

## Evaluation of chemical and microbial properties of Iranian white cheese using kefir, yogurt and commercial cheese culture as a starter

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### Abstract

In this research, the chemical and microbiological characteristics of white brined cheeses produced with five different starter cultures, namely: traditional kefir grain, commercial kefir, commercial yogurt, traditional yogurt and commercial cheese starter culture, were examined during 60-day ripening period. Results of statistical analysis showed that the type of starter culture had a significant effect on pH, acidity, fat, protein, moisture, enterobacteria, total counts, mold & yeast as well as on lactococcus ( $p < 0.01$ ), and lactobacillus level ( $p < 0.05$ ). pH, acidity, fat, protein, total count, mold & yeast, and lactobacillus level ( $p < 0.01$ ) were significantly affected by starter culture type during ripening period. However, moisture, enterobacteria and lactococcus level in all cheese samples were not affected by ripening period. Whereas other parameters including pH, fat and protein content showed decreasing trend during ripening. Among chemical analyses, cheese produced with traditional kefir had highest pH and cheese produced using commercial kefir showed highest acidity and moisture. Among microbial parameters cheese produced with commercial kefir starter had the lowest total microbial count and after that cheese using traditional kefir starter. It is concluded that traditional kefir grain can be used as a starter culture in production of functional white brined cheese.

**Keywords:** White brined cheese, kefir, and starter culture, chemical and microbial profiles

### Introduction

Iranian white cheese which categorized as a brined cheese is the most popular cheese consumed in Iran with annual production over 267.7 thousands tones in 2011 (FAO, 2011). Its characteristic flavor, body and texture are developed at the ripening period of several weeks to months (Kourkoutas, *et al.*, 2006). It is a close textured brined cheese, resembling Feta cheese but differs from Feta in the way it is made. It is, for example, manufactured without dry salting of curd and slime formation on the curd surface before brining, which are essential for the development of characteristic Feta flavor during ripening (Khosrowshahi, *et al.*, 2006).

The importance of starter culture addition in cheese making is related to some factors including the inhibition of growth of undesirable bacteria, improved whey drainage, production of characteristic flavors and aromas and controlled acid development (Goncu, and Alpkent, 2005).

Starter cultures used in the production of white brined cheeses usually contain *Lactobacillus casei*, *Enterococcus faecium*, as well as *Lactococcus lactis* spp. *lactis* and *L. lactis* spp. *cremoris*. In some countries like Greece and Turkey, cheese manufacturers use yogurt as a starter culture in production of Feta like cheeses (Goncu, and Alpkent, 2005).

Kefir has some probiotic and functional properties due to the presence of various Lactic Acid Bacteria (LAB) and yeasts including *Lactobacillus brevis*, *Lb. helveticus*, *Lb. kefir*, *Leuconostoc mesenteroides*, *Kluyveromyces marxianus* and *Pichia fermentas* alive in the final product. These microorganisms produce lactic

acid, ethanol, antibiotics and bacteriocins, which can inhibit the development of spoilage and pathogenic microorganisms (Goncu, and AlpKent, 2005). This feature has encouraged some workers to use traditional kefir grain in production of some probiotic dairy products as a health promoting foods such as probiotic cheese and yogurt (Goncu, and AlpKent, 2005).

Kourkoutas et al. (2006) evaluated the freeze-dried kefir co-culture as starter in Feta-Type cheese. They realized that kefir co-culture extended the shelf life of unsalted cheese. All types of cheeses produced with kefir as a starter were approved and accepted by the panel during sensory evaluation compared to commercial feta-type cheese (Kourkoutas *et. al.*,2006).

Since to the best of our knowledge no such work has been performed on the Iranian white brined cheese, this study was performed.

The aim of this study was to investigate the effect of five different starter cultures namely, commercial cheese starter culture, commercial yogurt starter culture, traditionally produced yogurt, commercial kefir starter culture and traditionally produced kefir grain as an undefined culture on the chemical and microbiological properties of cheeses during ripening period.

## Materials and methods

### Materials

#### Milk

In this study, cheese was prepared with commercial pasteurized bovine milk. Milk used in the manufacture of cheese trials was obtained from Animal Husbandry Department of Hasheminejad Higher Education Center (Mashhad, Iran).

