



Original article

## Effects of Starter Culture Combination on the Characteristic of White Cheese <sup>1</sup>

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### Abstract

In this study, the effects of using mesophilic and thermophilic cheese cultures at different ratios on White cheese yield, physico-chemical, textural, sensory and microbiological properties were investigated. Yeast, mold, *Enterobacter spp.*, and *Staphylococcus aureus* counts were determined. In sample with high ratio of thermophilic culture, there was slightly increase in fat, salt, fat and salt in dry matter content. All textural parameters were found significantly different. The number of *Enterobacter spp.* was found lower in cheese with high ratio of thermophilic culture, while the number of yeast was high. As a result, it has been observed that starter culture ratios, which contain different bacterial strains, can affect the technological and functional properties of freshly consumed cheese.

**Keywords:** White Cheese, Starter Culture, Texture.

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## INTRODUCTION

Heat treatment of the milk in cheese production reduces the level and diversity of raw milk microflora, inactivates enzymes and changes the biochemical and microbiological properties of the cheeses. By the way, microbial characteristics of cheese are influenced by different factors such as the microflora of the raw milk, starter cultures and *cross contamination* from *unhygienic* conditions during processing. It is well known that reduction in milk pH due to acidification by different starter cultures at the appropriate ratio are the important factor in the manufacture of a good quality cheese. The use of starter cultures containing thermophilic/mesophilic lactic acid bacteria is an essential requirement for cheeses. Their major function is to produce lactic acid and flavour compounds. In the production of different cheese types, according to taste, aroma and texture of cheese, different types of microorganisms are used (Fox et al. 2000; Sulejmani et al. 2014).

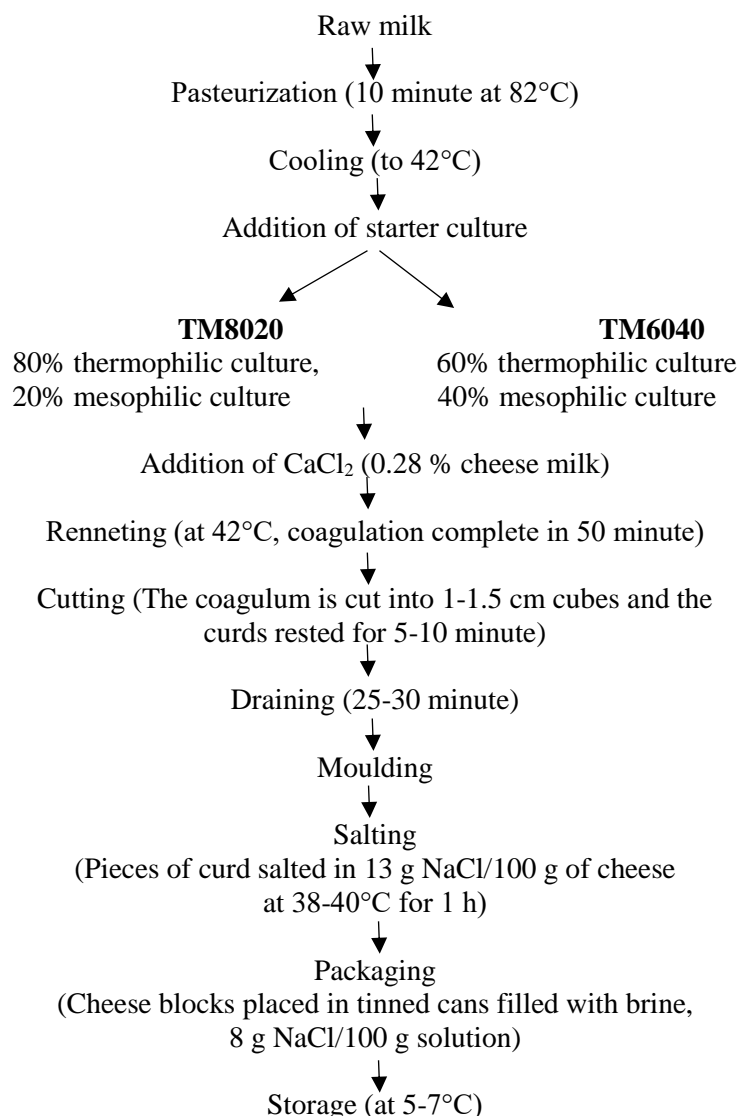
Cheese starter cultures may be classified in a number of ways according to optimal growth temperature. Mesophilic starters comprise *Lactococcus* species, *Leuconostoc cremoris*, *Lactobacillus casei* and have an optimal growth temperature of ca. 30°C, while thermophilic starters comprise the more widely used *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *helveticus*, *Lactobacillus delbrueckii* subsp. *lactis* and have an optimal temperature of 40- 45°C (Kayagil 2006).

