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Physicochemical and rheological characteristics of alcohol-free probiotic boza produced using *Lactobacillus casei* Shirota: estimation of the apparent viscosity of boza using nonlinear modeling techniques

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Abstract: The effects of fermentation with 2 different starter culture mixtures composed of lactic acid bacteria (LAB) on some physicochemical, microbiological, sensorial, and rheological characteristics of boza, a traditional fermented Turkish beverage, during storage for 10 days at 8 °C were investigated. *Lb. casei* Shirota adapted in boza well and provided the boza with a probiotic property. At the beginning of storage, the *Lb. casei* Shirota count of boza was 6.83 log cfu mL^{-1}, while total LAB counts were 8.01 and 8.11 log cfu mL^{-1} for S1 and S2 samples, respectively. A decrease in pH values was observed when the counts of LAB and *Lb. casei* Shirota increased during the storage period. Sensory acceptability scores were high for all boza samples but extending storage caused a decrease in sensory scores. Adaptive neuro-fuzzy inference system (ANFIS) and artificial neural network (ANN), 2 important nonlinear modeling techniques, were used to construct the predictive models for the estimation of apparent viscosity during storage. All boza samples showed pseudoplastic flow behavior. Apparent viscosity decreased with increasing of shear rate, storage period, and measurement temperature. ANFIS showed better fitting performance with a higher coefficient of determination ($R^2 = 0.995$) compared to ANN ($R^2 = 0.980$).

Key words: Boza, *Lb. casei* Shirota, rheology, probiotic, alcohol-free, modeling

1. Introduction

Boza is a traditional low-alcohol cereal-based fermented beverage made from millet, maize, and wheat and/or rice semolina/flour (Arıcı and Dağlıoğlu 2002). It dates back to the ancient Anatolian and Mesopotamian civilizations, while its preparation method was introduced to other populations by the Ottomans through their conquests (Todorov 2010). Today, it is commonly consumed in some regions of Turkey and Bulgaria (Uysal et al. 2009). Boza is traditionally produced through several steps: preparation of raw materials, cooking, cooling, straining, sugar addition, and fermentation at 30 °C for 24 h (Akpınar-Bayizit et al. 2010). Fermentation of boza is generally carried out naturally by lactic acid bacteria (LAB) and yeasts (Zorba et al. 2003) after inoculation with a starter culture, sourdough, or yogurt (Todorov 2010). A number of LAB (*Lactobacillus fermentum*, *Lb. sanfrancisco*, *Lb. rhamnosus*, *Lb. plantarum*, *Lb. pentosus*, *Leuconostoc paramesenteroides*, *Leu. mesenteroides* subsp. *mesenteroides*, *Leu. raffinolactis*, *Lactococcus lactis* subsp. *lactis*, *Oenococcus oeni*, *Weissella paramesenteroides*, and *W. confusa*) and yeasts (*Saccharomyces cerevisiae*, *S. uvarum*, *S. carlsbergensis*, *Candida glabrata*, *C. tropicalis*, *Geotrichum candidum*, and *G. penicilatum*) isolated from boza have been reported (Hancıoğlu and Karapınar 1997; Velitchka et al. 2000; Gotcheva et al. 2000; Arıcı and Dağlıoğlu 2002; Todorov and Dicks 2006).

Probiotics are defined as live microorganisms that have benefits for the health of consumers by maintaining or improving their intestinal microbial balance, when they are found in adequate amounts (Fuller 1989). Probiotic organisms stimulate the growth of desirable microorganisms, inhibit potentially harmful bacteria, and promote the body's natural immune system (Saarela et al. 2000). Probiotic bacteria mostly include *Lactobacillus* and *Bifidobacterium* strains. However, several *Lactococcus*, *Enterococcus*, *Saccharomyces*, and *Propionibacterium* strains are also considered probiotic bacteria (Vinderola and Reinheimer 2003). Boza inherently includes a number of probiotic species such as *Lb. rhamnosus*, *Lb. plantarum*, *Lb. fermentum*, and *Lb. pentosus* (Todorov et al. 2007). In previous research, potential probiotic characteristics and survival of *Enterococcus mundtii* in boza were evaluated (Todorov et al. 2009). Probiotic microorganisms inhibit

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the growth of various foodborne pathogens in boza by means of their bacteriocin produced (Todorov and Dicks 2004, 2006, 2007; Todorov 2010). The acidic property of boza (about 4.0 pH) also contributes to the inhibition of pathogens (Hancıoğlu et al. 1999; Güven and Benlikaya 2005). *Lb. casei* Shirota, known as a probiotic LAB, is a native member of Yakult (a traditional Japanese drink) and is recognized for its therapeutic effects (Shah 2007). Survival of *Lb. casei* Shirota was previously investigated in different products such as a milk-based dessert containing cranberry sauce (Magarinos et al. 2008) and ice cream (Sagdıç et al. 2012). However, boza has not been evaluated as a probiotic carrier food for *Lb. casei* Shirota.

The rheological properties of a particular food are parameters of special interest in terms of food acceptability, food processing, and food handling (Tabilo-Munizaga and Barbosa-Canovas 2005). A few studies are available on the rheology of boza. Hayta et al. (2001) investigated the effect of fermentation temperature on the viscosity and protein solubility of boza, while Genc et al. (2002) showed the pseudoplastic behavior of boza and reported that the consistency coefficient of boza could be used as a predictor for sensory properties. LAB, which are commonly used in food fermentations, convert sugars into organic acids, and therefore contribute to the organoleptic and rheological properties of the products (Edema and Sanni 2008).

