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Low Salt Soft Cheese; Compositional Quality and Incidence of Aerobic Spore Forming Bacteria

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ABSTRACT

Soft white cheese is the principal type of cheese available to all in huge quantities in Egyptian markets .Consumption of low salt soft cheese increased through the last decades due to the adverse effects of salt .The aim of this study was to evaluate compositional quality and incidence of aerobic spore forming bacteria of low salt soft cheese .A total of fifty four samples of Egyptian low salt soft cheeses were collected randomly during the period between June and December 2019 from different localities in Alexandria and El Behera Governorate. The respective mean values of pH were (5.83±0.52, 5.40±0.70); acidity% (0.53±0.31, 0.67±0.35); moisture% (57.95±3.54, 56.76±4.91); Fat/DM% (23.72±5.76, 25.45±12.99) and salt% (1.80±0.49, 1.78±0.62) for Alexandria and El-Behera Governorate, respectively and comparison of these results with Egyptian standard .While mean values of incidence of aerobic spore formers were 8.63×10^2 $\pm7.87\times10^2$, $5.71\times10^2\pm4.10\times10^2$ respectively for samples from Alexandria and El-Behera Governerate. There was no significant difference between chemical composition of low salt soft cheese samples from both Governerate, and significant variation of results of aerobic spore formers count in examined soft cheese samples existed between Alexandria and El Behera Governorates. All suggested measures should be taken to improve the quality of low salt soft cheese.

1. INTRODUCTION

Cheese is one of the most wide spread foods globally, and cheese especially soft cheese is the main dairy product worldwide, with the high adding value for dairy industry (IDFA., 2010).Tallaga cheese characterized mainly by elevated moisture content and soft creamy consistency as well low salty taste. Tallage means in Arabic refrigerator, so this cheese must be ripened in low temperature storage conditions. Low percent of salt was added to cheese milk as it affects various aspects of cheese including the shelf life, enzyme activity, flavor, casein hydration and microbial proliferation during ripening. (Hamad, 2015).

In recent years, there was an increasing trend in the demand of low salt food including cheese especially for people suffering from hypertension (Albernethy, 1979 and Anonymous 1981)

The principal objective of cheese manufacturing has always been and remains to convert milk, which is perishable into a product with extended shelf life with keeping most of its nutrients (Ahmed et al., 2010). Differences in temperature used in soft cheese manufacturing may affect chemical composition of cheese since temperature monitors removal of moisture from curd during cheese making and differences in moisture content could be a significant impact on cheese. (Yun et al., 1993). Time of storage period affects the chemical cheese analysis because conditions of storage and temperature influence on the properties of cheese (Effat et al., 2018).

The quality and shelf- life of soft cheese mainly rely on their microbial content, storage temperature, transportation and technological treatments which can be mainly applied to destroy the pathogenic microorganisms to improve the hygienic quality of the end product. Application of cold storage methods has been successfully applied to prevent spoilage and prolong the shelf life of cheese during transportation in markets (El-Shamery, 2014).

Spore-forming bacteria such as Bacillaceae family are common contaminants of dairy products

mainly soft cheese and their germination may lead to food spoilage or pathogenic cases due to food borne illness. They are of great importance in dairy industry due to their chemical and thermal resistance in their dormant state (Ranieri and Boor, 2009; Egan et al. 2016).

Milk and dairy derivatives contamination with spore forming bacteria involves two routes of entrance: raw milk route and post pasteurization route. Raw milk in the tank of farm is contaminated through the external of the cattle's teats and via inefficiently cleaned milking facilities contamination from soil, bedding material and faeces .Soil is the primary and a direct contamination origin of spore-forming bacteria into foods because it is a major source of these microorganisms. On the other side, the post pasteurization contamination of milk with spores is associated with the dairy industry or to the biofilms of spore-forming bacteria present in processing lines that can finally be widely spread by releasing into the system of milk production (Heyndrickx, 2011).

Spore formers have the ability to produce a group of various enzymes as proteases, lipases and phospholipases that affect adversely on the texture of dairy products and cause typical off-flavors defects (Lücking et al. 2013).The presence of proteases can result in uncontrolled or undesirable proteolysis, negatively affecting on food quality and flavour through the formation of bitter peptides from milk proteins (McClure, 2006).

Bacillus cereus is the most important species since its classification as a common food contaminant involved in both food spoilage (Yusuf et al. 2018) as well as food poisoning that usually occurs in two types of illness: the emetic and diarrheal syndromes. The emetic syndrome is primarily manifested by nausea and vomiting (1–5) hrs, after consumption. It is due to a small molecular weight a heat-stable toxin (Cereulide) usually preformed in food and it is highly resistant to heat treatments, proteolytic enzymes and extreme pH conditions (Organji et al., 2015; Owusu-Kwarteng et al., 2017). While the diarrheal syndrome results from the production of enterotoxins and it's usually illustrated by abdominal cramps and diarrhea following 8 to 16 hrs, incubation period (Amin, 2018).

