

ORIGINAL ARTICLE

Using optimization method for determining lactic acid bacteria counts in white cheese with different salt concentrations

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Abstract

Total lactic acid bacteria, *Lactococcus* spp., yeast, and mold counts in white cheese were determined during 55 days of storage in brine solutions with 4%, 6%, 8%, 10%, 12%, 14%, and 16% (w/v) salt concentrations at 4°C. In addition to salt content, dry matter, acidity, protein, fat contents, and sensory evaluations of cheese were also determined. *Lactococcus* spp. numbers in cheese salted with 4% and 14% brines (w/v salt concentrations) were 9.32 and 8.48 log cfu/g at the 55 days of storage, respectively. The lowest total lactic acid number was 7.24 for cheese in brine with 16% (w/v) salt concentration after 55 days of storage. Yeast numbers were 7.01 and 6.71 log cfu/g for cheese in brines with 4% and 16% (w/v) salt concentrations. Cheese samples salted in 12% and 14% (w/v) concentrations of brines have the highest sensory scores. Analysis results were interpreted mathematically by “fuzzy soft set” modeling.

Novelty impact statement: This research is a novel study detailing mathematical modeling related to optimizing the salt concentration to preserve the viability of lactic acid starter culture bacteria in the white cheese. Also, it covers the first optimizing method for process control in the cheese industry with the application of “fuzzy soft set theory.”

1 | INTRODUCTION

According to the Turkey Statistical Institute, “white cheese” includes 60% of the total cheese production in Turkey. Establishing a production standardization of white cheese is very important because of its economic value and the highest production capacity of white cheese (Cakmakci, 2011).

During the production of white cheese, the starter culture is added to the cheese because of its technological benefits. Protection vitality of lactic acid bacteria, textural, and sensory properties of cheese are the most important factors for high-quality cheese production. The factors that sustain the viability of microorganisms are salt concentration in brine solution, cheese and brines acidity, and cheese dry matter (Salih & Abdalla, 2020).

Lactic acid bacteria may influence the quality and variety of dairy products. Among these lactic acid bacteria, *Lactobacillus*, *Bifidobacterium*, *Lactococcus*, *Carnobacterium*, *Enterococcus*,

Streptococcus, *Leuconostoc*, *Pediococcus*, *Propionibacterium*, and *Bacillus* spp. are the most common species. It was also explained that these lactic acid bacteria contribute to ripening changes in white cheese by their acidifying, proteolytic, lipolytic, and inhibitory activities (Kalkan, 2020).

Some thermophilic and mesophilic starter culture combinations, including *Lactococcus lactis* ssp. *lactis*, *Lc. lactis* ssp. *cremoris*, *Lc. lactis* ssp. *lactis* biovar. *diacetylactis*, *Str. thermophilus*, *Lb. sake*, *Lb. casei*, *Lb. plantarum*, *Lb. helveticus*, have been used in white cheese production until nowadays. However, low salt resistance of these culture microorganisms makes it necessary to find alternatives in production (Irkin, 2017).

The salting process has some effects on cheese microflora, pH, water contents, and microbial enzymes. Because of the salt addition effects on starter and nonstarter microorganisms, the acid production in cheese and the ripening period can be affected. Salt decreases the water content of cheese and is related to the salt

content; microflora, pH, and microorganism's enzymes are affected (Pachlova et al., 2019). It was explained that the salt resistance of bacteria can vary according to the species and subspecies. Lactic acid bacteria numbers decreased rapidly in high salt concentrations and reported the inhibitory effects on these bacteria (Koçak et al., 2011). In cheese production, dry or brine salting is preferred to control the proliferation of spoilage and undesired microflora but will obviously have an impact on the viability of starter bacteria as well (Hickey et al., 2018). In addition, some unwanted yeast varieties, such as *Candida* spp., *Kluyveromyces lactis*, *Pichia amethionina* biovar. *amethionina*, and *Debaryomyces hansenii*, can grow in brine solutions and caused blowing, putrefaction, and bitter aroma in cheese (Geronikou et al., 2020).

Cheese starter culture of *Lactococcus* spp. can increase rapidly in low NaCl concentration-contained brine solutions, but higher than the 5% (w/v) NaCl concentration shows inhibitory effects on the bacteria. Sometimes subspecies of bacteria can be variously affected by the salt concentrations. For example, *Lc. lactis* subsp. *lactis* can grow at 4% (w/v) NaCl concentration effectively and *Lc. lactis* subsp. *cremoris* can grow at 2% (w/v) but cannot grow at 4% (w/v) concentration (Gursoy & Kesencas, 2011).

Today, predictive mathematical modeling studies have become widespread to optimize the inhibition of pathogens and parameters in order to determine the shelf life of food in scientific studies (Azevedo et al., 2014; Kodogiannis & Alshejari, 2014; Rojo et al., 2014).

Gomes da Cruz et al. (2009) studied the viability of probiotic bacteria in cheese and reported that probiotic bacteria are very sensitive to salt conditions in cheese.

Zadeh (1965) was first introduced the concept of a fuzzy set. Then, Molodtsov (1999) defined the concept of a soft set, and Maji et al. (2001) gave the notion of a fuzzy soft set. On the other hand, there exist some applications of the notions of "soft set" and "fuzzy soft set." For example, Yüksel et al. (2013) gave an application of soft sets to diagnose the prostate cancer risk. Kalachelvi and Malini (2011) studied a new application of fuzzy soft sets to investment decision-making problems. Özgür and Taş (2015) presented a new approach to investment decision-making problems by means of the notions of the period, soft set, and matrix form.

Accordingly, the aims of this research were to produce and store white cheese in brine solutions with different salt concentrations, and in all the storage days, the changes of total lactic acid bacteria, *Lactococcus* spp., yeast, and mold counts in white cheese were determined and a mathematical modeling study was conducted according to the results. From the obtained data, it was applied a statistical method, first. Results were evaluated statistically. Mathematical modeling was constructed using the "fuzzy soft set theory." The advantage of this modeling is the optimization of the results with adequate parameters and degrees of membership functions, was determined the optimum concentration by giving the appropriate decision-making function. Also, dry matter, acidity, fat, protein contents, and sensory evaluations of cheese were determined.