Boza has a number of nutritive constituents arising from the cereals used in its production and fermentation as well as viable beneficial microorganisms (Arıcı and Dağlıoğlu 2002). However, boza is a food restricted for Muslims because of its alcohol content resulting from yeast fermentation as the consumption of alcoholic drinks is completely prohibited in Islam; foods or drinks that contain even a small amount of alcohol cannot be considered halal (Riaz and Chaudry 2004). Demand among Muslims for halal certificated foods has increased in recent years. For instance, trade in halal products is worth about 150 billion dollars and halal certification has become a requirement for products to be imported in most countries (Egan 2002; Regenstein et al. 2006).

Nonlinear modeling techniques such as adaptive neuro-fuzzy inference system (ANFIS) and artificial neural network (ANN) have been commonly used for the estimation of nonlinear parameters in food science and technology. ANN is known to be a mathematical algorithm and modeling method having the ability to relate input and output parameters. ANN does not require the relations of the process parameters and computational models inspired by structure or functional aspects of biological neural network systems. Because ANN is a nonlinear statistical data modeling tool, it can also be used in the modeling of nonlinear systems having complex relationships between inputs and outputs (Jeyamkondan et al. 2001; Ramadan 2011; Yılmaz 2012). ANFIS is a combination of ANN and fuzzy inference system (FIS). A significant weakness of FIS is its weak adaptability to changing external environment (Vassilopoulus and Bedi, 2008). To construct a better modeling technique, ANN, which is a very efficient technique, can be combined with FIS because their combination can result in synergy, which improves speed, fault tolerance, and adaptiveness (Jang 1993; Setlak 2008). Many studies have been carried out on the modeling of food characteristics using the FIS in the food engineering field. Abu Ghoush et al. (2008) reported that physicochemical and rheological properties such as viscosity or stability of foods are regarded as complex nonlinear systems and these parameters can be modeled effectively using ANFIS or ANN. Many studies have been carried out in the literature related to application of ANFIS and ANN modeling in food science (Perrot et al. 1999; Kavdir and Guyer 2003; Abu Ghoush et al. 2008; Lee et al. 2008; Karaman and Kayacıer 2011; Karaman et al. 2011; Yalçın et al. 2011).

In the present study, it was aimed (a) to produce a new alcohol-free boza fermented with probiotic bacteria containing no yeast for observant Muslims, (b) to determine the rheological characteristics and evaluate the survival of probiotic starter cultures during storage, and (c) to construct a predictive model for the estimation of apparent viscosity, an important rheological parameter for sensorial acceptance and the production process, using 2 important and commonly used nonlinear modeling techniques (ANFIS and ANN).

2. Materials and methods

2.1. Materials and bacterial strains

The raw materials used for boza production, including wheat, maize, and rice flours and table sugar were purchased from local markets in Kayseri, Turkey.

Lactobacillus casei Shirota, *Lb. acidophilus*, *Lb. plantarum*, *Weisella paramesenteroides*, and *Leuconostoc mesenteroides* subsp. *mesenteroides* were used in starter culture combinations for boza fermentation. *Lb. casei* Shirota was isolated from Yakult, a commercially available drink. Other bacterial strains were obtained from the Food Control Laboratory Directorate (Kayseri, Turkey). These bacteria were selected for boza fermentation since they have been isolated in boza by different researchers.

Stock bacterial cultures were maintained separately in MRS agar (Merck, Germany) including glycerol (30% v/v) at <–20 °C and activated twice in MRS broth (Merck, Germany) for 24 h at 30 °C prior to experimental usage (Arıcı et al. 2004). Activated bacterial cultures were prepared to be fresh bacteria cells in 50 mL of MRS broth for 18 h at 30 °C. Then these cultures were centrifuged at 4000 × *g* for 10 min and the supernatant was removed. The cells were washed a few times with 0.85% (w/v) NaCl

solution and suspended in 50 mL of solution to use for the fermentation.

2.2. Production of boza wort

Figure 1 illustrates the process flow chart for boza production. The method of Zorba et al. (2003) was used for the production of boza wort with some modifications. First of all, maize, whole wheat, and rice flours were mixed in the ratio of 2:1:1, respectively. One liter of water was incorporated into the flour mixture and the mixture was heated on a medium heating system with continuous