#### Starters

The 5 types of starters were used as a culture in this study including:

- (a) commercial cheese starter culture (FRC 65, as a control),Chr.Hansen, Denmark
- (b) commercial yoghurt starter culture (Lactina),Bulgaria
- (c) traditionally produced yogurt
- (d) commercial kefir (DG 500 L), Danisco,Biolacta, Poland
- (e) traditionally produced kefir grain (as an un-defined starter culture)

#### Rennet

Standard rennet; chy-Max, chr.Hansen Inc., Denmark; 183 International milk clotting units(IMCU)/mL (International Dairy Federation,1997), was used.

## Methods

### Cheese manufacture

The production diagram (flow chart) of white brined cheese is presented in Fig. 1. White brined cheese trials were analyzed at 15-day intervals during a ripening period of 60 days.

### Chemical analysis

Titrateable acidity of milk was determined by the Dornic method(AOAC,1997) and pH of milk was measured using a digital pH meter (model JENWAY 3020 pH Meter).cheese was analyzed for total solids or dry matter by drying 5 g of cheese at 105°C for 2.5h (AOAC,1997).

The fat content of milk and cheese samples was determined by Gerber method and their total protein content were determined by measuring total nitrogen using the kjeldahl method (AOAC,1997) and multiplying the result by 6.38 to get the protein content. Cheese acidity was determined as lactic acid by titration with 0.1 N NaOH in presence of phenolphthalein as an indicator (Kourkoutas, *et al.*, 2006). All chemical measurements were performed in duplicate.

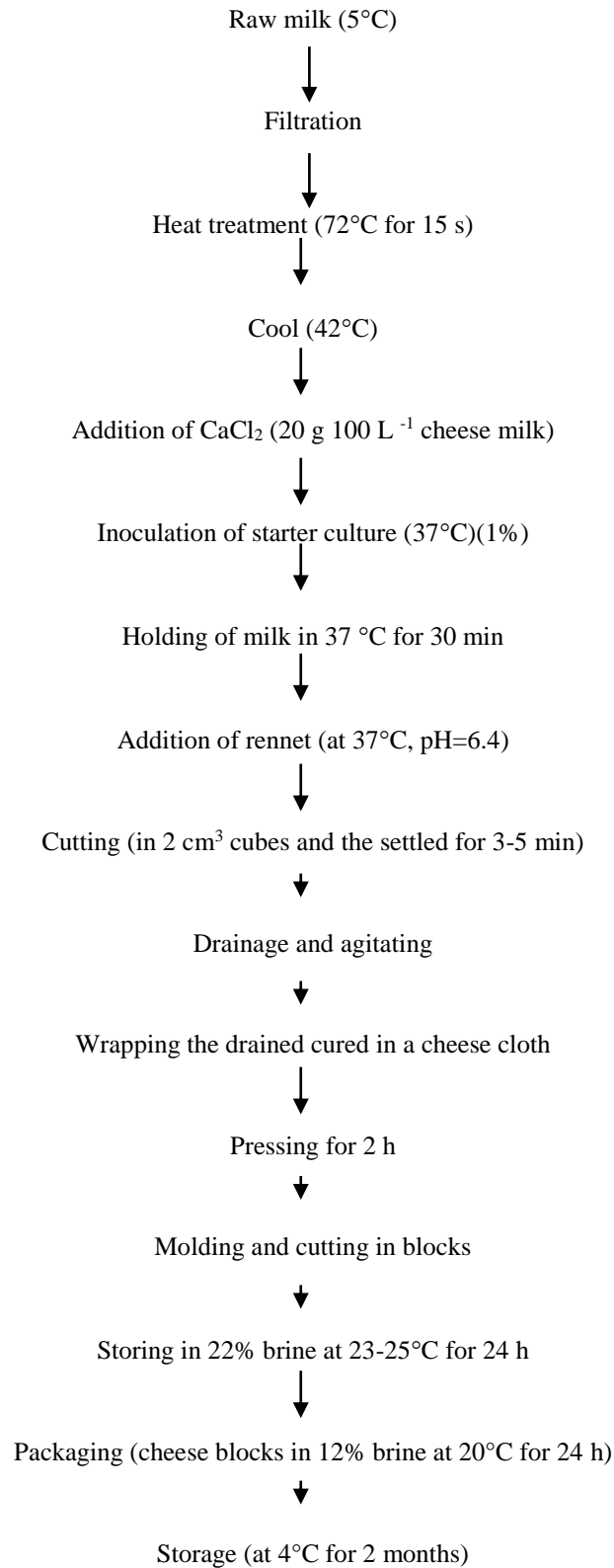


Fig. 1. The production diagram (flow chart) of white brined cheese.