2 | MATERIALS AND METHODS

2.1 | White cheese production

Whole raw cow's milk analysis (fat %, dry matter %, protein %, pH, acidity %, and total aerobic bacteria) was determined, and milk was pasteurized at 75°C/15 s and then cooled to 32°C. Starter culture (Clerici Sacco, MOS 062D, Cadorago, Italy) (*Lactococcus lactis*–*Lactococcus cremoris*–*Streptococcus thermophiles*) was activated at 32°C/5–6 hr in sterilized milk. The activated culture was added to the cheese milk about 2% (w/v). After half an hour, calcium chloride as an additive for cheese texture (Tito, FIN) was added to the milk (20 g CaCl₂/100 L milk) and waited about 15 min. Calf rennet (REN-NA are chymosin and bovine pepsin, TR) was added (23 ml/100 L milk) to the cheese milk for providing a 90-min coagulation period. After the coagulation of cheese curd, it was cut into about 1 cm³ in size. Collected cheese whey at the surface of the curd was separated and then cheese curd was pressed under the pressure in a cheesecloth for 3 hr (Ucuncu, 2004).

The cheese was cut into about 4 × 4 × 4 cm³ size and grouped according to the rock salt (NaCl) concentration in brine solutions. Each group of cheese has waited in different concentrations (4, 6, 8, 10, 12, 14, and 16 w/v) of sterilized (90°C/15 min) and cooled (25°C) brine solutions for 8 hr. After that cheese was collected from the brine solutions and put into the glass jars and completed with 8% (w/v) salt concentration brine solutions, cheese samples were stored at 4°C during 55 days.

Total lactic acid bacteria, *Lactococcus* spp., yeast, and mold counts were determined on 1, 2, 3, 5, 8, 13, 21, 34, and 55 days (the Fibonacci sequence was preferred). Also, sensory analysis and acidity changes were reported these days.

2.2 | Physicochemical analysis

Fat %, dry matter %, salt %, and acidity % contents were determined according to Cesur (2014). Protein % contents were determined by using Velp NDA 701, Nitrogen Dumas Analyzer. The pH of cheese was measured using a pH meter (Hanna HI221 Microprocessor, Hanna Instruments Inc., Woonsocket, Rhode Island).

2.3 | Microbial analysis

Samples were diluted in (1:10) in buffered peptone water (BPW) and homogenized for 20 s in a stomacher (Bag mixer, Interscience, FR). Then, a decimal dilution series was made in BPW, and enumeration was performed by pour plate or spread plating techniques.

Counts of total lactic acid bacteria were determined on MRS (deMann, Rogosa and Sharpe Agar, pH 5.2) agar at 30°C for 3 days (Whitley et al., 2000).

Lactococcus spp. in cheese samples were enumerated on M17 agar with added 5% (v/v) sterilized lactose solution (10% w/v) at 37°C for 48 hr (Cesur, 2014).

Total yeast counts were enumerated on Yeast Extract Glucose Chloramphenicol Agar at 25°C for 3 days (Gonzales-Fandos et al., 2000).

The number of colony-forming units (cfu) on plates was calculated per gram of sample and converted to log₁₀ unit.

2.4 | Statistical analyses

Results were converted to logarithms and three replicated trials were applied for each duplicate experiment. Data were subjected to analysis of variance (ANOVA) and Duncan's multiple tests as well as general multivariate analysis tests SPSS 16.0 (SPSS Inc., Chicago, IL, USA) to determine if there were significant differences.

2.5 | Mathematical modeling

The results were optimized using the notion of a "fuzzy soft set." First, we give some definitions from the fuzzy soft set theory.

Let X be a universal set, E be a set of parameters, and $A \subseteq E$. Let $F(X)$ denotes the set of all fuzzy subsets of X . Then a pair (F, A) is called a fuzzy soft set over X , where F is a mapping from A to $F(X)$ (Maji et al., 2001).

It can be introduced by the following algorithm for the optimization of the viability of lactic acid bacteria in "Turkish White Cheese" under the storage of different salt concentrations:

- Step 1. Define a universal set X and a parameter set E .
- Step 2. Construct the fuzzy soft sets (F_i, E) , $i \in \{1, 2, 3, 5, 8, 13, 21, 34, 55\}$, according to the results.
- Step 3. Give table presentations of the fuzzy soft sets (F_i, E) , $i \in \{1, 2, 3, 5, 8, 13, 21, 34, 55\}$.
- Step 4. Construct the total table of beneficial bacteria according to days and parameters.
- Step 5. Draw the graphic of the total table using MATLAB (Curve Fitting Toolbox 2015).
- Step 6. Define the decision-making function.
- Step 7. Construct the comparison table using the decision-making function.
- Step 8. The highest score is the result of our decision-making problem.
- Step 9. Draw the decision-making graphic using the distance function.

2.6 | Sensory analysis

The sensory characteristics of cheese samples were carried out on each day of sampling. A panel composed of five experienced trained members from Balikesir University was used to evaluate the cheese groups according to some flavor and texture characteristics of cheese samples and overall impression with a point scale-ranking

score test from 0 to 5 (0 spoiled sample and unfit for human consumption; 5, very good). Cheese samples were compared with each other groups (IDF, 1995).

3 | RESULTS AND DISCUSSION

Some physicochemical analysis results of milk and cheese samples are given in Table 1. Proximate analysis of raw milk gave average dry matter $10.8 \pm 0.7\%$ (w/v), protein $3.8 \pm 0.6\%$, fat $3.8 \pm 0.7\%$ (w/v), pH 6.49, acidity 0.168% lactic acid, and total aerobic bacteria count was 40,000 cfu/ml at 30°C for three batches, respectively.