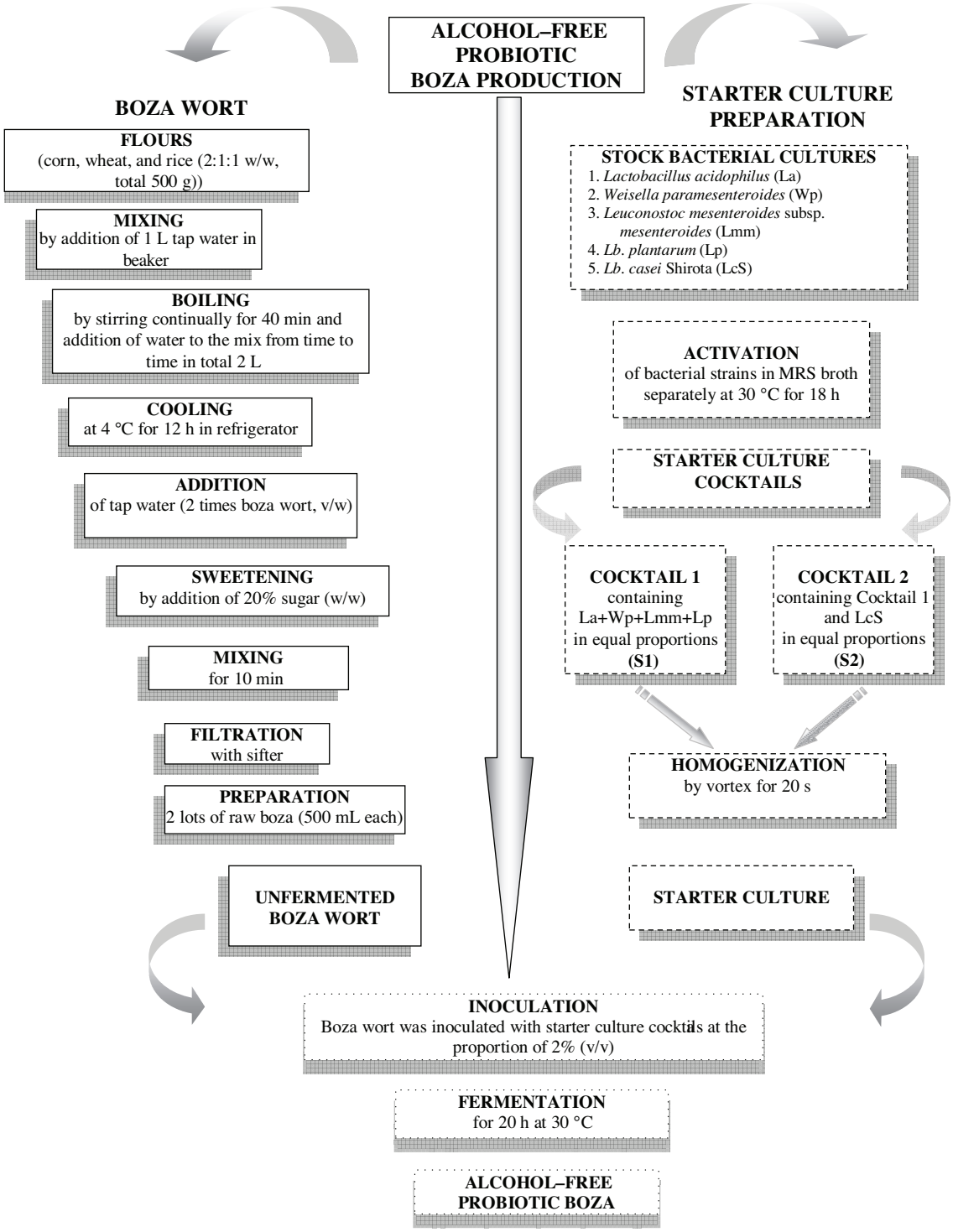


Figure 1. Process flow chart for production of alcohol-free probiotic boza.

stirring for 40 min. Because the mixture absorbed the water, hot water (4 times the total flour weight (v/w)) was incorporated into the mixture during the heating process. Afterwards, the final mixture was cooled at 4 °C for 12 h and tap water (2 times the mixture weight (v/w)) was added commensurately. Sugar (20% w/w) was added and stirred in. Finally the mixture was filtered by a sifter to remove the ungelatinized parts of the boza wort.

2.3. Boza fermentation and storage

Boza wort was divided into 2 lots (500 mL each) in sterile volumetric flasks and selected bacterial cocktails were inoculated into each lot at 2% (v/v) concentrations. Two bacterial cocktails were prepared as follows: (S1) *Lb. acidophilus* + *W. paramesenteroides* + *Leu. mesenteroides* subsp. *mesenteroides* + *Lb. plantarum* in equal proportions; (S2) *Lb. casei* Shirota + cocktail 1 containing *Lb. acidophilus*, *W. paramesenteroides*, *Leu. mesenteroides* subsp. *mesenteroides*, and *Lb. plantarum*. After inoculation of bacterial cocktails, the final boza samples were incubated at 30 °C for 24 h (Figure 1). After the fermentation, boza samples were stored at 8 °C for 10 days. Analyses were carried out on days 0, 5, and 10 of the storage period.

2.4. Physicochemical analyses

Brix, pH, water activity (a_w), and total acidity values of the boza samples were measured after fermentation during the storage period. An automatic refractometer (Reichert AR 700, USA) was used for the measurement of Brix values at 20 °C. pH values were determined using a pH-meter (inoLab, WTW, Weilheim, Germany). a_w values were determined using an automatic a_w-meter (Decagon, USA). Total acidity was determined by titration using 0.1 N standardized NaOH (Merck, Germany) in the presence of phenolphthalein (Merck, Germany) and the results were expressed as % lactic acid.

2.5. Microbiological analysis

2.5.1. Enumeration of total LAB on MRS agar

Ten milliliters of each boza sample was transferred into 90 mL of sterile maximum recovery diluent solution (Merck, Germany). Then appropriate 10-fold dilutions were prepared and spread-plated on petri plates with MRS agar. The petri plates were incubated at 30 °C for 48 h in anaerobic conditions and colonies were counted at the end of incubation (Sagdıç et al. 2004). The results were expressed as log cfu mL^{-1}.

2.5.2. Enumeration of yeast and mould

Total yeasts and moulds were enumerated using the spread plate technique. After preparation of serial dilutions, they were spread onto dichloran rose-bengal chloramphenicol agar (DRBC, Merck, Germany) and the plates were incubated at 25 °C for 5 days. The results were expressed as log cfu mL^{-1}.