### Microbiological analysis

10 g of cheese was taken from the cheese interior and was blended with 90 ml of sterilized Ringer solution and subjected to serial dilutions.

The following microbiological analyses were performed:

- (1) Determination of total aerobic counts on plate count agar at 30°C for 48h,
- (2) Enumeration of enterobacteria after incubation on Violet Red Bile glucose Agar at 30°C for 24h,
- (3) Enumeration of yeasts and molds after incubation on Yeast Glucose Chloramphenicol Agar (YGC) at 23°C for 3-5 days,
- (4) Enumeration of staphylococcus after incubation on Corom Agar staphylococcus at 37°C for 24h, (5) Enumeration of *Salmonella sp.* after incubation on Corom Agar Salmonella at 37°C for 24h, (6) Enumeration of Lactococci after incubation on M-17 at 42°C for 48h anaerobically, (7) Enumeration of Lactobacilli after incubation on **MRS agar** at 42°C for 48h anaerobically. Results were

presented as the log of the mean number of CFU on plates containing between 30 and 300 cfu/g of cheese.

### Statistical analysis

The treatments had a factorial structure including five types of starter culture and storage time during 60 days of ripening with 15-day interval. All data (results) were analyzed by analysis of variance using the General linear models procedure of Minitab system software, Version 14.2. Tukey multiple comparison tests were applied for data.

### Results and Discussion

#### Chemical composition

The effect of 5 types of starter culture on chemical parameters including pH, acidity, fat, protein and moisture content of white brined cheese samples is presented in Table 1. The effect of starter culture was significant on pH, acidity, fat, protein and moisture ( $p < 0.01$ ).

Table 1. Effect of starter culture of white brined cheese samples on chemical parameters.

Type of starter culture	pH	Titrateable acidity	Fat(%)	Protein(%)	Moisture(%)
Commercial kefir	5.411±0.36	0.501±0.13	14.57±1.59	10.595±2.49	0.68±0.028
Commercial cheese starter culture(FRC-65)	5.517±0.37	0.457±0.14	16.17±1.05	12.287±2.95	0.617±0.023
Commercial yogurt(Lactina)	5.306±0.21	0.341±0.21	17.53±1.34	12.82±3.39	0.605±0.137
Traditional kefir grain(un-defined)	5.793±0.37	0.458±0.19	16.58±1.34	10.88±2.16	0.623±0.026
Traditional yogurt	5.148±0.21	0.333±0.17	19.43±1.57	14.736±3.09	0.557±0.017

#### pH and acidity

Cheese samples produced using traditional kefir grain had the highest pH, followed by cheese produced with commercial cheese starter culture. This might be due to the different micro-flora presented in these starter cultures. Such finding is in agreement with the work of Goncu and Alpkent (2005) who found that kefir cheese had the highest pH. In contrast, cheeses produced with commercial kefir starter culture

showed the highest acidity (Goncu and Alpkent 2005). Kourkoutas, *et al.* (2006) showed that addition of a freeze-dried kefir starter increased the cheese acidity (Kourkoutas, *et al.*, 2006).

Cheese samples produced using traditional yogurt had the highest fat content. Ferit Atasoy, A. *et al.* (2008) showed that cheese made with thermophilic starter cultures had higher fat content than its counterpart (Ferit Atasoy, *et al.*, 2008).