In relation to the physicochemical characteristics, it was observed that the values for pH and acidity (%) levels in the different salt concentrated cheese remained between 5.11%–5.70% and 0.18–0.432% (l.a.), respectively, during the 55 days of storage at 4°C (Table 1). The decline of the pH resulted in all cheese samples with 4%, 6%, 8%, 10%, 12%, 14%, and 16% (w/v) brine conditions.

The study of Jesus et al. (2016) showed that a decrease in salt concentration causes accelerates the metabolism of both starter culture and lactic acid bacteria flora. As a result, organic acids such as lactic and acetic acids are produced, which leads to a fall in the pH of the cheese. It can be said that using starter culture causes a rapid decrease in the pH and an increase in acidity. However, the reduction in pH is an inhibitory factor for the growth and survival of other microorganisms' groups.

Salt treatment had some effects on pH, acidity % (l. a.), salt % (w/w), dry matter % (w/w), fat % (w/w), and protein % (w/w) contents of cheese. This result was expected since these parameters depend mainly on milk characteristics and the processing technology, which was the same for all cheese. Dry matter contents % (w/w) of cheese samples were found between 37.81% and 46.74% (w/w) during the storage. Dry matter contents % (w/w) were found higher in cheese which was salted in 16% (w/v) concentrated brines than the 4% (w/v). Salt % (w/w) contents of cheese samples decreased during the storage and the contents were min. 4.68% (w/w) and max. 10.06% (w/w) for cheese salted in 4% (w/v) and 16% (w/v) concentration brines, respectively. Fat % (w/w) and protein % (w/w) contents of cheese samples varied between 17.3%–18.8% (w/w) and 14.06%–15.60% (w/w) during the storage, respectively. Some research results about the cheese physicochemical properties can be seen in the below paragraphs.

Total dry matter % (w/w), protein % (w/w), and acidity % (l. a.) values were found as 40%–43%, 14%–16.2%, and 1.5%–2.7%, respectively, in Turkish white cheese ripened in 12% brine solution after being waited in 16% brine (12 hr) (Yaman et al., 2022). It was determined that the total dry matter contents changes between 37.81% and 46.74% were higher, protein contents of 14.11% and 15.60% were similar, and acidity of 0.18% and 0.432% l.a. were lower in this study. Oner et al. (2006) determined higher results with total dry matter (39.42%–51.42% w/w) and lower pH values (4.96–4.88) during 105 days ripening period of artisanal Turkish white cheese samples which were salted with 14%

TABLE 1 Some physicochemical analysis average results of cheese during storage of 55 days at 4°C

	1	2	3	5	8	13	21	34	55
pH	4	5.64 ± 0.7	5.62 ± 0.9	5.60 ± 0.5	5.58 ± 0.2	5.37 ± 0.8	5.35 ± 0.2	5.26 ± 0.8	5.11 ± 0.6
	6	5.68 ± 0.3	5.66 ± 0.6	5.63 ± 0.7	5.60 ± 0.7	5.39 ± 0.6	5.35 ± 0.3	5.28 ± 0.9	5.13 ± 0.5
	8	5.69 ± 0.9	5.66 ± 0.5	5.66 ± 0.8	5.64 ± 0.9	5.40 ± 0.5	5.36 ± 0.5	5.32 ± 0.6	5.17 ± 0.8
	10	5.68 ± 0.4	5.67 ± 0.6	5.65 ± 0.9	5.64 ± 0.8	5.41 ± 0.6	5.38 ± 0.6	5.36 ± 0.4	5.20 ± 0.5
	12	5.68 ± 0.6	5.67 ± 0.8	5.65 ± 0.8	5.65 ± 0.2	5.42 ± 0.8	5.40 ± 0.6	5.38 ± 0.3	5.25 ± 0.8
	14	5.65 ± 0.8	5.67 ± 0.3	5.65 ± 0.6	5.66 ± 0.4	5.44 ± 0.9	5.43 ± 0.5	5.40 ± 0.6	5.29 ± 0.4
	16	5.70 ± 0.3	5.68 ± 0.4	5.67 ± 0.3	5.66 ± 0.6	5.50 ± 0.4	5.45 ± 0.7	5.42 ± 0.9	5.32 ± 0.5
Acidity (l.a.) %	4	0.324 ± 0.6	0.324 ± 0.2	0.360 ± 0.7	0.360 ± 0.6	0.360 ± 0.2	0.396 ± 0.3	0.396 ± 0.8	0.432 ± 0.5
	6	0.252 ± 0.3	0.252 ± 0.4	0.252 ± 0.6	0.288 ± 0.4	0.288 ± 0.4	0.288 ± 0.5	0.324 ± 0.4	0.360 ± 0.3
	8	0.252 ± 0.9	0.252 ± 0.5	0.252 ± 0.4	0.288 ± 0.3	0.288 ± 0.8	0.288 ± 0.8	0.288 ± 0.5	0.360 ± 0.6
	10	0.252 ± 0.3	0.252 ± 0.9	0.252 ± 0.8	0.288 ± 0.2	0.288 ± 0.9	0.288 ± 0.9	0.288 ± 0.9	0.324 ± 0.2
	12	0.216 ± 0.5	0.216 ± 0.3	0.216 ± 0.3	0.252 ± 0.5	0.252 ± 0.4	0.252 ± 0.6	0.288 ± 0.2	0.324 ± 0.7
	14	0.216 ± 0.5	0.216 ± 0.6	0.216 ± 0.6	0.252 ± 0.9	0.252 ± 0.6	0.252 ± 0.3	0.252 ± 0.4	0.288 ± 0.4
	16	0.18 ± 0.6	0.18 ± 0.3	0.18 ± 0.7	0.18 ± 0.5	0.180 ± 0.8	0.216 ± 0.4	0.216 ± 0.9	0.288 ± 0.5
Dry matter content % (w/w)	4	38.21 ± 0.8	38.29 ± 0.6	38.61 ± 0.5	38.67 ± 0.3	37.81 ± 0.4	38.22 ± 0.5	38.80 ± 0.4	39.45 ± 0.2
	6	39.42 ± 0.7	38.40 ± 0.8	39.22 ± 0.4	39.48 ± 0.7	38.82 ± 0.2	38.40 ± 0.3	39.40 ± 0.2	39.80 ± 0.7
	8	39.26 ± 0.8	39.87 ± 0.4	40.31 ± 0.6	40.72 ± 0.5	40.63 ± 0.8	40.23 ± 0.8	41.22 ± 0.7	40.88 ± 0.3
	10	42.83 ± 0.6	42.91 ± 0.5	43.08 ± 0.7	43.21 ± 0.8	43.50 ± 0.6	43.29 ± 0.4	43.38 ± 0.5	43.56 ± 0.8
	12	44.10 ± 0.6	43.18 ± 0.4	43.52 ± 0.2	44.25 ± 0.8	45.23 ± 0.5	45.19 ± 0.7	45.33 ± 0.8	44.87 ± 0.7
	14	44.22 ± 0.5	44.57 ± 0.8	45.22 ± 0.9	46.30 ± 0.2	45.29 ± 0.3	45.34 ± 0.6	44.86 ± 0.5	44.26 ± 0.5
	16	45.23 ± 0.5	46.48 ± 0.9	45.29 ± 0.5	45.31 ± 0.3	46.34 ± 0.9	46.74 ± 0.5	45.19 ± 0.6	45.87 ± 0.1
Salt % (w/w)	4	6.43 ± 0.7	7.95 ± 0.7	7.95 ± 0.7	6.01 ± 0.7	5.61 ± 0.7	5.38 ± 0.7	5.38 ± 0.7	4.68 ± 0.7
	6	9.12 ± 0.7	8.65 ± 0.7	8.65 ± 0.7	8.42 ± 0.7	6.01 ± 0.7	5.61 ± 0.7	5.61 ± 0.7	5.38 ± 0.7
	8	9.36 ± 0.7	9.12 ± 0.7	9.12 ± 0.7	7.95 ± 0.7	6.55 ± 0.7	6.38 ± 0.7	6.01 ± 0.7	5.61 ± 0.7
	10	9.59 ± 0.7	9.36 ± 0.7	9.36 ± 0.7	9.36 ± 0.7	9.12 ± 0.7	8.65 ± 0.7	8.42 ± 0.7	8.42 ± 0.7
	12	9.82 ± 0.7	9.59 ± 0.7	9.59 ± 0.7	9.36 ± 0.7	9.36 ± 0.7	9.12 ± 0.7	9.12 ± 0.7	8.65 ± 0.7
	14	9.82 ± 0.7	9.82 ± 0.7	9.82 ± 0.7	9.59 ± 0.7	9.59 ± 0.7	9.59 ± 0.7	9.59 ± 0.7	9.36 ± 0.7
	16	10.06 ± 0.7	10.06 ± 0.7	10.06 ± 0.7	9.82 ± 0.7	9.82 ± 0.7	9.82 ± 0.7	9.59 ± 0.7	9.59 ± 0.7