2.5.3. Enumeration of *Lb. casei* Shirota on *Lb. casei* (LC) agar

LC agar, which is a selective medium developed for enumeration of *Lb. casei*, was used to determine *Lb. casei* Shirota counts in boza samples according to the method described by Ravula and Shah (1998). Following the preparation of dilution solutions, they were spread-plated on petri plates with LC agar. Plates in duplicate were incubated in anaerobic conditions using Anaerocult A kits (Merck, Germany) at 27 °C for 72 h. The results were expressed as log cfu mL^{-1}.

2.6. Rheological analysis

The rheological properties of boza samples were characterized using a controlled stress rheometer (Thermo-Haake, Rheostress 1, Germany) equipped with a temperature-control unit (Haake, Karlsruhe Germany). A cone-plate configuration with a cone radius of 35 mm, an angle of 4°, and a gap of 0.140 mm between the cone and plate was utilized for the analysis. The measurements were carried out in the shear rate range of 1–100 s^{-1} at 3 different temperatures (10, 20, and 30 °C). For this purpose, 0.85 mL of boza sample was placed between the cone and the plate. In total, 35 data points were recorded in the shear rate range at 10-s intervals during the shearing. After measurements, the shear stress and apparent viscosity values of samples as a function of shear rate were calculated using a power law model (Eq. (1)). Rheowin Data Manager Software (ver. 2.96) was used for calculating the coefficients of the power law equations:

$$\eta_a = K\dot{\gamma}^{n-1} \quad (1)$$

where η_a is the apparent viscosity (Pa s), K is the consistency coefficient (Pa s^n), $\dot{\gamma}$ is the shear rate (s^{-1}), and n (dimensionless) is the flow behavior index.

The effect of temperature on the apparent viscosity and consistency coefficient was determined by using the Arrhenius model (Eq. (2)):

$$\eta_{50} = \eta_0 \exp(E_a/RT) \quad (2)$$

$$K = k_0 \exp(E_a/RT) \quad (3)$$

where η_{50} is the apparent viscosity at 50 s^{-1} (Pa s), K is the consistency coefficient (Pa s^n), η_0 or k_0 is the constant, E_a is the activation energy (J mol^{-1}), R is the universal gas constant (8.14 J mol^{-1} K^{-1}), and T is the absolute temperature (Kelvin). Rheological measurements were performed in replicate with 5 repetitions.

2.7. Sensorial analysis

Sensorial analysis of the boza samples was carried out by 8 trained panelists using a sensorial rating scale (1–9, bad–excellent). The panelists were selected due to their interest in boza. Sensorial analysis was conducted in a room of

appropriate temperature in an open sitting. The panelists consumed deionized water to cleanse their palates prior to proceeding to the next sample. Before starting the sensory analysis session, the panelists were trained with relation to analysis and the rating scale. The color, consistency, taste, mouth feel, strange flavor/odor, and overall acceptability of boza samples were evaluated during storage by the panelists. Boza samples of 20 g were served to the panelists in a glass at 10 ± 1 °C.

2.8. ANFIS modeling procedure

The fuzzy logic toolbox of MATLAB 7.0.1 was used for the modeling of the ANFIS procedure. To construct a fuzzy-based predictive model, apparent viscosity data of samples obtained from rheological analysis were classified into 3 different groups: training, testing, and validation. The training data matrix was composed of half of the selected data (108 data points). Forty-four data points, which are different from the training data, were used for the testing and validation of the fuzzy model system separately. For the determination of the best fit in the fuzzy model, different combinations that change depending on the input parameter number were trained. Within this scope, 4 different ANFIS models that have the same number of membership functions (3) and types of membership function for input (Gaussian), and similar membership functions for output (constant) were constructed. To construct the best fuzzy model for apparent viscosity, ANFIS and Sugeno-type fuzzy inference system were used using the best combinations (giving the highest coefficient of determination and lowest root mean square error and mean absolute error) among the tested parameters. To build a fuzzy model, sample type, storage period, shear rate, and measurement temperature values were selected as input while the apparent viscosity was output. Ten learning epochs and a radius of 0.5 were used for the development and implementation of the models.

2.9. ANN modeling procedure

The neural toolbox of MATLAB 7.0.1 was used for ANN modeling of the apparent viscosity of boza samples. ANN has one or more hidden layers, whose computation nodes are called hidden neurons. In this modeling technique, the estimated outputs are compared to the experimental outputs, and the errors are back propagated to obtain the appropriate weight adjustments necessary to minimize the errors. The ANN was trained using the Levenberg–Marquardt technique in the present study since this technique is more powerful and faster compared to the conventional gradient descent technique (Yılmaz 2012).