A possible relation of aerobic spore formers to another cheese safety problem, biogenic amines production since amine-producing property has been known for a few of *Bacillus* strains (Gopal et al. 2015).

The aim of this work was to evaluate the compositional quality of low salt soft cheeses collected from Egyptian markets, and enumeration of aerobic spore forming bacteria to ensure its compliance to quality and safety standards.

2. MATERIAL AND METHODS

2.1. Cheese samples:

Fifty four samples of Eg yptian low salt white soft cheese were purchased randomly from Alexandria (24 samples) and El-Behera (30 samples) Governorates from various localities. Cheese samples were collected randomly during the period between June and December 2019.Samples were obtained as sold to the public and transferred in insulated icebox as soon as possible to the laboratory within 4 hours to be examined for microbiological and compositional quality.

2.2. Compositional quality evaluation of examined white low salt soft cheese

:2.2.1. Determination of pH value (AOAC, 2005)

The pH of white low salt soft cheese samples was determined using digital pH meter Martini Milwaukee made in Italy. The pH meter was calibrated using buffers of pH 4 and 7. The cheese samples were stirred, mixed well and the pH value was recorded.

2.2.2. Determination of titratable acidity content (AOAC, 2005):

Nine gram dispersed in 10 ml of distilled water at 40°C in conical flask, and then six drops of phenolphthalein indicator were added. The sample was then titrated with 0.1N sodium hydroxide until a stable faint pink color was formed.

2.2.3. Determination of moisture content (AOAC, 2005):

In an oven 80°C, metallic dishes were dried for 20 minutes, cooled in desiccator then weighed. 3gm of cheese sample were placed in dish, weighed dish with sample placed in oven with temperature 102 ± 2°C for 5-6 hours transferred to desiccator to cool weighed immediately with minimum exposure to atmosphere. Loss of weight of cheese sample during drying is the moisture content. The method was repeated till reach a constant reading.

2.2.4. Determination of fat content (APHA&AOAC, 1978):

In clean dry cheese butyrometer add 10 ml Gerber sulphuric acid, 1 ml Iso-amyl alcohol, 3gm cheese sample then add warm distilled water till reach shoulder of butyrometer, neck of butyrometer dried by filter paper, , rubber stopper fixed firmly then invert butyrometer 3-4 times till complete dissolving of cheese sample. Butyrometer placed in Gerber Centrifuge 1500 rpm for 3-4 minutes. Fat column appear as pale yellow colour. The reading was recorded.

2.2.5. Determination of Dry matter of low salt soft white cheese (AOAC, 2005).

Cheese dry matter (DM) was calculated as 100 - % moisture.

2.2.6. Determination of Salt content (AOAC, 2005):

0.5 gm of sample was weighed and placed into 250 ml conical flask; also, 100 ml boiling water were added. Then, swirled for 10 min and cooled to 50 - 55°. Titrated against silver nitrate (N/20) till the color of indicator potassium chromate (10%) changed from pale yellow to buffered brownish red color.

2.3. Microbiological examination

2.3.1. Preparation of low salt soft cheese samples:

Using aseptic technique, 5 grams of low salt soft cheese sample were transferred by sterile spatula to sterile polyethylene bag then adding 45 ml sterilized Sodium citrate 2%, bags were placed in stomacher for shaking at 160 rpm for 5 min, then serial dilutions 1/100 and 1/1000 using sterilized Sodium citrate 2% were prepared.

2.3.2. Enumeration of aerobic spore forming bacteria (Wehr and Frank, 2012)

All prepared dilutions were heated in water bath at 80°C for 10 min then cooled suddenly to 30°C before transferring one ml aliquots into sterilized petri dishes containing nutrient agar. The duplicated plates were incubated in inverted position at 32°C for 48 hours. Mesophilic aerobic spore forming bacteria were enumerated as cfu/g.

3. RESULTS AND DISCUSSION

3.1. Physicochemical evaluation of examined soft cheese samples:

3.1.1. pH value :

Results recorded in Table (1) revealed that mean values of pH of examined low salt soft cheese samples were 5.83±0.52 in Alexandria and 5.40±0.70 in El-Behera.

These obtained results were in the same range with Kapoor et al., (2007) who mentioned that pH of examined white soft cheese ranged between (4.4-6.3). While, lower results of pH 4.7 in soft cheese was reported by Ibrahim (2003).

pH gives a true picture of reactions which occur in cheese during ripening period. There are many widespread spoilage-causing flora related to low pH products which include yeasts and molds, spore formers, psychrotrophs which are responsible for deteriorating the product quality, especially causing flavor and textural problems (MacBean , 2009).

