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Properties of Low Salt Soft Cheese Supplemented With Probiotic Cultures

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Abstract

In recent years upsurge of interest for developing functional dairy products containing probiotics. Soft cheese is a very popular product worldwide and a good alternative for probiotic delivery into the gastrointestinal tract. So, the objective of the present study was to manufacture five treatments of low salt soft cheese. One of them without probiotic cultures keep as a control and others supplemented with probiotic cultures and four others were prepared by adding *Lactobacillus casei* NRC AM2 (T1), *Pediococcus pentosaceus* NRC AM4 (T2), *Lactobacillus rhamnosus* NRC AM6 (T3) and *Pediococcus acidilactici* NRC AM8 (T4). The resultant cheeses were analyzed for chemical, microbiological and sensory evaluation during refrigeration storage period. The results indicated that the viability of *Lactobacilli* and *Pediococcus* in all cheese treatments were increased at the first two weeks of storage then decreased till the end of storage period but still above a threshold level (10^6 CFU/g). The obtained results confirmed that the type of starter and/or time of storage period affected the chemical and microbiological cheese analysis. Also, *Salmonella*, psychrotrophic bacteria, *Enterococci*, coliforms and thermoduric bacteria were not detected in all cheese treatments. Also, there were variations found in fatty acid concentrations in treatments manufactured by adding probiotic cultures. In conclusion, this study demonstrated that the addition of probiotics to soft cheese made the product more acceptable in sensory properties, enhanced the shelf life and can be produced cheese with high quality and health aspects.

Keywords: Probiotic cultures, soft cheese, functional dairy products, fatty acid

Introduction

Functional foods are products containing health promoting components beyond the traditional nutrients. One way for modifying cheese to become functional is by incorporation of probiotics. Freshly fermented dairy products such as cheese, yoghurt, ice cream, desserts and cultured milks are the most popular food delivery systems for probiotics (**Shah 2007 and Karimi et al., 2012**). The supplementations of dairy products with probiotics are able to produce natural antimicrobial substances in order to inhibit undesirable microorganisms (**Vinderola et al., 2002**). These bacteria are responsible for the fresh acidic flavour of unripened cheese. In addition, probiotics play many essential roles in the production of volatile flavour compounds such as diacetyl and aldehydes, synthesis of proteolytic and lipolytic enzymes involved in the ripening of cheese. Therefore, these cultures used in cheese manufacture are very important for enhancement the quality of cheese (**Leroy and De Vuyest, 2004**). Cheese is one of the oldest dairy products with the best nutritional value and health care function. It is widely popular in many countries worldwide with good taste and diverse flavour (**Awad et al., 2012**). The ingestion of cheeses supplemented with probiotics has been associated with several benefits to human health, such as improving lactose intolerance, immune system functions, intestinal health and inhibition of pathogens (**Karimi et al., 2012 and Albenzio et al., 2013**). Many researches were shown that *Lactobacillus* genera, *Streptococcus thermophilus* and *Bifidobacterium* spp. were used as probiotics in many fermented dairy products such as yoghurt, soft cheeses, hard cheeses and cream cheese (**Mahrous et al., 2015**). Cheese provides a very important alternative vehicle for probiotic delivery, due to certain potential advantages. It creates a buffer against the high acidic environment in the gastrointestinal tract and thus creates a more favorable environment for probiotic survival throughout the gastric transit due to higher pH value**.** Probiotics must remain viable in food products above a threshold level (10^6 CFU/g or ml) until the consumption. Probiotic bacteria must be able to survive the unfavorable environment of GIT, which benefits resistance to acid and bile. Upon arrival in the intestine, the bacteria must have the potential of colonization in the GIT. However, besides the essential characteristics, the organisms should preferably show health benefits with functional properties. Nowadays, many new functional characteristics have been developed, including exclusion of pathogens (**Isolauri et al., 2001; Tsai et al., 2005 and Tkhruni et al., 2013**). The goal of the present study was planned to produce synbiotic low salt soft cheese supplemented with probiotic cultures and investigate the chemical, microbiological and organoleptic properties of the cheese during refrigeration storage period.