2.10. Selection of the optimal model

To compare the constructed models and decide on the best one among them, estimation performances of the models were evaluated using root mean square error (RMSE), mean absolute error (MAE), and coefficient of determination (R^2), which are common comparison criterions in modeling. The 2 error measurement equations used were as follows:

$$\mathrm{RMSE} = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(Yi_{\mathrm{observed}} - Yi_{\mathrm{predicted}})^2} \quad (4)$$

$$\mathrm{MAE} = \frac{1}{N}\sum_{i=1}^{N}\left| Yi_{\mathrm{observed}} - Yi_{\mathrm{predicted}} \right| \quad (5)$$

in which N is the number of data and Yi is the oxidation parameter used as output (Yılmaz 2012).

$$R^2 = \left(\frac{\sum_{i=1}^{n}(xi-\bar{x})(yi-\bar{y})}{\sqrt{\sum_{i=1}^{n}(xi-\bar{x})^2 \sum_{i=1}^{n}(yi-\bar{y})^2}} \right)^2 \quad (6)$$

Determination coefficient is the square of the correlation coefficient between 2 variables, x and y, whose n pairs are available. As is known, the lower the RMSE and MAE are, the better the accuracy of the model to predict the parameter is. Moreover, the highest R^2 values indicated that the model performed the best (Yin and Ding 2009).

2.11. Statistical analysis

The Statistical Analysis System (SAS) (SAS 2000) was used for the 2-way analysis of variance (ANOVA), carried out using the general linear model procedure. Duncan's multiple range test was utilized for the determination of differences among mean values with the significance level of $P < 0.05$.

3. Results

3.1. Selection of starter cultures

In the preliminary studies, bacterial cultures used as starter culture for boza production were determined. For this purpose, different studies in the literature were used to determine the bacteria commonly encountered in boza (Arıcı and Dağlıoğlu 2002; Todorov and Dicks 2006). Eventually, *Lb. acidophilus*, *Lb. plantarum*, *W. paramesenteroides*, and *Leu. mesenteroides* subsp. *mesenteroides* were used as the main starter culture. *Lb. casei* Shirota, which is not an inherent member of traditional boza, was also combined with the starter culture mix.

3.2. Physicochemical properties

Changes in pH, total acidity, Brix, and water activity levels of boza samples during the storage are tabulated in Table 1. pH values of S1 and S2 boza samples were found to be 3.73 and 3.48 at the end of the fermentation for 30 h (on the first day of storage), respectively. Total acidity of S1 and S2 increased to 0.77% and 0.92% from 0.46% and 0.62%, respectively, in accordance with the change in pH values throughout the storage (Table 1). On the other hand, the positive change in total acidity and negative change in pH level indicate the ongoing multiplication of LAB in this period.

Table 1. Changes in physicochemical properties of boza samples during storage.

Physicochemical properties	Sample	Storage period (day)		
		0	5	10
pH	S1	3.73 ± 0.01Aa	3.39 ± 0.02Ba	3.23 ± 0.01Ca
	S2	3.48 ± 0.01Ab	3.21 ± 0.01Bb	3.12 ± 0.01Cb
Total acidity (%)	S1	0.46 ± 0.02Cb	0.59 ± 0.01Bb	0.77 ± 0.01Ab
	S2	0.62 ± 0.01Ca	0.77 ± 0.01Ba	0.92 ± 0.01Aa
Water activity (a_w)	S1	0.97 ± 0.00Aa	0.96 ± 0.00Ba	0.96 ± 0.00Bb
	S2	0.97 ± 0.00Aa	0.96 ± 0.00 Ba	0.97 ± 0.00Aa
Brix	S1	14.94 ± 0.03Ca	15.12 ± 0.09Ba	15.30 ± 0.02Aa
	S2	14.67 ± 0.02Bb	14.80 ± 0.06ABb	14.87 ± 0.09Ab

A–C: differences in uppercase letters indicate significant difference ($P < 0.05$) in a row; a–b: differences in lowercase letters indicate significant difference ($P < 0.05$) in a column.

a_w is one of the restricting or promoting factors for microbial growth in a nutritious medium or food. Boza can be regarded as a unique medium for the growth of both beneficial and pathogenic bacteria due to its relatively high a_w.

Brix value gives an idea about the water soluble dry matter content of a food. It is clear from Table 1 that significant ($P < 0.05$) increments were observed in Brix values of the samples during storage. However, S1 sample had a higher ($P < 0.05$) Brix value compared to S2 both at the beginning and at the end of storage. This finding supports the idea that *Lb. casei* Shirota consumed the water soluble sugar molecules present in boza and converted them to lactic acid during this period. As can be seen from Table 1, Brix values are in accordance with both the pH and total acidity parameters. The highest Brix value was determined in the boza sample that had the lowest total acidity and the highest pH values, which means that the growth of LAB caused a decrease in Brix value because of the consumption and conversion of sugars into lactic acid.

3.3. Microbiological profile

Total LAB counts of boza samples and their change during the storage period are illustrated in Figure 2. Initial total LAB counts of S1 and S2 were 8.01 and 8.11 log cfu mL^{-1}, respectively. Significant ($P < 0.05$) increases were observed in total LAB counts of the boza samples from the beginning to days 5 and 10 of the storage period. The increase in total LAB count of S2 was more rapid than that of S1, while total LAB counts of S1 and S2 did not differ significantly from each other ($P > 0.05$).

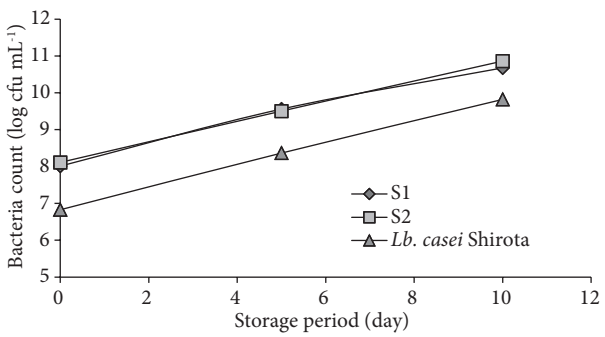


Figure 2. Changes in total LAB (S1 and S2) and *Lb. casei* Shirota (S2) counts of boza samples during storage.