3.1.2. Acidity percent:

The mean value of acidity percent in soft cheese samples collected from different localities in Alexandria and El-Behera Governerates were 0.53±0.31 and 0.67±0.35 respectively (Table 1).

Our results are in line with Salwa and Galal, (2002), Ismail and Osman, (2004) and Ismail et al., (2011) who mentioned that titratable acidity in soft cheese were in the range between 0.20% - 0.73%, 0.18% -1.86% and 0.27% - 2.06% respectively, while lower than results reported by Sulieman et al., (2005) who showed that titratable acidity in soft cheese samples was 1.70.

It is well known that cheese manufacture depends on elevate acidity by different methods for curdling of casein, so that it may be explained that why there is no standard for cheese acidity.

The acidity of cheese is due to many various factors such as cheese proteins and their degradation byproducts, conversion of lactose into lactic acid, as well as salt content that may affect the acidity of cheese. Cheese is characterized by elevated titratable acidity value and low pH values which affected the structure of cheese (Savić, 2015).

The relative variation in acidity percent of the examined soft cheese may be attributed to manufacturing process, ripening period, temperature of storage and or age of cheese samples. Generally, delayed manufacture process, prolonged ripening period, warm storage temperature increase the acidity of cheese (Mohamed, 2016).

3.1.3. Moisture content:

Results given in Table (1) illustrated that mean value of moisture percent of examined soft cheese samples collected from different localities from Alexandria and El-Behera Governerates were 57.95±3.54, 56.76±4.91 respectively.

Our results agreed with Salwa and Galal , (2002), Ismail and Osman, (2004) and Ismail et al., (2011) who reported that moisture percent in soft cheese samples was in the range between (57.18% - 61.14%) , (50.68 %-62.70%), (51.48 % - 64.46%) respectively.

Difference of moisture of cheese may be due to temperature variance during cooling or storage, salt percent of cheese (Bradley and Vanderwarn, 2001), and may be a result of the composition of milk used for cheese making and technique of manufacture (Abdalla and Eldin, 2018).

Moisture content is one of the most important considerations regarding the safety and shelf life of cheese. Setting maximum moisture content ensures that the consumer will receive a minimum amount of solids (Weiser, 2016).

According to Egyptian standards stipulated for cold stored cheese (ES: 1008-5/2005) which

stated that moisture percent in half cream cold stored cheese should be not more than 65%.it was found that 4.17% of soft cheese samples collected from Alexandria not complied with the standard, while all samples collected from El-Behera localities were complied with ES(1008-5/2005).

3.1.4. Fat/DM:

Results summarized in Table (1) revealed that mean values of Fat/DM percent of examined soft cheese samples were 23.72±5.76 and 25.45±12.99 in Alexandria and El-Behera Governerates respectively.

Our results are lower than data mentioned by Salwa and Galal, (2002) and Ismail et al., (2011) who reported 46.21% and 57.46 % fat to dry matter in soft cheese, respectively.

The variation in results of fat content of examined soft cheese samples due to properties of milk used for cheese manufacturing which include several factors such as breed of animal, lactation stage, seasonal fluctuation, level of feeding, type of feed, hygienic condition, age of animal and milking intervals storage condition, temperature and period of storage also play a role in increasing the weight of cheese and as a result the fat content (Abd El-Wahab, 2008).

The difference in results of dry matter content of examined soft cheese samples may be due to some factors, as the close relation between moisture content and total solids, preservation technique applied and temperature along storage period (Kardak, 2012).

Egyptian Standards of cold stored cheese (ES: 1008-5/2005) stated that the Fat/DM percent in half cream-cold stored cheese should be not less than 25%, so only 50% and 46.67% of examined cheese samples collected from Alexandria and El-Behera Governorates, respectively were compatible with this standard. (Table 2).

3.1.5. Salt content:

Cheese is regarded as the most important dairy derivative involving the application of salt (sodium chloride) in its manufacture. Salt is considered a preservation technique as it lowers the water activity leading to prevention of most harmful microorganisms' proliferation and growth (Abdalla and ElZubeir, 2006).

Results given in Table (1) illustrated that mean value of salt percent of examined soft cheese samples was 1.80±0.49 in Alexandria while in El-Behera was 1.78±0.62.

The obtained results were lower than those obtained by Sulieman et al., (2005), Ismail et al., (2011) and Hamad, (2015) who reported that salt content of examined soft cheese were 4.76 %, 6.19% and 6.19% respectively.