White cheese, the soft or semi-hard cheese, is manufactured from sheep's or cow's milk or their mixture using specific starter culture (Ozcan and Eren-Vapur, 2013). In the production of feta-white cheese milk has to be processed at the critical temperature (32-45 °C), which is a critical temperature after pasteurization, so cross contaminations that may occur during the process should be considered. This product is an important criterion on the cultural activity added as it is important for the quality of microbiological quality. In this study, the effects of using mesophilic and thermophilic cheese cultures at different ratios on White cheese microbiological, technological properties were investigated.

### Material and Methods

#### *White Cheese Manufacturing*

Materials whole cow's milk that was used in cheese making was daily provided from the cow farm of Bursa region. Rennet and starter culture (Thermophilic culture, Valiren C1T; Mesophilic culture, Valiren C1M) was purchased from Mayasan Food Industry and Trade Anonymous Company (Istanbul).



**Figure 1.** White cheese production

### Physico-chemical and Textural Analyses

Dry matter, protein, fat and lactose contents of milk and whey samples were determined using the MilkoScanTM FT 120 (Foss a/S, Hillerød, Sweeden). Before physico-chemical analysis, the cheese samples were ground and homogenized to achieve homogeneity. Dry matter, protein, fat and lactose contents of the cheese samples were determined using *FoodScan Dairy Analyzer* (Foss, Electric A/S, Hillerød, Denmark). The pH of milk, whey and cheese samples was determined using a pH meter. The total yield (kg of cheese/kg of milk) x 100 was determined so as to evaluate the yield of cheese making. SH values and salt content were evaluated according to the method by Ozcan and Eren-Vapur (2013).

Texture properties of cheese samples were determined on replicated samples with a Texture Analyser TA-XT Plus (Stable Micro Systems) texturometer, using a two bite compression of cylindrical

samples of 36 mm of diameter using texture parameters hardness (maximum force required to compress the cheese), adhesiveness (force required to remove the cheese from the probe), cohesiveness (strength of internal bonds of cheese), gumminess (required force to swallow cheese), springiness (elasticity of cheese after force is removed), chewiness (required energy to masticate cheese) and resilience (re-deformation capacity) (Dimitreli et al., 2017).

### ***Sensory Analyses***

In sensory tests, the panel was asked to describe the sensory attributes of appearance and color, texture, taste, salinity, odor, and elasticity of the cheese samples; by assigning a liking score on a 9-point hedonic scale. The descriptions used for the hedonic scale were: 9=excellent, 8=very good, 7=good, 6=fairly good, 5=indifferent, 4=fairly poor, 3=poor, 2=very poor, 1=extremely poor. Approximately 50 g of cheeses at 10 °C was placed in plastic cups coded with random 3-digit numbers for identification. Unsalted crackers and mineral water were provided to cleanse the palate between tasting periods (Andreatta et al., 2009).

### ***Microbiological Analyses***

In order to determine the microbial counts of cheese samples, the total yeast and mould (Y and M), total *Enterobacteriaceae* group bacteria (EG), and total *Staphylococcus aureus* (SA) counts were carried out using the instructions described by Roberts and Greenwood (2003). Yeast Extract Glucose. Chloramphenicol Agar (YGC, Merck, Germany), Violet Red Bile Dextrose Agar (VRBD, Merck, Germany), Baird Parker Agar (BPA, Merck, Germany) and were used for enumeration of Y and M, E, and SA respectively. After incubation, plates with 30 to 300 colonies were counted and the results were expressed as log cfu g<sup>-1</sup>.

### ***Statistical Analyses***

The results obtained from microbiological, physico-chemical, textural and sensory analysis were statistically analyzed by one way analysis of variance (ANOVA) in order to observe differences between cheese samples.

## **Results and Discussion**

### ***Physico-chemical and Textural Properties***

In this study, the physico-chemical changes occurring in fresh White Cheese and possible effects of starter culture (mesophilic/thermophilic ratio) were examined (Table 1).