3.4. Sensorial analysis

Table 2 shows the changes in sensory scores of boza samples during the storage period. In general, the panelists declared that the taste of boza samples was negatively influenced by the acidity development resulting from LAB growth during storage. Furthermore, boza samples were considered generally acceptable by the panelists. As expected, sensorial scores of the boza samples showed a continuous decrease throughout the storage period, indicating declining general acceptability. Although the general acceptability of S1 was higher than that of S2 prior to storage, differences between those scores for S1 and S2 were not statistically significant ($P > 0.05$). It was concluded that the storage period caused a significant decrease ($P < 0.05$) in the sensory scores of S2 boza sample because of the development of high acidity due to the growth of LAB. Until day 5 of storage, differences among the sensory scores given for S2 by the panelists were not significant ($P > 0.05$) for the sensory parameters except for mouthfeel, but, after day 5, the scores given to the boza sample (S2) decreased significantly ($P < 0.05$). Additionally, the changes in sensory scores given for S1 boza sample were found to be insignificant depending on storage ($P > 0.05$).

3.5. Rheological analysis

Figure 3 illustrates the flow behavior curves of the boza samples after different storage periods. Both boza samples showed non-Newtonian pseudoplastic flow behavior in which apparent viscosity decreases with increment of shear rate because the flow behavior index was found to

Table 2. Changes in sensory characteristics of boza samples during storage.

Sensory characteristics	Sample	Storage period (day)		
		0	5	10
Color	S1	7.2 ± 1.47Aa	6.5 ± 2.07Aa	6.3 ± 2.22Aa
	S2	7.3 ± 0.96Aa	7.0 ± 1.67Aa	5.8 ± 1.71Aa
Consistency	S1	6.0 ± 2.76Aa	5.2 ± 2.93Aa	4.0 ± 2.16Aa
	S2	7.3 ± 0.50Aa	6.5 ± 2.51Aa	4.0 ± 1.41Ba
Taste	S1	6.0 ± 1.26Aa	5.7 ± 2.58Aa	4.8 ± 1.50Aa
	S2	5.3 ± 0.96Aa	6.2 ± 1.47Aa	3.5 ± 0.58Ba
Mouthfeel	S1	5.7 ± 2.16Aa	5.7 ± 2.58Aa	4.8 ± 1.50Aa
	S2	5.0 ± 1.41Ba	6.5 ± 1.76Aa	4.0 ± 0.82Ba
Strange flavor and odor	S1	5.5 ± 2.59Aa	6.7 ± 1.97Aa	7.5 ± 0.58Aa
	S2	6.3 ± 1.26Aa	6.3 ± 2.94Aa	7.3 ± 0.96Aa
General acceptability	S1	6.6 ± 1.69Aa	6.0 ± 2.19Aa	4.5 ± 1.00Aa
	S2	5.8 ± 1.26Aa	6.2 ± 1.60Aa	4.0 ± 0.82Ba

A–B: differences in uppercase letters indicate significant difference ($P < 0.05$) in a row. a: differences in lowercase letters indicate significant difference ($P < 0.05$) in a column

be lower than unity, which means that the decrease in flow behavior index provides greater departure from Newtonian behavior (Hayta et al. 2001). As can be seen from the figure, the samples showed similar flow behaviors. Pseudoplastic behavior of boza has been reported previously (Genc et al. 2002; Zorba et al. 2003).

3.6. Modeling of apparent viscosity using nonlinear techniques

Nonlinear modeling techniques such as ANFIS and ANN, which are novel and effective, have been commonly used for the modeling of nonlinear parameters in food science with high precision and accuracy. In the present study, sample type (S1 or S2), storage period (0, 5, and 10 days), temperature (10, 20, and 30 °C) and shear rate (1, 25, 50, 75, and 100 s^{-1}) were used as input parameters and model performance was evaluated using RMSE, MAE, and R^2.

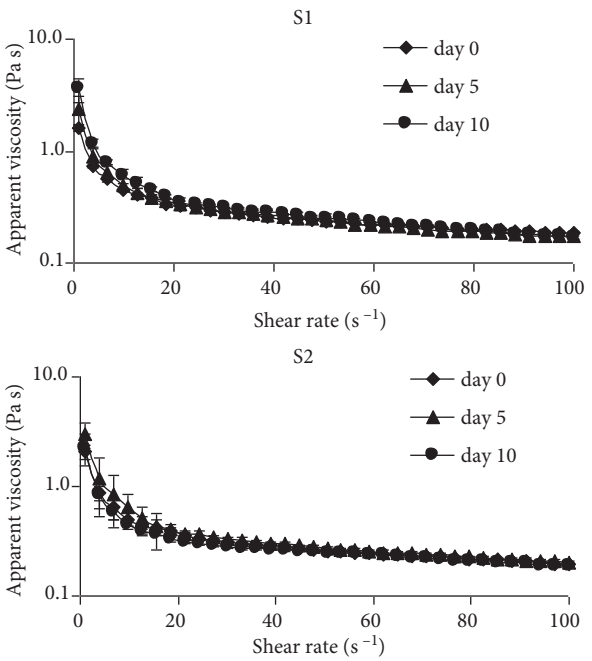


Figure 3. Apparent viscosity change in boza samples with shear rate at 20 °C after different storage periods.