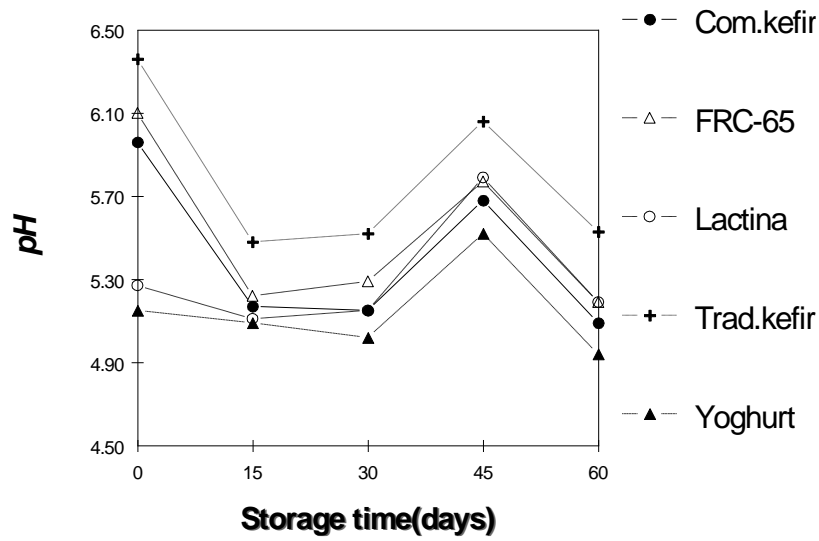


Fig. 2.a. Interaction effect of starter culture and ripening period on pH

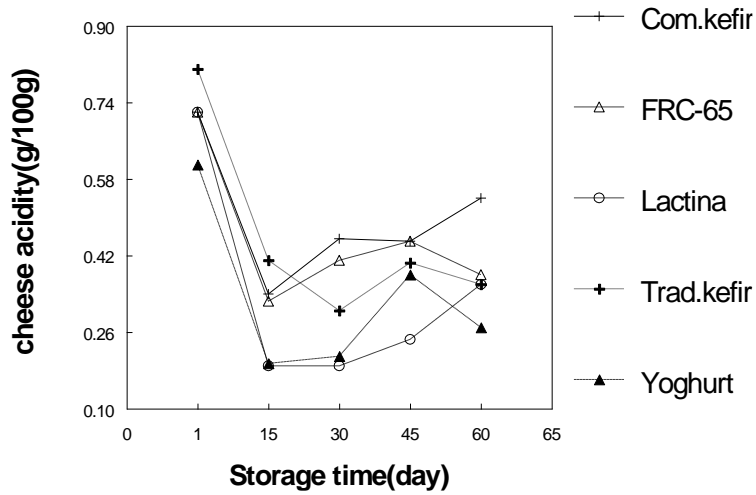


Fig. 2.b. Interaction effect of starter culture and ripening period on acidity

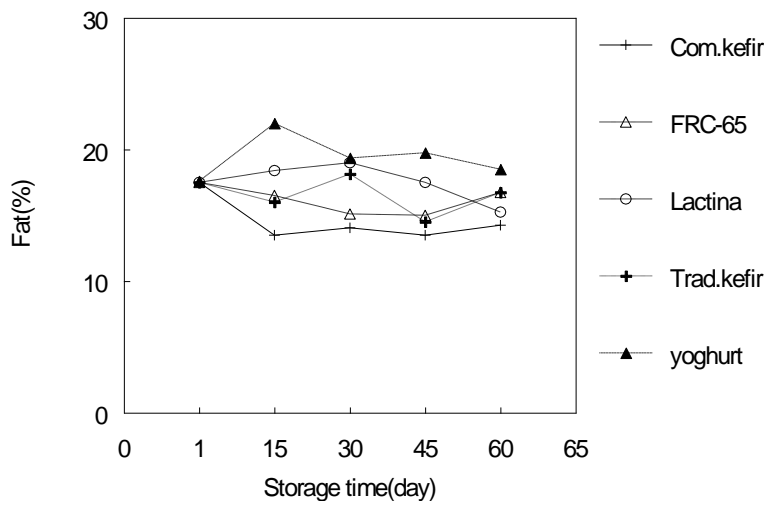


Fig. 2.c. Interaction effect of starter culture and ripening period on Fat

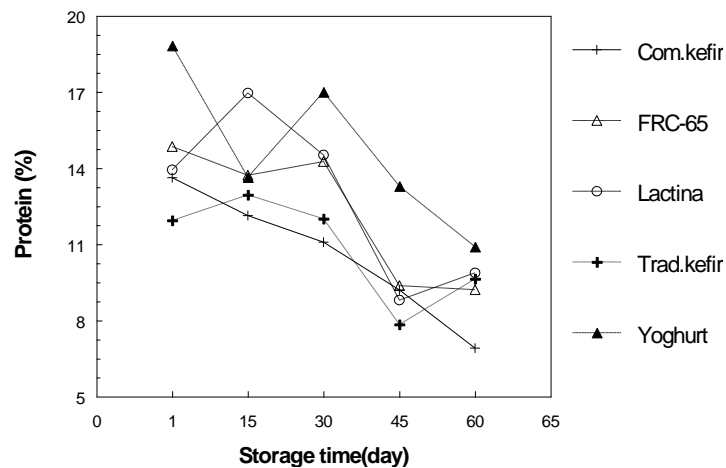


Fig. 2.d. Interaction effect of starter culture and ripening period on protein

According to Table 1, the highest protein content is belonged to cheese produced by addition of traditional yogurt starter culture as well as commercial yogurt starter culture respectively. This is may be due to the fact that in two type of cheeses whey protein more entrapped in protein network than the other cheese types because the fat content in these two type of cheeses, as presented in table 1, is more and fat globules can act as "plugs", blocking the flow of whey through channels in the curd (Green, and Grandison, 1993). As shown in Table1, cheese samples made with commercial yogurt starter culture had lowest moisture content because this type of cheese has the lowest pH and thus the moisture loss or syneresis is more intense in this cheese. The rate of moisture loss is inversely related to pH (Fox, *et al.*, 2000), with increasing the concentration

of hydrogen ions during acidification, the repulsive forces decreases, and the casein micelles begin to aggregate. Souza *et al.*(2009) showed similar results for Minas fresh cheese during storage (Souza, et al. 2009).

Table 2 shows the effect of ripening storage on chemical composition. Statistical analysis showed that effect of ripening period on pH, acidity, fat and protein ( $p < 0.01$ ) was significant. The maximum rate of change occurs during the first month of storage, which corresponds to the maximum growth of cheese micro flora and after that the changes occurred at slower rates (ABD EL-Salam, *et al.*, 1993). El-Abd et al showed that the composition changes progressively in Domiati cheese (ABD EL-Salam, *et al.*, 1993).