Materials and Methods

Original of strains

The strains were isolated from traditional fermented dairy products, characterized and identified by **Mabrouk *et al*., (2014) and Hassan *et al*., (2016).**

Probiotic low salt soft cheese manufacture

Cheese was manufactured according the method described by **Fahmi and Sharara (1950)** with some modifications. The milk was heated to 80^{o}C for 5 min, then cooled to starter addition temperature 40^o C and divided into 5 equal portions (4 kg for each portion). The first portion was regarded as a control treatment without starter and the others were inoculated with probiotic starters at the level of 2 % as the following:
Control: Without probiotic culture

Treatment (1): Addition of *Lactobacillus casei* NRC AM2.
Treatment (2): Addition of *Pediococcus pentosaceus* NRC AM 4.
Treatment (3): Addition of *Lactobacillus rhamnosus* NRC AM6.
Treatment (4): Addition of *Pediococcus acidilactici* NRC AM8.

The resultant cheese was withdrawn when fresh and after 7, 15, 21 and 28 days of refrigeration storage period (7^o C) for chemical, bacteriological and organoleptic evaluation.

Chemical analysis

pH values

Values of pH of all probiotic low salt soft cheese samples were also measured by using a digital pH meter (JENWAY, Mode 3510).

Titratable acidity

Titratable acidity and moisture contents in cheese were determined by method of **AOAC**, (**1990**).

Total protein (%) and water soluble nitrogen (WSN %) contents

Total protein and water soluble nitrogen contents of cheese were determined by Kjedahl nitrogen method according to the method of **AOAC**, (**2007**).

Determination of fatty acids

Fatty acids of cheese samples were determined according to the method described by **Collins et al., (2003)**. Fatty acid methyl esters were analyzed using gas chromatography spectrometer (GC-MS QP2010 Shimadzu, Japan).

Microbiological analysis

Total viable bacterial counts

Total viable bacterial counts were determined by using standard plate count agar medium (Oxoid) according to **Houghtby et al., (1992)**.The plates were incubated at 32 ±2° C for 48 h.

Viability of *Lactobacillus* and *Pediococcus* counts

Lactobacillus and *Pediococcus* counts were determined by using MRS (Oxoid) medium according to **De Man et al., (1960)**. The plates were anaerobically incubated at 30° C for 48 h.

Mould and yeast counts

Mould and yeast counts of all probiotic low salt soft cheese samples were determined by using Rose Bengal Chloramphenicol agar medium (Oxoid) according to **Jarvis, (1973)**. The plates were incubated at 25° C for 3-5 days.

Aerobic spore forming bacteria, *Salmonella* and Psychrotrophilic bacteria

Aerobic spore forming bacteria, *Salmonella* and Psychrotrophilic bacteria were detected according to **Stulova et al., (2010)**. The plates were aerobically incubated at 37° C for 24 h for aerobic spore forming bacteria and *Salmonella* and at 7° C for 10 days for psychrotrophic bacteria.

Enterococci counts

Enterococci were estimated using bile esculin azide agar according to the method described by **Mennane et al., (2007)**. The plates were anaerobically incubated at 37° C for 24 h.

Total coliform and fecal coliform counts

Total coliform and fecal coliform counts were enumerated on Violet red bile agar medium (VRBA) according to the method described by **El-Nasri et al., (2012)**.

Thermoduric bacteria counts

Thermoduric bacteria were enumerated according to the method of **Marshall, (1993)** using nutrient starch agar. The plates were aerobically incubated at 55° C for 48 h.

Sensory evaluation

The resultant probiotic low salt soft cheese samples were randomly coded and organoliptically assessed according to **Pappas et al., (1996)**.