There was slightly increase in fat, salt, fat and salt in dry matter content in samples TM8020. The increase in these properties may be related with activity of thermophilic culture. Cheese by using % 80 thermophilic + % 20 mesophilic culture (TM8020) had higher pH the other cheese. Kehegias et al. (1995) mentioned that the highest pH found in white cheeses produced by using thermophilic starters.

**Table 1.** Physico-chemical composition of cheese samples

Parameters	TM8020	TM6040	Level of Significance
Dry matter %	32.89 <sup>a</sup>	32.90 <sup>a</sup>	ns
Fat %	9.53 <sup>a</sup>	9.91 <sup>ab</sup>	**
Fat in dry matter%	28.97 <sup>ab</sup>	30.12 <sup>a</sup>	**
Salt %	1.71 <sup>a</sup>	1.51 <sup>ab</sup>	**
Salt in dry matter%	5.19 <sup>a</sup>	4.58 <sup>ab</sup>	**
SH	65.00 <sup>a</sup>	65.00 <sup>a</sup>	ns
pH	4.64 <sup>a</sup>	4.60 <sup>a</sup>	ns

\*\*Significant at  $P < 0.01$ ; ns not significant

**Table 2.** The whey values and cheese yields taken at different times throughout the production.

Parameters	TM8020			TM6040		
	After cheese curd cutting	Pre-press	Post-press	After clot cutting	Pre-press	Post-press
Fat %	0.19 <sup>b</sup>	0.16 <sup>a</sup>	0.07 <sup>a</sup>	0.26 <sup>a</sup>	0.10 <sup>ab</sup>	0.05 <sup>a</sup>
Protein %	0.52 <sup>a</sup>	0.65 <sup>a</sup>	0.83 <sup>a</sup>	0.49 <sup>b</sup>	0.54 <sup>ab</sup>	0.70 <sup>b</sup>
Lactose %	5.03 <sup>a</sup>	5.14 <sup>b</sup>	5.83 <sup>a</sup>	4.84 <sup>b</sup>	5.24 <sup>a</sup>	5.18 <sup>b</sup>
Dry matter %	6.33 <sup>a</sup>	6.57 <sup>a</sup>	6.52 <sup>a</sup>	6.34 <sup>a</sup>	6.41 <sup>b</sup>	6.40 <sup>b</sup>
Non-fat dry matter %	6.14 <sup>a</sup>	6.41 <sup>a</sup>	6.45 <sup>a</sup>	6.08 <sup>a</sup>	6.31 <sup>b</sup>	6.35 <sup>b</sup>
pH	6.11 <sup>a</sup>	5.85 <sup>b</sup>	5.41 <sup>b</sup>	6.14 <sup>a</sup>	6.17 <sup>a</sup>	5.76 <sup>a</sup>
Cheese yield (kg/L)	5.55 <sup>a</sup>			5.33 <sup>b</sup>		

\*\*Significant at  $P < 0.01$ ; \*Significant at  $P < 0.05$ ; ns not significant

As seen in Table 2, whey samples taken at different stages of production had the highest protein, lactose and non-fat dry matter values in TM8020 sample after cheese curd cutting.

While the value of the dry matter of whey is almost same after cutting of the clot, the dry matter value of the whey of the TM8020 is the higher pre-press and post-press of curd. Banks (2007) refers to casein and fat, which are the main constituents of the active ingredient in the cheese yield, and that these constitute 94% of the dry matter in the cheese. Similarly, the pre-press and post-press losses of the TM8020 have been the most common. It is thought that the rapid acidity of the TM8020 strain, which is called TM8020 strain, causes the proteolysis to occur faster and causes the loss of dry matter to whey. Similarly, Banks (2007) reported that casein disruption may occur during curd formation, which may affect cheese yield. Depending on the acidity of the culture used, the degree of casein loss may range from 0.7-6.6%, which is related to the cultured proteolytic activity in the degree of casein hydrolysis.

When we looked at the cheese yield, the TM6040 with the best performance came out on the sample. The difference between the two cheese yields was found to be 0.22%. For a company that processes 50 tons of feta cheese, this is equal to 110 liters of milk per day and 3300 liters of milk per month. In this sense, every figure after a conviction is a big precaution in the calculation of cheese yield.