Table2. Effect of ripening storage on chemical composition.

Ripening period(day)	pH	Titrateable acidity	Fat(%)	Protein(%)	Moisture(%)
1	5.77±0.53	0.716±0.07	17.52±0.04	14.6378±2.56	0.5946±0.08
15	5.21±0.15	0.292±0.09	17.28±3.16	13.884±1.83	0.615±0.05
30	5.22±0.19	0.314±0.11	17.13±2.40	13.78±2.32	0.642±0.12
45	5.76±0.19	0.386±0.08	16.05±2.53	9.708±2.09	0.617±0.04
60	5.19±0.21	0.382±0.09	16.3±1.62	9.312±1.47	0.616±0.04

The pH value of cheese samples decreased until day- 15 due to the production of organic acids (especially lactic acid) by lactic acid bacteria which considered the main source of sugar

fermentation. The pH trend after two weeks tends to increase mainly as a result of secondary proteolytic activity by bacteria and yeasts and formation of free amino acids as well as ammonia

(Ferit Atasoy, et al., 2008). El-Abd showed that the pH of Domiati cheese reaches as low as 3.3. Acidity developed brings the pH of the cheese close to the isoelectric point of casein and partially solubilizes the colloidal calcium which causes shrinkage of the cheese matrix and exudation of cheese serum into the brine (ABD EL- Salam, et al., 1993). Similar results were reported by Marcelino kongo, J et al. (2009).

Cheese acidity decreased until the day 15, and then increased during the rest of the storage period. This increasing trend is due to lactic acid fermentation. The high moisture content of fresh cheese which retains a high level of lactose and whey is a rich source of lactose for bacterial fermentation within cheese through diffusion. Ferit Atasoy, and Turkoglu (2008) presented similar results for Urfa cheese (a white-brined Turkish cheese) (Ferit Atasoy, and Turkoglu, 2008).

