonella sp* and *S. aureus* in the present research samples of yoghurt and cheese may be linked to the normalized values of samples acidity on the one hand; this is approved by the research of (Poueme, 2006). It was found that *Salmonella* cannot resist a pH between 4.6-4.8, and reduced handling of the product yoghurt, on the other hand, to the adequate heat treatment (pasteurization/sterilization) of milk for yoghurt applied in the yoghurt manufacturing process. Chye *et al.*, (2004) reported such no contamination of a post-manufacture of dairy products. Knowing that the microbiological quality of milk as a raw material is important for its transformation (Guinot-Thomas *et al.*, 1995), Bonhof *et al.*, (2002) found *S. aureus* 3.2 x 10^4cfu/g and 1.2 x 10^2cfu/ml for yoghurt and fermented milk respectively. This could result in a very poor hygiene or high contamination of dairy products during preparation.

It should be noted that *S. aureus* represent a real risk to public health in transformed products. It ranks first in the etiology of food borne disease, as may produce in certain conditions, there most able enterotoxin that can withstand the heat treatments. They are also capable of forming stable biofilm, which is constituted do playas an important virulence factor (Mack *et al.*, 2004).

Absence of contaminants and pathogenic microorganisms, clearly evidence in favor of hygiene indicator, and they are related with compliance for good manufacturing practice and hygiene. Absence of both fecal streptococci in the analyzed products reflects compliance with good hygienic practices and pasteurization of milk used as raw material for the preparation of dairy products. These results are also in agreement with our series of study on the consumption milk (Benyagoub and Ayat, 2013). Even though *Enterococci* are widespread in the environmental sphere of the animal but are only marginally or not pathogenic, they are revealed at the level of 1.2 x 10^5cfu/g and 8.7 x 10^4cfu/ml for yoghurt and fermented milk respectively. As a consequence, they are not included in the criteria adopted by the legislation of raw milk or dairy products (Ghazi and Niar, 2011). The ratio thermo-tolerant coliform/fecal streptococci is generally greater than 1. This indicates that there is a man-made contamination.

The acquired data to enumeration of yeasts and molds in melted cheese samples were on average 0.2±0.29 x 10^3cfu/g, and for yoghurt samples, the result approximates to results reported by (Bonfoh *et al.*, 2002). This phenomenon can be influenced by both the age of the product as by transformation methods. This is due to the effect of fermentation (Gadaga *et al.*, 2000), which dominates the fungal flora to be able to grow in these acidic environments. (Ndiaye, 1991) in Senegal found that 42% of yoghurt samples are contaminated by yeasts and molds. Poueme (2006) found the origin of the contamination of milk powder in the bag opening after fractional use, and (Bonfoh*et al.*, 2002), due to the storage period of milk powder.

Microbiological parameters of samples analyzed are in accordance with the standard (NA 35, 1998) indicating sat is factory quality. Obtaining reconstituted milk of good hygienic quality is the first step of the good quality of the raw material (Benyagoub and Ayat, 2013), the sterilization step, compliance rules and hygiene measures at the local manufacturing involving continual maintenance of optimal condition of cleanliness. Factors of quality depend on individual hygiene for manipulators and prevention of exogenous recontamination (Hamama *et al.*, 1995), and in addition to the effectiveness of the cleaning and disinfection with the CIP "clean in Place" before and after the production of UHT sterilized milk. The results obtained are in agreement with the work of (Ould Moustapha *et al.*, 2012) which presents the operational and technological application for a good hygienic quality of milk.

Identification and analysis of hazards

The prerequisite programs (PRP) represent the conditions and basic activities needed to maintain a hygienic environment for the production, storage and the provision of safe finished products throughout the process (Wallace and Williams, 2001). The prerequisites section, in this study focused on efforts on education and personnel training on hygiene behavior, organized movement of raw materials, waste and personnel to avoid cross contamination.

However, the hazard analysis has been established only after the manufacturing diagram has been verified. It was carried out step by step beginning from the receipt of raw materials to the shipment of the finished product: First, we began by identifying hazards that are classified into three general types: biological (contaminants and pathogenic microorganisms), chemical (toxic substances) and physical (foreign bodies).

A 'HACCP' plan bringing together the key information of hazard identification revealed the existence of six critical points at different stages of production of raw milk, UHT sterilized milk and the production process of dairy products, in which hazards are either biological 'microbial origin'. They are firstlyat; receipt and storage of raw materials 'raw milk and milk powder'; secondly at Pasteurization/Sterilization; thirdly at Inoculation and maturation; fourthly at packaging; fifthly at refrigeration and storage of finished product and finally at cleaning and disinfecting. It is imperative to implement preventive and corrective measures so that the dairy industry enterprise must undertake activities to prevent the frequency of microbiological hazards' appearance causing out breaks of food borne disease among consumers.

Conclusions

The microbiological quality of flavored yoghurt, dessert cream and melted cheese indicate that most of the results are below the values indicated by the standard with absence of pathogens germs, and that 40% of samples of fruity yoghurts have a relatively high level coliforms contamination. This is simply explained by insufficient hygiene during the manufacturing process, lack of physical parameters control such as 'time and temperature' and also the conditioning step 'adding fruit'. Although the heat treatment of raw milk or reconstituted milk intended for the manufacture of dairy products to be performed, but we can observe that there are still critical points at the level of the conditions of manufacturing cheese and yoghurt. Finally, it should nevertheless create a quality policy with the popularization of good production practices and hygiene ensured the safety and suitability of milk and milk products.

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