Results and Discussion

Chemical analysis of probiotic low salt soft cheese

pH value:

Figure (1) illustrates the pH values of all cheese treatments which gradually decreased by extending of storage period. The control treatment made without adding starters had the higher pH value after 7 days and till the end of storage period than other treatments (**Buriti et al., 2007**; **Mahmoud et al., 2013** and **Elsamani et al., 2014**).

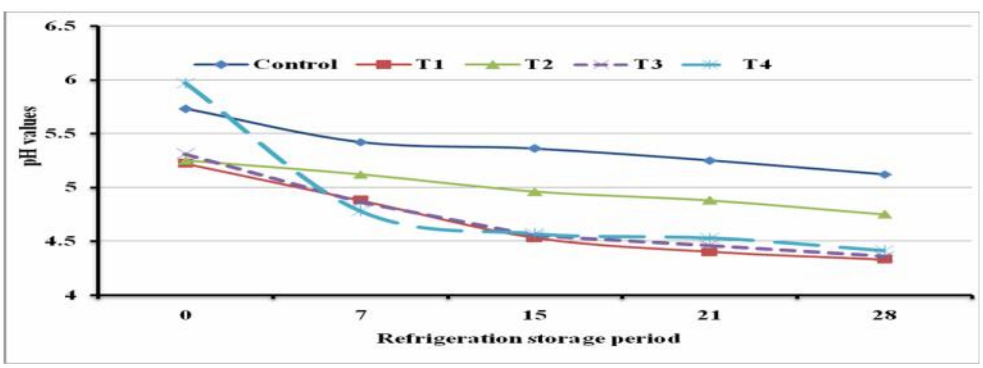

Figure 1. pH values of probiotic low salt soft cheese during refrigeration storage period.
Control: cheese without starters
T1: *Lactobacillus casei* NRC AM2
T2: *Pediococcus pentosaceus* NRC AM4
T3: *Lactobacillus rhamnosus* NRC AM6
T4: *Pediococcus acidilactici* NRC AM8

Titratable acidity (%)

The changes of titratable acidity (%) had an opposite trend to the pH values. Meanwhile, the data presented in Figure (2) confirmed that all cheese treatments exhibited higher acidity values than the control cheese as a result of starter activities and conversion of residual lactose in cheese into lactic acid **Elnemr *et al.*,** (**2013**) and **Mahmoud *et al.*,** (**2013**).

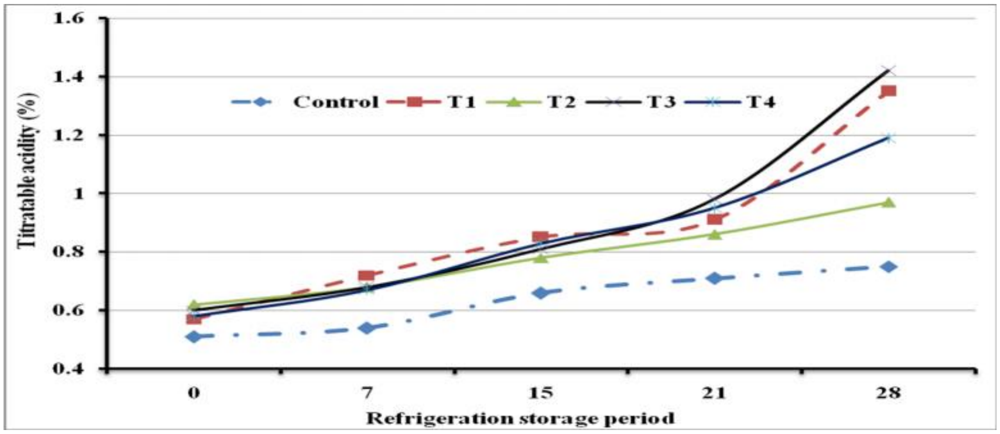

Figure 2. Titratable acidity (%) of probiotic low salt soft cheese during refrigeration storage period.