TABLE 1 (Continued)

	1	2	3	5	8	13	21	34	55	
Fat % (w/w)	4	17.4 ± 0.5	17.6 ± 0.7	17.3 ± 0.3	17.7 ± 0.2	17.8 ± 0.3	17.7 ± 0.7	17.7 ± 0.4	17.8 ± 0.4	17.8 ± 0.1
	6	17.8 ± 0.2	17.8 ± 0.8	17.9 ± 0.5	17.9 ± 0.7	18.1 ± 0.4	18.3 ± 0.6	18.2 ± 0.6	18.5 ± 0.3	18.4 ± 0.3
	8	17.9 ± 0.7	17.6 ± 0.3	17.7 ± 0.9	17.7 ± 0.8	17.8 ± 0.5	18.1 ± 0.7	18.3 ± 0.7	18.4 ± 0.4	18.6 ± 0.4
	10	18.1 ± 0.8	18.2 ± 0.4	18.2 ± 0.4	18.4 ± 0.9	18.4 ± 0.5	18.5 ± 0.4	18.7 ± 0.8	18.6 ± 0.7	18.7 ± 0.4
	12	18.3 ± 0.5	18.3 ± 0.6	18.5 ± 0.3	18.5 ± 0.3	18.6 ± 0.6	18.6 ± 0.9	18.7 ± 0.6	18.7 ± 0.4	18.8 ± 0.5
	14	18.2 ± 0.9	18.5 ± 0.7	18.6 ± 0.6	18.7 ± 0.4	18.6 ± 0.3	18.7 ± 0.8	18.8 ± 0.3	18.5 ± 0.8	18.7 ± 0.6
	16	18.4 ± 0.2	18.7 ± 0.1	18.6 ± 0.9	18.6 ± 0.4	18.7 ± 0.2	18.7 ± 0.3	18.8 ± 0.5	18.7 ± 0.4	18.8 ± 0.2
Protein % (w/w)	4	14.30 ± 0.5	14.21 ± 0.5	14.18 ± 0.6	14.12 ± 0.3	14.43 ± 0.4	14.38 ± 0.8	14.60 ± 0.7	14.27 ± 0.2	14.21 ± 0.3
	6	14.63 ± 0.6	14.34 ± 0.5	14.38 ± 0.7	14.76 ± 0.5	14.63 ± 0.6	14.55 ± 0.6	14.22 ± 0.1	14.20 ± 0.5	14.17 ± 0.3
	8	14.67 ± 0.7	14.32 ± 0.8	14.28 ± 0.8	14.36 ± 0.2	14.27 ± 0.7	14.32 ± 0.4	14.30 ± 0.6	14.11 ± 0.4	14.06 ± 0.1
	10	14.75 ± 0.4	14.66 ± 0.9	14.31 ± 0.7	14.27 ± 0.2	14.30 ± 0.8	14.25 ± 0.7	14.20 ± 0.6	14.18 ± 0.6	14.22 ± 0.6
	12	15.08 ± 0.3	14.89 ± 0.3	14.80 ± 0.7	14.67 ± 0.9	14.57 ± 0.8	14.55 ± 0.6	14.40 ± 0.4	14.32 ± 0.7	14.26 ± 0.4
	14	15.17 ± 0.3	15.12 ± 0.7	14.84 ± 0.4	14.76 ± 0.4	14.68 ± 0.2	14.77 ± 0.7	14.63 ± 0.2	14.59 ± 0.8	14.62 ± 0.9
	16	15.60 ± 0.6	15.47 ± 0.4	15.40 ± 0.3	15.18 ± 0.7	15.22 ± 0.1	15.12 ± 0.9	15.09 ± 0.3	15.22 ± 0.2	15.10 ± 0.5