Goncu and Alpkent (2005) showed that the titratable acidity increased and pH decreased due to the production of organic acid with LAB responsible for most of the sugar fermentation changes in the patterns of pH and titratable acidity observed in their study were similar with the previous findings of Akin et al.(2003). Kourkoutas, Y et al.(2006) showed that ripening in brine with no surface pre-salting resulted in a statistically significantly lower lactic acid concentration and a higher pH at the end of the process. This might be due to the facts that the cheese with no surface salting lacked the necessary hardness and was easily grated and thus the produced lactic acid might have extracted by the brine solution. A rapid decrease in pH could be due to the temperature of curd formation favoring the action of lactic acid bacteria rather than yeasts. The rising in pH could be related to the action of yeasts. A rise in pH due to the action of yeasts causes the growth of non-acid-resistant surface growing bacteria and their proteolytic and lipolytic activities. The difference in cheese pH caused by starter cultures may be due to different rates of acid production and the salt tolerance of

the different starters (Kourkoutas, et al., 2006). In contrast to our results, Souza et al. (2009) showed that the decreasing trend of pH and increasing trend of acidity during 21 days of ripening. Regarding to effect of starter type on pH and acidity, they showed that the presence of *S.thermophilus*, which produces small amount of CO<sub>2</sub> and formic acid from lactose, therefore increasing the acidity. Similarly, in our study the cheese samples produced using the commercial kefir and commercial cheese starter cultures (FRC) which contain *S.thermophilus* in their composition showed the highest acidity.

Rosa, et al.(2008) reported that the pH declined significantly during the first week of ripening, probably due to lactic acid production by lactic bacteria. The increase in pH between 15 and 45 th day (table 2), could be due to metabolic activity of mould and yeast, which use lactic acid as a source of carbon. Also, proteolytic process can lead to pH increase because of releasing nitrogen-containing alkaline compounds.

As seen in Table 2, fat content of cheese samples had decreasing trend during ripening. This fact is probably due to the lipolytic activity of microorganisms that resulted in formation of volatile free fatty acids. Goncu and Alpkent (2005) and Ferit Atasoy, and Turkoglu (2008) showed similar results in their respective studies (Goncu, and Alpkent, 2005; Ferit Atasoy, and Turkoglu, 2008).

Protein content of cheese samples decreased during ripening (Table2). This could be due to degradation of cheese proteins by endogenous proteinases, residual coagulant and proteinases from starter and non-starter microorganisms. Generally, the total nitrogen content of cheese gradually decreases while the levels of the soluble nitrogen fractions increase continuously during storage, indicating a continuous proteolytic process. Transfer of degradation products to the pickling solution by diffusion explains the decrease in Total N during storage. Goncu and Alpkent (2005) and Ferit Atasoy, and Turkoglu (2008) obtained similar trend in their study (Ferit

Atasoy, and Turkoglu (2008); Goncu, and Alpknnet, 2005). Ferit Atasoy, and Turkoglu (2008) reported that nitrogenous compounds in brine increased during ripening of Urfa cheese Özer *et al.* (2002) showed the continuous decrease in total solids and nitrogen content of Urfa cheese throughout cold storage (Özer *et al.* 2002). Pereira *et al.* (2008) showed similar results for model Portuguese cheeses (Pereira *et al.* 2008). The moisture content of cheese samples increased during early storage period and decreased thereafter (Table 2). During storage at low temperature, a decrease in the volume of pickle occurs during the early storage period through swelling of cheese protein and an increase in its moisture content, but further storage is accompanied by an increase in pickle volume and decrease in cheese moisture content (ABD EL-Salam, *et al.*, 1993). Goncu and Alpknent (2005) showed the same trend in white pickled cheese produced by different kinds of starter culture (Goncu and Alpknent, 2005). Ferit Atasoy and Turkoglu (2008) showed that in Urfa

cheese the total solids declined and consequently, the moisture content increased during ripening period (Ferit Atasoy and Turkoglu, 2008). This moisture increase may be due to breaking of peptide bonds and releasing short peptides and amino acids having higher water binding capacity (ABD EL-Salam, *et al.*, 1993).

Ozer *et al.* (2000,2002), Atasoy *et al.* (2003), and Ozer *et al.* (2004) reported that the total solids content of Urfa cheese decreased during storage due to proteolysis (Atasoy, *et al.*, 2003; O`zer, *et al.*, 2000; O`zer, *et al.*, 2002; O`zer, *et al.*, 2004). The combined effect of starter culture and ripening period on pH, acidity, fat ( $p<0.01$ ) and protein ( $p<0.05$ ) were also significant (data not shown).