Moisture contents (%)

Moisture contents of probiotic low salt soft cheese were showed in Figure (3). Gradually decreased in moisture contents as storage period proceeded in all cheese treatments because of acidity development and expulsion of the whey from the curd **Elnemr et al.,** (**2013**); **Hussein and Shalaby,** (**2014**) **and Kebary et al.,** (**2015**)**.**

Total protein contents (%)

Figure (4) illustrate the slight increase in total protein contents (%) of probiotic low salt soft cheese during storage period which could be attributed to the partial loss in moisture **Mahmoud et al.,** (**2013**).

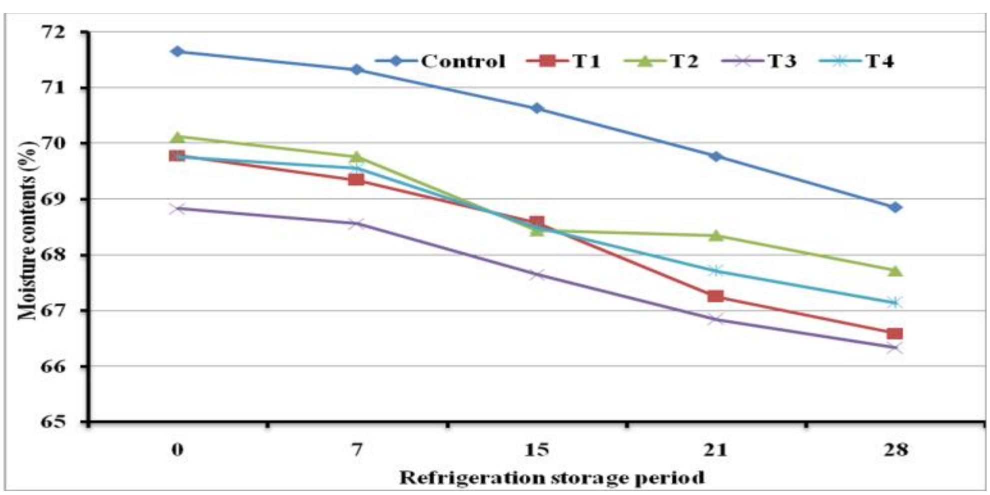

Figure 3. Moisture contents (%) of probiotic low salt soft cheese during refrigeration storage period.

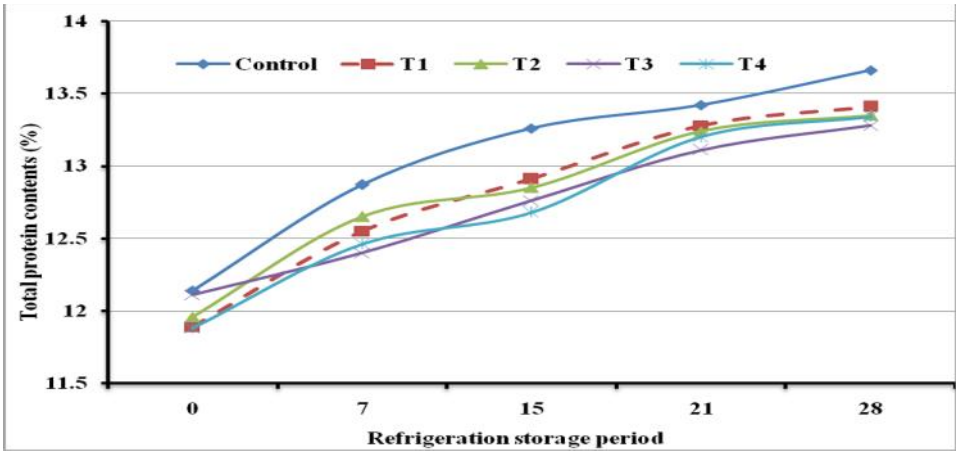

Figure 4. Total protein contents (%) of probiotic low salt soft cheese during refrigeration storage period at 7 °C for 28 days.