(w/v) brine solution in their research. Cuffia et al. (2015) produced Argentinean sheep's milk cheese using brine with the following concentrations: 20%, 15%, 10%, 5% (w/v) and they found higher dry matter contents of 55% and 58.5% (w/w) and protein amounts of 39.8% and 37.1% (w/w) in their research. In Kamleh et al. (2015) study, they produced Akkawi cheese with 10%–12% (w/w) brine solution and they found lower (38.97%–42.03%, w/w) dry matter contents, higher amounts of fat (19.5%–25.18%, w/w), similar amounts (15.74%–14.75%, w/w) of protein, and higher (6.49–5.85) pH values after 2 and 8 week periods. Balabanova et al. (2017) researched the same kind of white cheese from Bulgarian and shaped curd was put into a solution containing 22% NaCl (w/v) for 12–15 hr. After the prebrining period, the cheese was packaged in plastic cups (1 kg) containing brine 8% NaCl (w/v), and the results of the study it was found similar levels of dry matter and protein contents; 40%–46% (w/w), 14.3%–15.3% (w/w), lower pH values (4.18–4.75), higher fat amounts (21%–23%) in cheese.

3.1 | Microbial analysis results

Figure 1a shows the growth of *Lactococcus* spp. in storage. As can be seen from the figure at the beginning of the storage, maximum bacteria numbers were found at 8.60 log cfu/g and the minimum was 7.46 log cfu/g for cheese in brines with 4% and 14% (w/v) salt concentrations then 9.44 and 8.10 log cfu/g at the 55th day of storage, respectively. The highest numbers were found as 10.63 and 9.77 log cfu/g on 13 days for cheese salted in 12% (w/v) salt concentration. However, the lowest numbers were obtained as 7.46 log cfu/g on the first day for cheese salted in 14% (w/v) salt concentration.

According to the first-day results, it evaluated the numbers of *Lactococcus* spp. for different salted brine solutions in white cheese groups statistically then it was observed that there is no significant ($p > .05$) differences between the cheese groups with 4% and 6% (w/v) salted concentrations. On the last storage day, it was determined that the highest number of *Lactococcus* spp. (9.32 log cfu/g) was in the cheese group salted with brine 4% (w/v) while after the 13th storage day, it was found that the numbers of *Lactococcus* spp. increased in the cheese groups salted with brine 12%, 14%, and 16% (w/v).

Some research considers lactococci to be the group of LAB that plays a leading role in the acidification of milk at the beginning of cheese production (Luiz et al., 2017; Vidojevica et al., 2020). The ripening process of feta cheese seems to favor the growth of the *Lactococcus* group, which takes place in starter culture used for the production of this type of cheese (Spyrelli et al., 2020). Oner et al. (2006) determined *Lactococci* counts: 8.14–6.47 log cfu/g, *Lactobacillus* counts: 7.90–6.40 log cfu/g, and yeast-molds counts: 5.37–3.74 log cfu/g during the ripening period of artisanal Turkish white cheese samples in 14% (w/v) brine solution. In our study, lactic acid bacteria numbers were higher and about 10 cfu/g for 34 days of ripening in 14% (w/v) brine solution.