Egyptian standards (1008-5/2005) stated that salt addition during soft cheese manufacturing relies on good manufacturing practices applied in cheese plant. Salt is not only applied for flavor enhancement during cheese manufacturing; salt has been used as a natural preservative, aids in cheese ripening ,controls moisture and causes the curds to shrink by enhancement of whey expulsion from the curds (Dusterhoft et al., 2017).

parameter	place	No of samples	Mean±SD
pH	Alexandria	24	5.83±0.52
	El-Behera	30	5.40±0.70
Acidity	Alexandria	24	0.53±0.31
	El-Behera	30	0.67±0.35
Moisture	Alexandria	24	57.95±3.54
	El-Behera	30	56.76±4.91
Fat/DM	Alexandria	24	23.72±5.76
	El-Behera	30	25.45±12.99
Salt	Alexandria	24	1.80±0.49
	El-Behera	30	1.78±0.62

Table (1). Statistical analytical results of physicochemical evaluation of examined soft cheese samples collected from different sources in Alexandria and El Behera Governorates.

SD=standard deviation

There was no significant variation between all values of parameters of Alexandria and El-Behera governerates ($p>0.05$)

Table (2). Moisture %, Fat/DM% of examined low salt soft cheese samples in comparison with Egyptian Standard

parameter	place	No. of examined samples	Egyptian Standard (ES/2005)	Samples complied with standard		Samples not complied with standard	
				NO.	%	NO.	%
Moisture %	Alexandria	24	<65%	23	95.83	1	4.17
	El Behera	30	<65%	30	100	0	0
Fat/DM%	Alexandria	24	>25%	12	50	12	50
	El Behera	30	>25%	14	46.67	16	53.33

(1008-5/2005).

Table (3): Statistical analytical results of aerobic spore formers count in examined soft cheese samples collected from different sources in Alexandria and El Behera Governorates.

place	No of examined samples	Positive samples		Minimum	Maximum	Mean ± SD
		No.	%			
Alexandria	24	23	95.83	2.00×10^2	2.25×10^3	$8.63 \times 10^2 \pm 7.87 \times 10^2$ a
El Behera	30	30	100	2.8 x 10	2.43×10^3	$5.71 \times 10^2 \pm 4.10 \times 10^2$ b

3.2. Incidence of aerobic spore forming bacteria in examined soft cheese samples:

Spore formers are of a particular concern in the international dairy industry because of the pervasive and resistant nature of their spores in comparison to vegetative cells, surviving extreme environmental conditions such as heat, desiccation, freezing, thawing, and presence of organic solvents, oxidizing agents and UV irradiation, as well as predation by protozoa (Setlow, 2014).

Data presented in Table (3) illustrated that the mean value of aerobic spore forming bacteria count in Alexandria Governorate was $8.63\times10^2\pm7.87\times10^2$ While, in El-Behera Governorate was $5.71\times 10^2 \pm 4.10\times10^2$ with respective incidence of 95.83 and 100%.There was a significant variation between results of aerobic spore formers count in examined soft cheese samples collected from different sources in Alexandria and El Behera Governorates.

The obtained results of aerobic spore forming microorganisms' counts were lower than those obtained by Khater and Abdella (2017) who reported that minimum, maximum and the means of total aerobic spore formers counts in soft cheese samples were 1.2×10^3, 2.3×10^4 and 1.2×10^4, in a respective manner.

Contaminated raw milk may represent a vehicle for introducing spore forming bacteria into chain food production. Spore formers may enter this way through spores present in the environment and established in the form of biofilm. Therefore, the persistence for prolonged periods of time.

Aerobic spore forming bacteria could produce heat-resistant endospores which play an important role in bacterial persistence and biofilm establishment in dairy environment (Ostrov et al., 2019) which enable them to survive pasteurization. Thus, the limiting factor for the shelf life of pasteurized milk and are potential sources of contamination for milk-derived products (Shaheen et al., 2006).

The high incidence of aerobic spore forming bacteria in soft cheeses and other dairy products is a result of many factors such as elevated hydrophobicity of spores, the low charge of spore surface and the shape of spore which lead to strict adhesion to many surfaces and survival during preparation processes of milk and milk products (Iurlina et al., 2006).

4. CONCLUSION

There was no significant difference between samples from both Governorates in chemical composition but significant variation of incidence of aerobic spore formers in low salt soft cheese existed between both Governorates. To improve the safety of these product, efforts should be raising awareness of the importance of hygiene barriers and raw milk quality as well as improved process control can be suggested. We can recommend that the receiving of raw milk should be carefully monitored and only obtained from suppliers apply good manufacturing practices. From the other hands, the strict hygienic measures of cleaning and sanitization of all food contact surfaces as well as prevention of environmental contamination.

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