Soluble nitrogen content (%)

The soluble nitrogen (SN) content (%) of probiotic low salt soft cheese during storage period at 7 °C for 28 days is illustrated in Figure (5) The results showed that the soluble nitrogen contents of all probiotic cheese treatments were gradual increased with prolonging the storage period (**Degheidi et al., 2009**; **Effat et al., 2012** and **Mahmoud et al., 2013**).

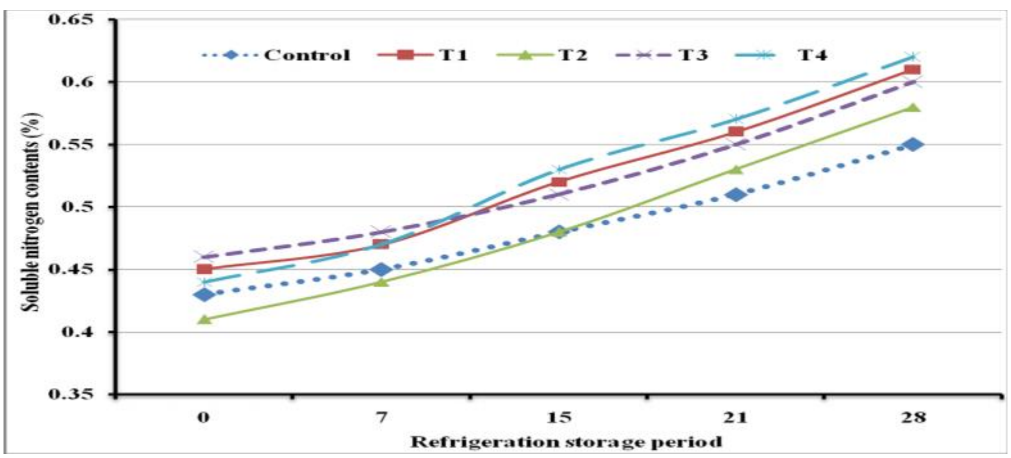

Figure 5. Soluble nitrogen contents (%) of probiotic low salt soft cheese during refrigeration storage period at 7 °C for 28 days.

Fatty acid contents of probiotic low salt soft cheese

Changes in fatty acid concentrations in probiotic low salt soft cheese were investigated when fresh and after 28 days of refrigeration storage period. The results presents in Figures (6 and 7) indicated that the fatty acid compositions of soft cheese were varied in all treatments at zero time. On the other hand and after 28 days of storage period the most fatty acid levels in all soft cheese were increased by extending the storage period and high levels of C18:2Δ9, 12 was the only detected after 28 days of storage in all cheese treatments manufactured by adding probiotic cultures. Generally, there were differences in fatty acid concentrations especially in cheese manufactures by adding probiotic starter cultures which responsible for producing lipolytic enzymes and increasing the levels of fatty acids (**Lavasani and Ehsani 2012** and **Rodrigues et al., 2012**).