The textural properties of samples obtained in this study has been depicted in Table 3. In this study, all textural parameters (hardness, adhesiveness, cohesiveness, springiness, gumminess, chewiness and resilience) were found significantly ( $P < 0.01$ ;  $P < 0.05$ ) different within the cheese samples depending on the differences in starter culture type and ratio. Based on the data presented, it can be confirmed that the higher values of textural properties were found for TM6040 sample than TM8020.

**Table 3.** Textural properties of cheese samples

<i>Parameters</i>	<i>TM8020</i>	<i>TM6040</i>	<i>Level of Significance</i>
<i>Hardness, (g)</i>	5204.00 <sup>b</sup>	5762.00 <sup>a</sup>	*
<i>Adhesiveness (g s<sup>-1</sup>)</i>	-27.88 <sup>a</sup>	-27.17 <sup>a</sup>	ns
<i>Gumminess (g s<sup>-1</sup>)</i>	1983.00 <sup>b</sup>	2978.00 <sup>a</sup>	**
<i>Chewiness (g mm<sup>-1</sup>)</i>	1738.00 <sup>b</sup>	2805.00 <sup>a</sup>	**
<i>Cohesiveness</i>	0.38 <sup>b</sup>	0.53 <sup>a</sup>	**
<i>Springiness (mm)</i>	0.87 <sup>b</sup>	0.94 <sup>a</sup>	**
<i>Resilience</i>	0.14 <sup>b</sup>	0.22 <sup>a</sup>	**

\*\*Significant at  $P < 0.01$ ; \*Significant at  $P < 0.05$ ; ns not significant

Proteolysis, glycolysis and lipolysis are usually regarded as the most important factors for the development of typical cheese flavor and texture. Proteins are partially hydrolyzed by rennet and other native microbial enzymes (the starter bacteria, and the non-starter microbiota) to produce lower molecular-weight compounds such as proteose, peptone, amino acids and amines (Martinez-Cuesta et al. 2001; Ozcan and Eren-Vapur 2013). Everett and Auty (2008) reported that texture of cheeses are influenced by factors that include casein-casein, casein-water, and casein-fat interactions, the state of water (either bulk, or bound to the casein matrix), pH and the state of calcium (either ionic or bound to the casein matrix), temperature, sodium chloride content, and the extent of proteolysis. Korish and Abd-Elhamid (2012) mentioned that the lowest values of hardness, springiness and chewiness in cheese, may be due to the increase in cheese moisture content. However, fat and moisture act as the filler in the casein matrix of cheese texture giving it elasticity and softness. Increasing the concentration of starter inoculated to milk also decreased cheese fracture stress and made the cheese body weaker (Madadlou et al., 2005). Awad et al. (2002) and Kaminarides et al. (2015) found that the hardness increased with decreasing the pH and increasing the salt, ash contents in cheese. Hussein and Shalaby (2014) mentioned that Kareish cheese made with thermophilic yoghurt starter had higher values of hardness, gumminess

and chewiness, but lower values of cohesiveness and springiness, as compared with cheese with probiotic starter.

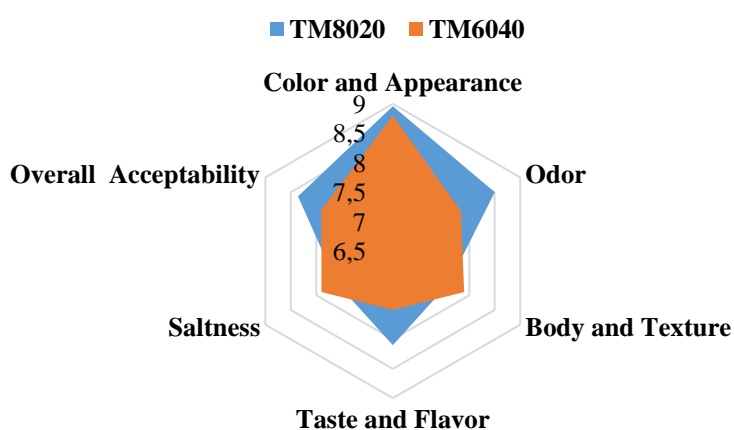
### **Sensorial properties**

Sensory evaluation has a great influence on consumer preference as it helps to improve the organoleptic attributes of a product, including appearance, flavor and texture. In addition, it can also provide the development technologist with useful information in order to achieve and control quality, at a level which is particularly acceptable to the consumers (Foegeding et al., 2003). The results of the sensory evaluation are shown in Table 4. Graphical representation of the sensory attributes evaluated through the acceptance test is presented in Figure 2. Based on the evaluation no significant differences were observed among the sensory properties of the samples. TM8020 sample received the higher scores for body and texture properties than TM6040.