Modeling performance of both ANFIS and ANN techniques is tabulated in Table 3. As can be seen from the table, increment in input number increased the estimation performance of the models. RMSE and MAE decreased with increasing input parameter number while coefficient of determination increased. It was impossible to construct a predictive model using sample type and storage period as input parameters since the coefficient of determination obtained from this model was close to zero. A model constructed with 3 input parameters for the estimation of apparent viscosity was not advisable ($R^2 = 0.424$). The best model was obtained by using all input parameters including sample type, temperature, storage period, and shear rate for the estimation of apparent viscosity. As can be seen from Table 3, the model constructed using ANFIS with 4 input parameters showed the highest accuracy with a high coefficient of determination ($R^2 = 0.995$) and very low RMSE (0.0008) and MAE (0.0044) values in the validation period. Compared to ANFIS, ANN resulted in a lower coefficient of determination and higher RMSE and MAE values.

4. Discussion

The pH results are comparable to those in previous studies, where values were between 3.40 and 4.00 (Hancıoğlu and Karapınar 1997; Hayta et al. 2001; Akpınar-Bayizit et al. 2010). In addition, a significantly ($P < 0.05$) higher decrease

Table 3. Testing performance of ANFIS and ANN models in estimation of apparent viscosity of boza samples.

ANFIS (b)										
	Constant	Training			Testing			Validation		
Input (a)	Number of MFs	RMSE(d)	MAE (e)	R^2	RMSE	MAE	R^2	RMSE	MAE	R^2
ST	3 Gaussian mf	0.0854	0.0687	0.0231	0.0848	0.0676	0.0108	0.0116	0.0681	0.0184
ST, SP	3 - 3 Gaussian mf	0.0841	0.0680	0.0537	0.0845	0.0673	0.0218	0.0115	0.0679	0.0411
ST, SP, T	3 - 3 - 3 Gaussian mf	0.0658	0.0533	0.4194	0.0647	0.0530	0.4228	0.0089	0.0528	0.4237
ST, SP, T, SR	3- 3 - 3 - 3 Gaussian mf	0.0087	0.0054	0.9900	0.0148	0.0094	0.9708	0.0008	0.0044	0.9949
ANN (c)										
	Number of nodes in layers									
ST	2 - 7 - 1	0.0854	0.0688	0.0232	0.0848	0.0678	0.0109	0.0852	0.0683	0.0184
ST, SP	2 - 3 - 1	0.0841	0.0680	0.0537	0.0845	0.0673	0.0218	0.0842	0.0679	0.0411
ST, SP, T	2 - 8 - 1	0.0658	0.0533	0.4194	0.0647	0.0530	0.4228	0.0652	0.0528	0.4237
ST, SP, T, SR	2 - 9 - 1	0.0141	0.0103	0.9739	0.0187	0.0133	0.9544	0.0124	0.0088	0.9802

(a) Inputs: ST; Sample type, SP; Storage period (day), T; Temperature (°C), SR; Shear rate (s^{-1}), (b) ANFIS: Adaptive neuro-fuzzy inference system, (c) ANN: Artificial neural networks, MF: Membership function, (d) RMSE: Root mean square error, (e) MAE: Mean absolute error, R^2: Coefficient of determination

was observed in the pH level of the S2 sample compared to S1 during the storage period. This indicates that starter culture mix containing *Lb. casei* Shirota contributed to the acidity development more. It was also obvious from Table 1 that a decrease in pH in both S1 and S2 samples was in progress during storage at 8 °C. In this study, water activity of the S1 sample decreased from 0.97 to 0.96 ($P < 0.05$) while no change was observed in the water activity of the S2 sample at the end of storage (Table 1).

LC agar has been developed for enumeration of *Lb. casei* populations from commercial yogurts and fermented milk drinks that may contain strains of yogurt bacteria (Ravula and Shah 1998). In the present study, this medium was utilized for the determination of the change in *Lb. casei* Shirota numbers in boza. The *Lb. casei* Shirota count was 6.83 log cfu mL^{-1} at the beginning of storage (after fermentation), while it reached 8.37 log cfu mL^{-1} and 9.82 log cfu mL^{-1} on days 5 and 10 of fermentation, respectively. The colony structure of *Lb. casei* Shirota on LC agar was distinctly distinguished while other LAB formed a few colonies in different structures. Ravula and Shah (1998) also successfully observed and counted *Lb. casei* colonies on LC agar while distinct *Lb. acidophilus* growth (<3 log cfu g^{-1}) was not observed. In the present study, probable yeast/mold contamination was examined during the storage and the yeast/mold count was lower than the detection limits (<2 log cfu mL^{-1}) in the boza samples. Therefore, it can be concluded that the boza samples were alcohol-free.