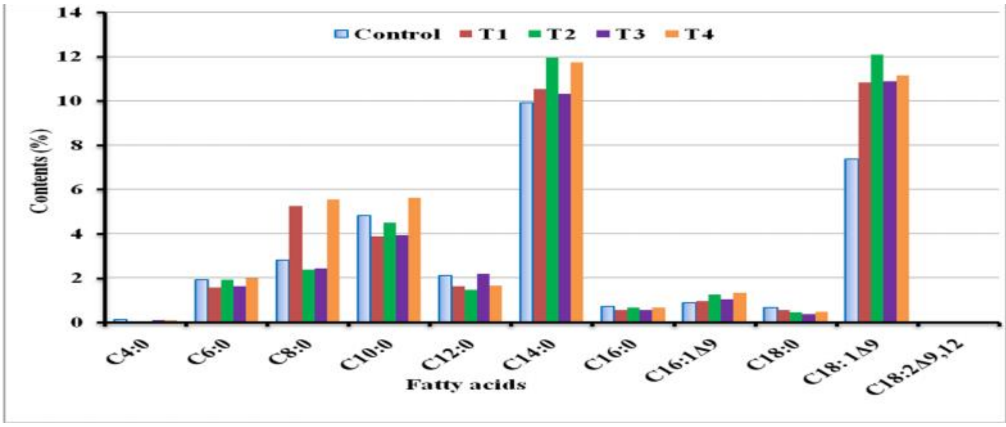

Figure 6. Fatty acid contents (%) in probiotic low salt soft cheese at zero time.

Fatty acids: C4:0= Butyric acid, C6:0 = Caproic acid, C8:0= Caprylic acid, C10:0= Capric acid, C12:0= Luric acid, C14:0= Myristic acid, C16:0= Palmitic acid, C16:1Δ9=9- Palmitolic acid, C18:0= Stearic acid, C18: 1Δ9 =9- Oleic acid, C18:2Δ9, 12 = 9, 12 Linoleic acid

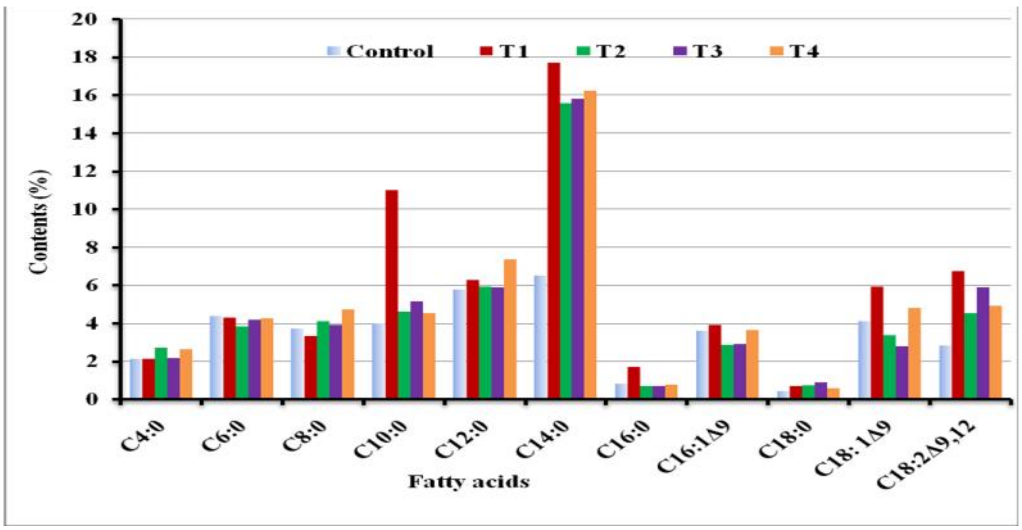

Figure 7. Fatty acid contents (%) in probiotic low salt soft cheese after 28 days.

Microbiological analysis of cheese:

Total viable bacterial counts

The results illustrates in Figure (8) indicated that the total viable bacterial counts of examined cheese increased during the first 15 days of storage period. Moreover, extending the storage the counts decreased in all treatments. The decrease in total viable bacterial counts in cheese might be attributed to the acidity development leading to the inhibition of bacteria in cheese **Sayed et al., (2011)**; and **Hathut et al., (2013)**.

Lactobacilli and *Pediococci* counts

The results showed that the counts of *Lactobacilli* and *Pediococci* in all cheese treatments were increased at the first two weeks of storage period then decreased till the end of storage period by 2 log cycle. The decrease in colony counts in all cheese treatments could be attributed to the accumulation of starter metabolites Figure (9) **Kholif et al., (2010)** and **Yerlikaya and Ozer (2014)**.