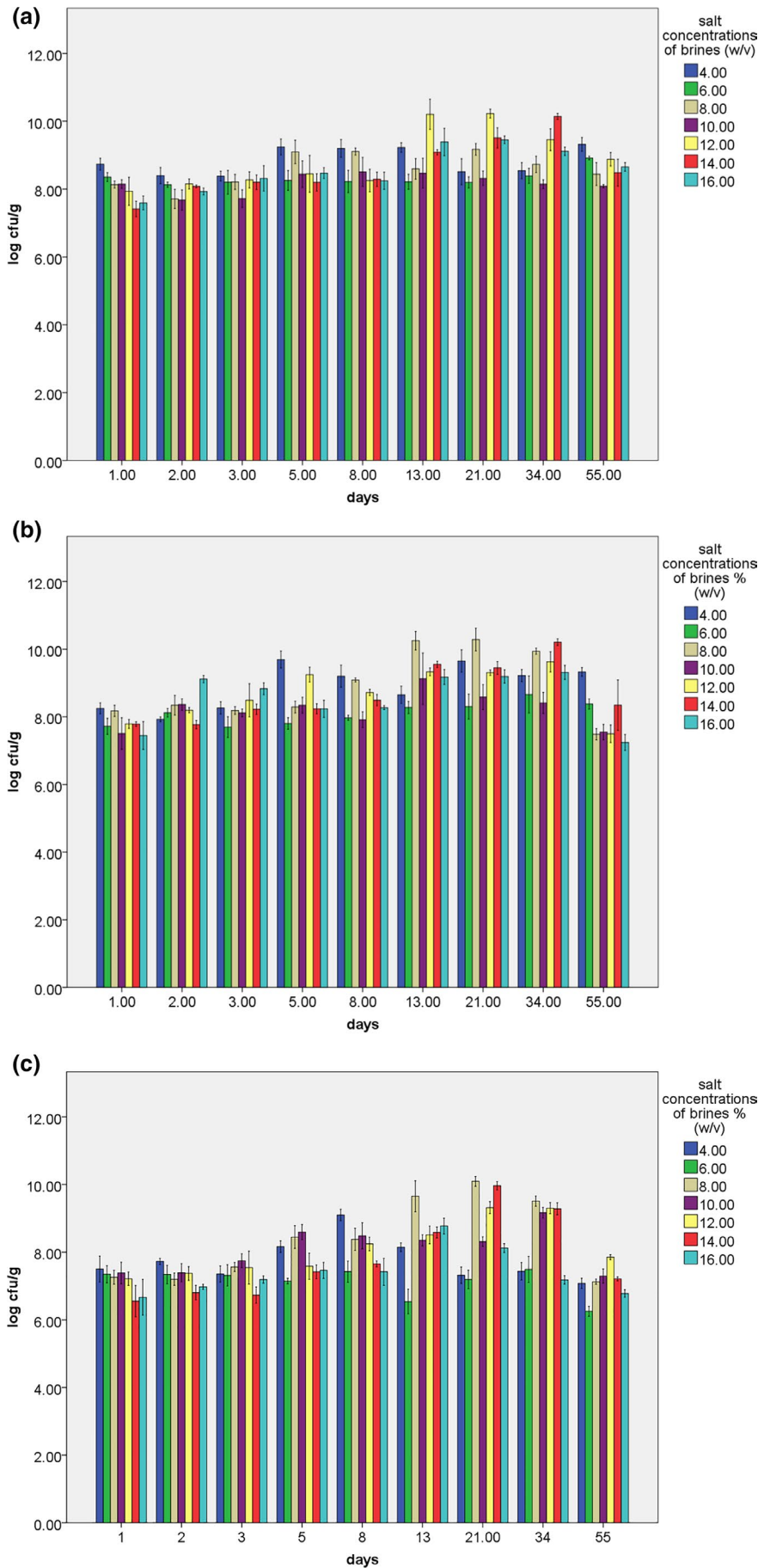


FIGURE 1 Growth rates of *Lactococcus* spp. cfu/g (A), total lactic acid bacteria log cfu/g (B), yeast-mold numbers log cfu/g (C) in white cheese samples during the storage period (55 days at 4°C) in brines with different salt concentrations (w/v). Error bars represent the standard deviation (SD) of means (n = 3)

Taracki and Tunctürk (2008) reported that using combined cultures of *Lc. lactis*, *Lc. cremoris*, *Str. thermophilus*, and *Lb. bulgaricus* in cheese with 14% salt (w/v) concentration brine showed additional flavor effects in cheese and also accelerated ripening time after 90 days of storage.

Total lactic acid bacteria numbers (Figure 1b) were 8.40 and 7.62 log cfu/g for cheese in brines with 4% and 16% (w/v) salt concentrations on the first day. The lowest number was 7.14 for cheese in brines with 16% (w/v) salt concentrations on 55 days of storage, and the highest numbers were 10.31 log cfu/g for cheese in brines with 8% (w/v) salt concentrations on the 13th and 21st days of storage.

It investigated the numbers of lactic acid bacteria in white cheese groups and that the lowest count of bacteria (7.24 log cfu/g) was in the brine with salted 16% (w/v) and the highest count of bacteria (9.32 log cfu/g) was in the brine with salted 4% (w/v) at the last storage day. In some cheese groups, it was observed that the numbers of lactic acid bacteria on storage days 13, 21, and 34 were higher than other days.

Lactic acid bacteria can contribute to the flavor and texture of the cheese besides being health promoters, with probiotic features (Luiz et al., 2017). Souza and Saad (2009) researched the viability of *Lactobacillus acidophilus* and *Streptococcus thermophilus* during storage of fresh Minas cheese, they determined *Lb. acidophilus* numbers were >6 log cfu/g but acidity and proteolysis values were higher in *Str. thermophilus* containing cheese.

Ahmed et al. (2021) reported lower numbers of about 6.6 cfu/g in low-fat feta cheese in 6%–8% brine solution during 14 days of storage compared with our study.

Rhoades et al. (2017) found about 8 cfu/g of total lactic acid bacteria and 5–6 cfu/g yeast and molds in Greek Type Feta brined cheese and in our study, lactic acid bacteria and mold yeast numbers were higher than these results.

Similarly, Moghanjougia et al. (2020) found a reason for the increase observed in the number of bacteria during storage could be the reduction of salt in brine in white-brined feta cheese, and they stated that the first population of probiotics bacteria was exposed to salt shock. The shock gradually decreased as a result of salt penetration into the cheese.

Soltani et al. (2015) found that higher salt concentration caused a decrease in the number of lactic acid bacteria in the Iranian white cheese samples produced. Similarly, in the ripening of traditional Iranian cheese regarding the lactic acid bacteria count, all samples showed a similar growth rate. The lactic acid bacteria loads of traditional Iranian cheese were about 10.01 log cfu/g (Abdolsattari et al., 2020).

Asteri et al. (2010) used *Lb. delbrueckii* subsp. *bulgaricus*, *Str. thermophilus*, *Lb. paracasei*, *Enterococcus faecalis* cultures for producing soft goat's cheese. The results of biochemical reactions of microorganisms such as proteolytic and acid-producing activities of *Lb. delbrueckii*, the acid-producing activity of *Str. thermophilus*, and lipolytic activities of *E. faecalis* in Feta cheese took high sensory scores in their study. In production, Feta cheese was dry-salted with 1% salt (w/w) and viabilities of microorganisms decreased at minimum levels

(from 9.26 to 8.96 log cfu/g for thermophilic *lactobacillus*, from 9.26 to 9.03 log cfu/g for mesophilic *lactobacillus*) for 30 days storage.

It is known that the quality and acceptability of food products can be affected by the spoilage and fermentation activity of yeast and molds. In our research, molds were not determined in the cheese samples during the storage. But yeasts were observed in cheese, and in Figure 1c, it can be seen that yeast counts were 7.77, 6.98, and 6.93 log cfu/g for cheese in brines with 4%, 16%, and 14% (w/v) salt concentrations on the first day. After 55 days, numbers were 7.01 and 6.71 log cfu/g for cheese in brines with 4% and 16% (w/v) salt concentrations. The minimum numbers were 6.39 log cfu/g on the 55th day of storage for cheese in brines 6% (w/v) and 7.17 log cfu/g for 14% (w/v).