### Microbiological analysis

According to statistical analysis the effect of the type of starter culture on the numbers of enterobacteria, total counts, mould and yeast, lactococci ( $p<0.01$ ) and lactobacilli ( $p<0.05$ ) was significant Table 3).

Table3. Effect of starter culture on microbiological parameters of cheese samples.

Type of starter	Coliform (logcfu/g)	Enterobacteria (log cfu/g)	Total count (log cfu/g)	Mold&yeast (logcfu/g)	Lactobacilli (logcfu/g)	Lactococci (logcfu/g)
Commercial kefir	3.48±1.2	4.04±0.39	6.84±0.29	6.05±1.03	2.2±1.22	3.82±0.6
Commercial cheese starter(FRC)	3.281±0.36	3.26±0.53	8.31±0.87	3.8±1.11	1.08±0.97	3.97±1.64
Commercial yogurt(Lactina)	1.777±1.54	2.21±1.27	8.6±0.71	4.72±0.5	3.03±1.14	7.25±1.02
Traditional kefir grain(undefined)	7.34±0.67	7.18±0.7	8.15±0.72	5.86±0.75	2.74±1.72	7.09±0.9
Traditional yogurt	1.99±0.88	2.03±1.13	9.62±1.45	5.59±1.02	2.79±2.008	6.3±2.38

As can be seen in table 3, cheese samples produced using traditional kefir grain and after that commercial kefir showed the highest enterobacterial counts, conversely cheese samples produced using yogurt starter culture showed the lowest level for the aforementioned microorganisms. Kourkoutas *et al.*, (2006) showed similar results in their study (Kourkoutas *et al.*, 2006). According to Table 3, cheese

samples produced using commercial kefir as well as traditional kefir grain showed the lowest total count respectively.

The cheese obtained with the commercial cheese starter (FRC-65) had the lowest mould and yeast and the cheeses obtained with commercial kefir and traditional kefir showed the highest mould and yeast counts. This may be due to the presence in cheese of some yeasts from kefir.



With respect to lactobacilli and lactococci, cheese samples produced by commercial yogurt as well as traditional kefir showed the highest lactobacilli. This is again related to the microflora composition of starter cultures used for these cheeses. Kourkoutas et al., (2006) showed that the kefir cheese (cheese produced with a freeze-dried kefir co culture) had more lactobacilli and lactococci than rennet cheese (2).

Statistical analysis showed that the effect of ripening period on microbiological profiles such as total counts, mould and yeast and lactobacilli ( $p < 0.01$ ) was significant. Table 4, presented the change of the microbial profiles during 60 days ripening period.

According to Table 4, all microbiological parameters showed an increasing trend during ripening. El. Abd- et al., (1993) showed that the total microbial count increased rapidly, reaching a maximum after a week of storage and then declined in Domiati cheese, while lactobacilli reach a maximum after 2-4 weeks and then decreased gradually in Domiati cheese. In Feta cheese, the numbers of aerobic bacteria, lactic acid bacteria and proteolytic bacteria reach  $\log_{10}$

counts of: 9.0, 7.7 and 8.0 at 5, 20 and 45 days, respectively. The maximum number of microorganisms correlates with a sharp increase of compounds formed by proteolysis and lipolysis during the early stages of ripening (ABD EL-Salam et al., 1993). In mature cheese (2-8 months old), the  $\log_{10}$  counts of total aerobic bacteria, lactic acid bacteria and yeasts range between 7.0-9.5, 7.0-8.0 and 2.5-4.5, respectively. With regard to the mould and yeast increase during storage, one reason that can be mentioned is the presence of strains of yeasts or mould in the composition of some starter cultures, such as those for commercial kefir and traditional kefir. Total viable counts reached their maximum value by 30 days of ripening, presumptive lactobacilli showed an increase of four log cycles within the first 30 days and yeast and moulds reached a maximum within the range  $10^5$ - $10^6$  cfu/g, by 90 days of ripening in Sao Jorge cheese (Marcelino Kongo, et al., 2009).

Fig 3 shows the interaction effect of starter culture and ripening period on the microbiological profile of cheese samples.

Table 4. Effect of ripening period on microbiological characteristics.