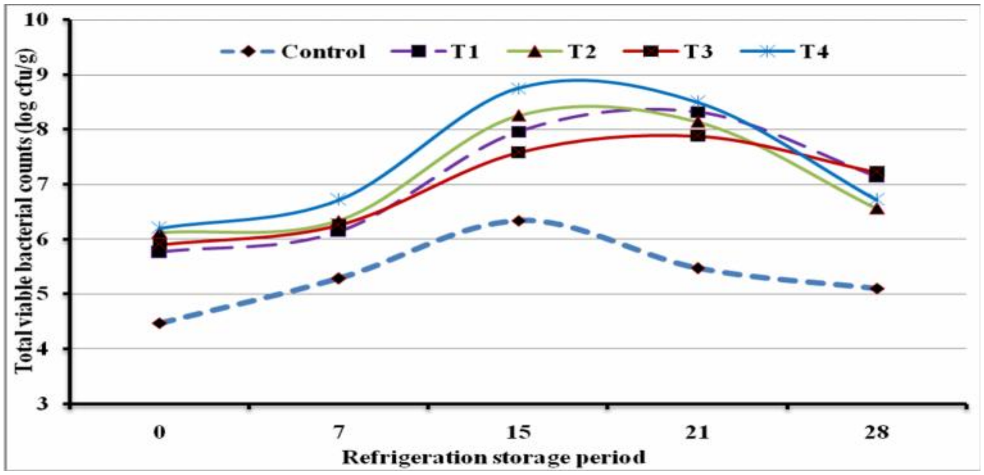

Figure 8. Total bacterial counts (log CFU/g) of probiotic low salt soft cheese during refrigeration storage period.

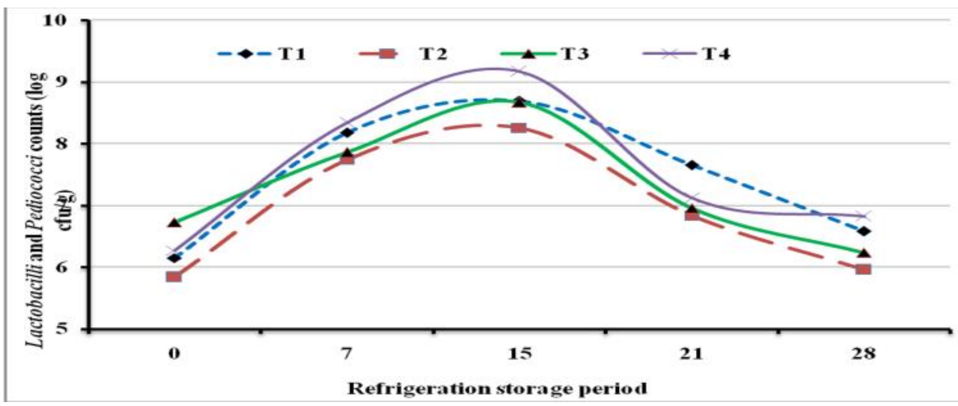

Figure 9. *Lactobacilli* and *Pediococci* counts (log CFU/g) of probiotic low salt soft cheese during refrigeration storage period.

Mould and yeast counts

Mould and yeast counts (log cfu/g) of probiotic low salt soft cheese are illustrated in Figure (10). The mould and yeast counts were detected in lower numbers after 7 days of storage period then started to increase by extending the storage period as a result acidity development. Generally, these microorganisms may be reached to the cheese samples from the manufacture environment (**Sayed et al., 2011** and **Derar and El Zubeir, 2013**).