It was evaluated the counts of yeasts which are unwanted groups of microorganisms and it was found that yeasts were significantly ($p < .05$) higher (7.88 log cfu/g) in the brine with salted 12% (w/v) than the other cheese groups at the last storage day in this study. Also, there was no observed correlation between the salt concentrations and the yeasts counts. Similarly, in research, the reduction of NaCl concentration of brine from 6% to 3% did not cause any microbial spoilage effects in goat milk cheese (Miloradovic et al., 2018).

3.2 | Sensory analysis results

The odor of all the cheese samples was the same during the storage (Figure 2). The highest taste scores were the cheese samples which were salted at 4%, 6%, and 8% (w/v) salt concentrations of brines than the other samples. Nevertheless, the best texture scores were directly proportional to high salt-containing cheese samples. In general, the overall impression of cheese samples, which were salted in 12% and 14% (w/v) concentration of brines, have the highest scores. In other research, it was emphasized that high salt concentrations can be decreased the degree of proteolysis and prevents the bitter taste of the cheese. Cuffia et al. (2015) found that cheese salted

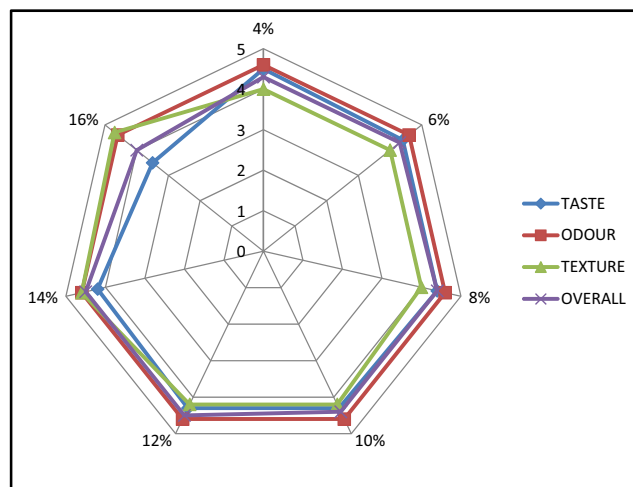


FIGURE 2 Sensory profile of cheese samples during the storage of 55 days

Day	$e_1 = 4\%$	$e_2 = 6\%$	$e_3 = 8\%$	$e_4 = 10\%$	$e_5 = 12\%$	$e_6 = 14\%$	$e_7 = 16\%$
1	1.70065	1.634	1.6236	1.5815	1.591	1.531	1.54265
2	1.6341	1.6085	1.6625	1.648	1.653	1.576	1.706
3	1.694	1.6225	1.614	1.608	1.743	1.618	1.7275
5	1.8205	1.639	1.669	1.616	1.741	1.6705	1.624
8	1.899	1.658	1.835	1.687	1.7615	1.689	1.679
13	1.814	1.684	1.8476	1.698	1.944	1.871	1.916
21	1.8245	1.651	1.888	1.647	1.984	1.9215	1.9195
34	1.811	1.76	1.881	1.668	1.943	2	1.848
55	1.887	1.747	1.6346	1.558	1.68	1.7604	1.591

TABLE 2 Total table of degrees of membership functions

with the concentrated brine 20% showed low moisture levels, and according to the sensory analysis results, the bitter taste was significantly higher than the less-salted Argentinean sheep cheese. Soltani et al. (2015) found that increasing salt concentrations caused a decrease in proteolysis, and cheese with 1% and 2.5% (w/w) salt was acceptable in odor and flavor.

3.3 | Optimization of the results

It was established fuzzy soft modeling to optimize the results. A universal set and a parameter set were defined as follows, respectively:

$$X = \{x_1 = \text{Lactococcus numbers}, x_2 = \text{Lactic acid bacteria numbers}, x_3 = \text{yeast - mold numbers}\}$$

and

$$E = \{e_1 = 4\%, e_2 = 6\%, e_3 = 8\%, e_4 = 10\%, e_5 = 12\%, e_6 = 14\%, e_7 = 16\%\}.$$

The fuzzy soft sets (F_i, E) , $i \in \{1, 2, 3, 5, 8, 13, 21, 34, 55\}$, were constructed by considering the results given in Figure 1a-c:

$$(F_i, E) = \left\{ \left(e_j \frac{x_k}{\mu_i(x_k)} \right) : i \in \{1, 2, 3, 5, 8, 13, 21, 34, 55\}, \right. \\ \left. j \in \{1, 2, 3, 4, 5, 6, 7\} \text{ and } k \in \{1, 2, 3\} \right\},$$

where $\mu_i(x_k)$ are the degrees of membership functions.

The fuzzy soft sets were defined according to days. The first fuzzy soft set is defined as follows:

$$(F_1, E) = \left\{ \begin{array}{l} \left(e_1 \frac{x_1}{0.8606} \right), \left(e_1 \frac{x_2}{0.84005} \right), \left(e_1 \frac{x_3}{0.7778} \right), \left(e_2 \frac{x_1}{0.839} \right), \left(e_2 \frac{x_2}{0.795} \right), \left(e_2 \frac{x_3}{0.757} \right), \\ \left(e_3 \frac{x_1}{0.805} \right), \left(e_3 \frac{x_2}{0.8186} \right), \left(e_3 \frac{x_3}{0.746} \right), \left(e_4 \frac{x_1}{0.804} \right), \left(e_4 \frac{x_2}{0.7775} \right), \left(e_4 \frac{x_3}{0.7365} \right), \\ \left(e_5 \frac{x_1}{0.80005} \right), \left(e_5 \frac{x_2}{0.79095} \right), \left(e_5 \frac{x_3}{0.7418} \right), \left(e_6 \frac{x_1}{0.747} \right), \left(e_6 \frac{x_2}{0.784} \right), \left(e_6 \frac{x_3}{0.693} \right), \\ \left(e_7 \frac{x_1}{0.7799} \right), \left(e_7 \frac{x_2}{0.76275} \right), \left(e_7 \frac{x_3}{0.698} \right) \end{array} \right\},$$

according to the results of the first day given in Figure 1a-c.