**Table 4.** Sensorial properties of cheese samples

<i>Parameters</i>	<i>TM8020</i>	<i>TM6040</i>	<i>Level of Significance</i>
<i>Color and appearance</i>	8.96 <sup>a</sup>	8.80 <sup>a</sup>	ns
<i>Odor</i>	8.50 <sup>a</sup>	7.84 <sup>a</sup>	ns
<i>Body and texture</i>	8.60 <sup>a</sup>	7.90 <sup>b</sup>	*
<i>Taste and Flavor</i>	8.10 <sup>a</sup>	7.50 <sup>a</sup>	ns
<i>Saltiness</i>	7.60 <sup>a</sup>	7.90 <sup>a</sup>	ns
<i>Overall Acceptability</i>	8.36 <sup>a</sup>	7.90 <sup>a</sup>	ns

\*Significant at  $P < 0.05$ ; ns not significant



**Figure 2.** Graphical representation of the sensory analysis

### **Microbiological Properties**

The effect of type and ratio of starter culture on microbiological properties of cheeses was presented in Table 5. The maximum number of yeast count was found to be 2.59 (sample TM8020) and minimum 1.30 (sample TM6040). The number of *Enterobacter* spp. was found lower in cheese with

high ratio of thermophilic culture, while the number of yeast was high. Insignificant growth was detected for mould and *Staphylococcus aureus* both of cheese. These results are possibly a reflection cross contamination and hygienic quality of production process.

Given the white cheese production process, there is a time between clotting and brine delivery of about 3.5 to 4 hours. Lactose, which is produced by the separation of the whey after the clotting process, is also used by starter bacteria as well as by non-starter bacteria. Culture will have positive effects on the milk in the vat as long as it finds the ideal working conditions, in terms of its activation and suppression of foreign flora.

The time required to develop a generative bacterium is highly variable and depends on both nutritional possibilities and genetic factors. Under ideal nutritional conditions, the *E. coli* bacterium can complete the cycle in about 20 min. Along with being a few bacteria that can multiply faster than *E. coli*, many multiply more slowly (Madigan and Martinko, 2006). Besides, cleaning and disinfection of every material entering the cheese vat after clotting is important and personnel hygiene is also of great importance. Curd is also in contact with the whey after the cutting clot for 2.5-3 hours. During this time, the number and activity of the cheese culture bacteria, which have been added as well as the number of bacteria which can be reached by milking and also by contamination, should be considered to increase rapidly. However, what is important in this environment is that the conditions that affect the activity of culture bacteria are not deteriorated. Here, the choice of fermentation temperature is very important. Culture bacteria are said to have the effect of seconder flora during and after the production process. Many cheeses contain secondary flora and these bacteria play a major role in the maturation process of the cheese. These secondary flora are usually natural contaminants and includes propionic acid bacteria, microcapsules, *Staphylococci*, *Corynoform* bacteria and yeasts (Bocelmann, 1999; Beresford, 2001; Frohlich et al., 2002; Sheehan, 2007; Beresford, 2007).

Based on this general description, an assessment based on the current cheese process will reveal that the culture microorganism in the web between the clot and the casein micel will start to increase lactase activity by separating the lactose after cutting the clot. The time required for the settlement of the curd after the cutting clot and the preparation phase of the press is approximately 30 minutes.

**Table 5.** Microbiological properties of cheese samples

<i>Parameters</i>	<i>TM8020</i>	<i>TM6040</i>	<i>Level of Significance</i>
<i>Yeast</i>	2.59 <sup>a</sup>	1.30 <sup>b</sup>	**
<i>Mould</i>	<1	<1	ns
<i>Enterobacteriaceae group</i>	1.48 <sup>b</sup>	2.18 <sup>a</sup>	**
<i>Staphylococcus aureus</i>	<1	<1	ns

\*\*Significant at P<0.01; ns not significant



## Conclusion

Considering physico-chemical, microbiological, textural and sensory evaluation results, it could be concluded that type and ratio of starter culture bacteria should be investigated in the production of white cheese to improve consumer acceptability and obtain food safety. During the process, important steps have been described for the process to ensure that non-starter bacteria in milk, clot, curd.

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