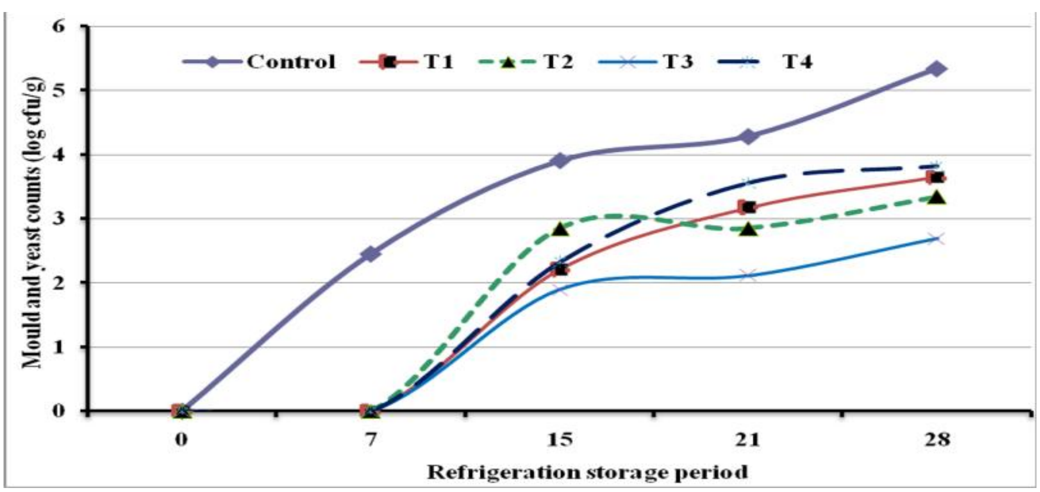

Figure 10. Mould and yeast counts (log CFU /g) of probiotic low salt soft cheese during refrigeration storage period.

Aerobic spore forming bacteria counts

Aerobic spore forming bacteria counts (log cfu/g) of probiotic low salt soft cheese are illustrates in Figure (11). The Aerobic spore forming bacteria counts (log cfu/g) were increased till 7 days of storage period then disappeared from all cheese treatments manufactured by adding probiotic starters at the end of storage period (**Mennane et al., 2007**).

Counts of *Salmonella,* psychrotrophic bacteria, *Enterococci* and thermoduric bacteria

It was clearly evident from the obtained results that counts of coliforms, *Salmonella*, psychrotrophic bacteria, *Enterococci* and thermoduric bacteria were not detected in all probiotic low salt soft cheese during refrigeration storage period were not detected in all cheese treatments either when fresh or during storage period (**Mennane et al., 2007** and **Kholif et al., 2010**).

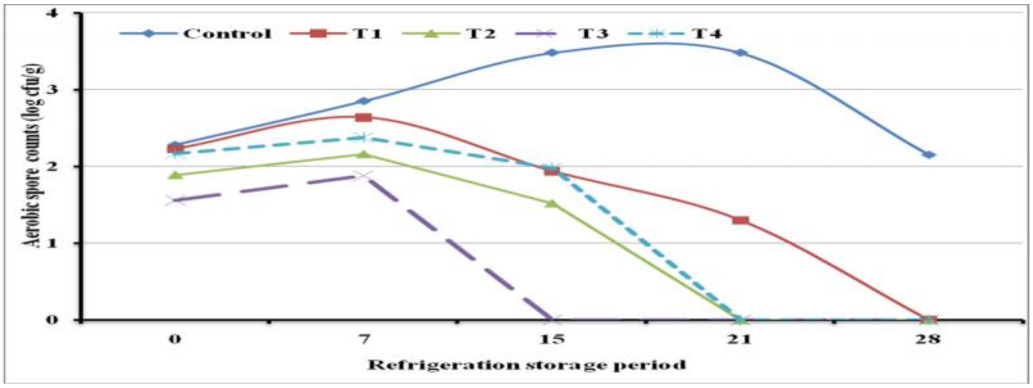

Figure 11. Aerobic spore forming bacteria counts (log CFU/g) of probiotic low salt soft cheese during refrigeration storage period.

Sensory evaluation

The results of sensory evaluation recommended that the addition of probiotic bacteria as starter or adjunct cultures in low salt soft cheese were enhanced the aroma of cheese, organoleptic properties and extending the shelf life **Karimi et al. 2012**; **El-Nemr et al.,** (**2013**) and **Hussein and Shalaby,** (**2014**).

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