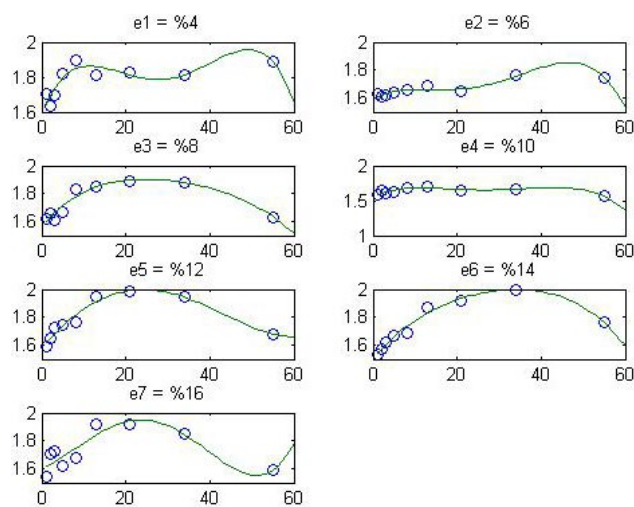


FIGURE 3 Appropriate functions for total degrees of membership functions

In a similar way, the other fuzzy soft sets (F_i, E) , $i \in \{2, 3, 5, 8, 13, 21, 34, 55\}$, and their table presentations could be constructed using the results in Figure 1a-c.

The total table of degrees of membership functions was formed according to the parameters for beneficial bacteria. In Table 2, values of $\mu_i(x_1) + \mu_i(x_2)$, $i \in \{1, 2, 3, 5, 8, 13, 21, 34, 55\}$, were written for each day.

Appropriate functions were drawn using Table 2 and MATLAB in Figure 3 (Curve Fitting Toolbox 2015).

Then the decision-making function was defined as follows:

$$\alpha_j = \max \{ \mu_i(x_1) + \mu_i(x_2) \}, \beta_j = \min \{ \mu_i(x_3) \}, S_j = \alpha_j - \beta_j,$$

and

$$D = \max_j \{ S_j \} = \max_j \{ \alpha_j - \beta_j \},$$

TABLE 3 Scores table for α_j and β_j

E	α_j	β_j	Score = S_j
e_1	1.899	0.7015	1.1975
e_2	1.76	0.64	1.12
e_3	1.888	0.718	1.17
e_4	1.698	0.7365	0.9615
e_5	1.984	0.7418	1.2422
e_6	2	0.693	1.307
e_7	1.9195	0.6715	1.248

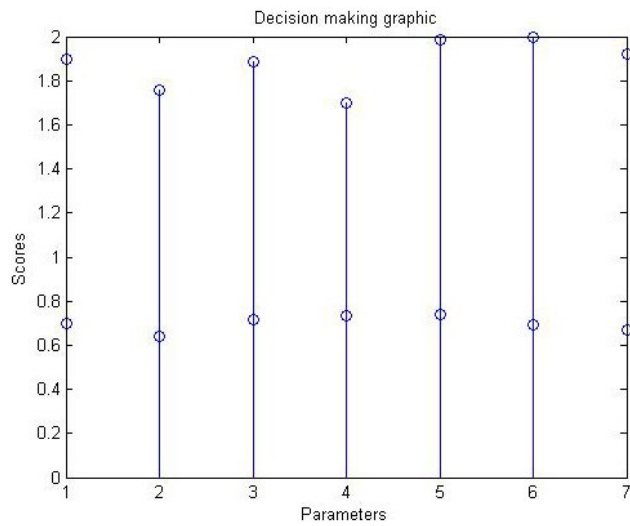


FIGURE 4 Optimum salt concentration

for $i \in \{2, 3, 5, 8, 13, 21, 34, 55\}$ and $j \in \{1, 2, 3, 4, 5, 6, 7\}$. Score table was constructed for α_j and β_j in Table 3.

The optimum salt concentration was the parameter $e_6 = 14\%$ as seen in Table 2. Also this result was seen in the decision-making graphic. For this purpose, the distance function was used. The highest distance score was the optimum salt concentration in Figure 4.

4 | CONCLUSION

Optimization of salt concentrations in cheese brine should always be taken into account in the production. Salt concentrations are often ignored in white cheese production in most countries, high concentrations of salt are generally preferred. However, cheese production with high salt concentrations not only affects our health but can also prevent the development of lactic acid bacteria, which can increase the bioavailability of the cheese produced. As a result, the increase of lactic acid bacteria in cheese, yeast, and mold growth can be limited. Therefore, there is a certain balance between salt, lactic acid bacteria, yeast, and molds in cheese, and the optimization of this balance is also important in terms of industrial production.

In this research, an optimization was tried to establish for white cheese brines salt concentrations by means of the “fuzzy soft set

theory.” For this purpose, an appropriate parameter set and a universal set were constructed and the decision-making function was defined. Consequently, 14% (w/v) salt concentration of brine was found optimum for white cheese production. It can be concluded that using “fuzzy soft set theory” in the food industrial area especially for the optimization of some processes is an effective and practical method.

If we investigate the numbers of microorganisms statistically, they can be only evaluated as separate groups. However, in our optimization model, it can be determined the useful microorganisms and unwanted microorganism groups together at the same time from the score table. This situation has great importance by means of both processing time and the exact decision-making. Even so, if it is needed the evaluation of microorganism groups separately, then it can be used statistical methods.

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AUTHOR CONTRIBUTIONS

Reyhan Irkin: Formal analysis; investigation; methodology; writing – original draft; writing – review and editing. **Nihal Yılmaz Ozgur:** Formal analysis; methodology; writing – review and editing. **Nihal Tas:** Formal analysis; methodology; writing – review and editing.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest in this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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