Time of storage	Coliforms (log cfu/g)	Total count (log cfu/g)	Mold & yeast (log cfu/g)	Lactobacilli (log cfu/g)
1	3.24±2.42	7.13±0.27	4.09±0.87	1.74±1.64
15	2.74±3.03	7.94±0.95	4.8±1.19	3.41±1.04
30	3.99±2.34	8.47±1.09	5.06±1.18	1.47±1.38
45	3.67±2.01	8.97±1.38	5.98±0.94	2.18±0.61
60	4.21±1.84	9.01±1.47	6.1±0.88	3.05±0.71

## Conclusion

Our results showed that cheese trials produced using traditional kefir grain (un- defined) had high acidity, low fat and protein in comparison with other cheese samples. With respect to microbiological properties, cheese samples produced using commercial and traditional yogurt starter culture showed lowest coliforms and enterobacteria count and cheeses with

commercial and traditional kefir starter culture presented the lowest total count. It is therefore can be introduced as a functional cheese with longer shelf life. Regarding to lactobacilli and lactococci, cheese with commercial and traditional yogurt obtained the first and second maximum among other cheese samples. All microbiological properties had increasing trend during ripening period.

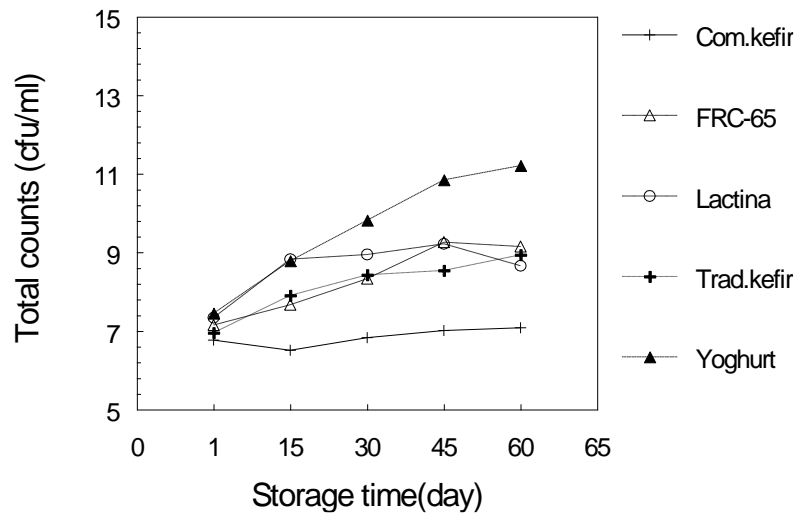


Fig. 3. Effect of starter culture and ripening period on total count of cheese samples.

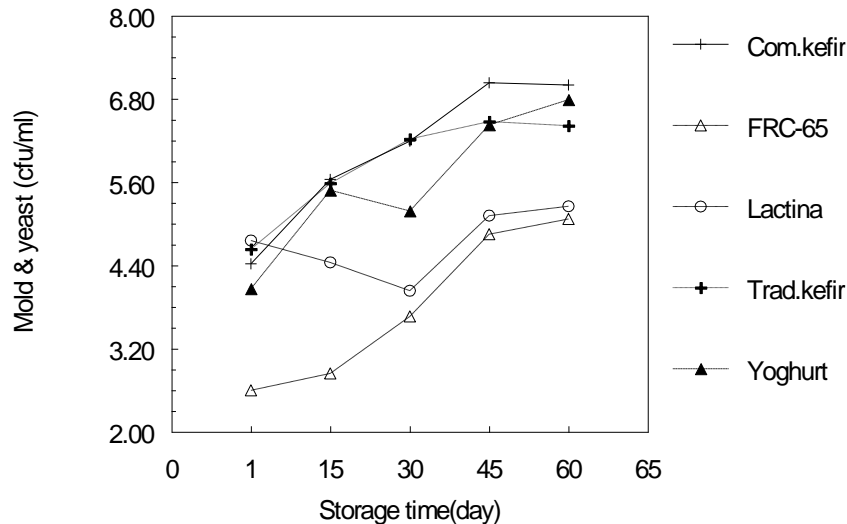


Fig. 4. Effect of starter culture and ripening period on mold and yeast count of cheese samples.

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