The decrease in apparent viscosity with increasing shear rate is related to the increment in alignments of the constituent molecules of the material (Rha 1975; Hayta et al. 2001). Increasing storage period caused a significant change in the apparent viscosity values ($P < 0.05$). Table 4 shows the apparent viscosity values of boza samples at the

Table 4. Change in apparent viscosity values of boza samples at 50 s^{-1} and different temperatures during storage.

	day 0			day 5			day 10		
Sample	10 °C	20 °C	30 °C	10 °C	20 °C	30 °C	10 °C	20 °C	30 °C
S1	0.342 ± 0.02Aa	0.232 ± 0.01 Ba	0.164 ± 0.00Ca	0.251 ± 0.01Ab	0.241 ± 0.03Aa	0.189 ± 0.01Bb	0.297 ± 0.01Ac	0.248 ± 0.00Ba	0.234 ± 0.02Bc
S2	0.384 ± 0.01Aa	0.264 ± 0.01Ba	0.172 ± 0.01Ca	0.334 ± 0.01Ab	0.273 ± 0.01Bab	0.237 ± 0.01Cb	0.301 ± 0.01Ac	0.251 ± 0.01Bb	0.231 ± 0.00Cc

A–C: differences in uppercase letters indicate significant difference ($P < 0.05$) in a row for each temperature. a–c: differences in lowercase letters indicate significant difference ($P < 0.05$) in a row for each storage period.

shear rate in the mouth (50 s^{-1}) at different temperatures during storage. It can be said that S2 generally showed higher apparent viscosity values compared to S1. Figure 4 shows the effect of temperature on the rheological flow curves. As can be seen from the figure, the lowest shear stress values were recorded at the highest measurement temperatures. Similarly, temperature caused a decrease in apparent viscosity in both samples. The apparent viscosity of S1 was 0.342 Pa s at 10 °C at the beginning of storage, while it decreased to 0.297 Pa s with increasing storage period. Similar results were observed in S2, of which the initial apparent viscosity was 0.384 Pa s at the same conditions, while it was 0.301 Pa s at the end of storage (Table 4). The decrease in the apparent viscosity values during storage may be explained by the reduction in sugar content with by LAB fermentation. Hayta et al. (2001) investigated the effect of fermentation on the viscosity and protein solubility of boza and found lower apparent viscosity values in fermented boza samples compared to those in unfermented ones. Similar results were also reported by Gotcheva et al. (2001), who observed a decrease in the apparent viscosity of boza produced from wheat flour.

Consistency coefficients and flow behavior index values of boza samples calculated for all measurement temperatures are tabulated in Table 5. It is clear that the power law model could describe the relationship between the shear rate and shear stress since the fitting process resulted in a high coefficient of determination ($R^2 > 0.977$). Change in apparent viscosity and consistency coefficient showed a significant ($P < 0.05$) difference depending on the boza type, measurement temperature, and storage period. The highest consistency coefficient (2.481 Pa s^n) was calculated for S2 boza sample at 10 °C at the beginning of the storage period, while the lowest (0.651 Pa s^n) was in S2 at 30 °C on day 10 of storage. Extending the storage period caused a significant decrease ($P < 0.05$) in the consistency coefficient and apparent viscosity. Increasing temperature provided a decrease in the consistency coefficient since it is a function of apparent viscosity parameters and has a positive correlation with them. Hayta et al. (2001) reported the consistency coefficients of unfermented and

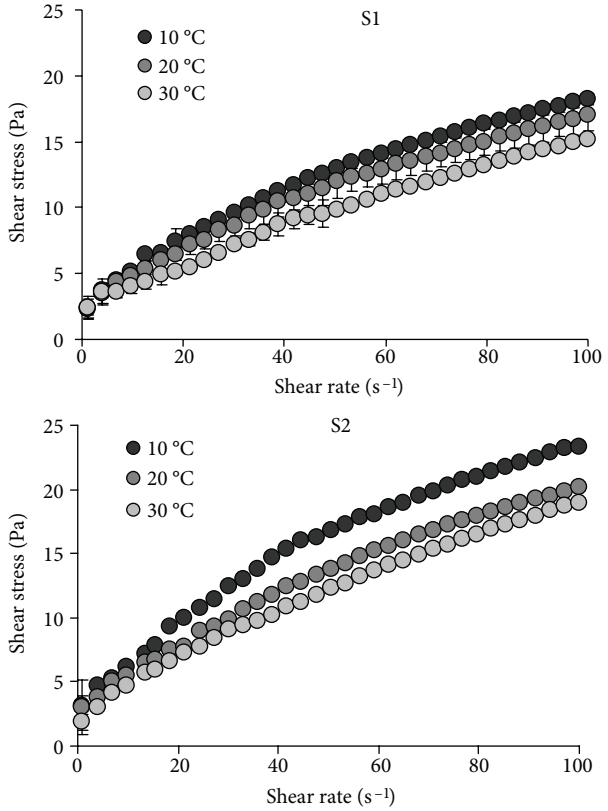


Figure 4. Shear stress versus shear rate curves of boza samples at different temperature on day 5 of storage.

Table 5. Consistency coefficient and flow behavior index of boza samples for different storage periods and temperatures.

		day 0			day 5			day 10		
Sample	T °C	K (Pa s^n) (a)	n (b)	R^2 (c)	K (Pa s^n)	n	R^2	K (Pa s^n)	n	R^2
S1	10	2.154 ± 0.09Ab	0.515 ± 0.01Ca	0.999	2.231 ± 0.00Aab	0.506 ± 0.02Aa	0.998	2.355 ± 0.12Aa	0.479 ± 0.03Aa	0.997
	20	1.048 ± 0.05Bb	0.621 ± 0.01Aa	0.999	1.686 ± 0.61Bab	0.505 ± 0.08Ab	0.980	1.939 ± 0.35Aa	0.477 ± 0.04Ab	0.990
	30	0.807 ± 0.04Cb	0.586 ± 0.01Ba	0.999	1.000 ± 0.23Bab	0.593 ± 0.05Aa	0.992	1.763 ± 0.63Aa	0.494 ± 0.09Aa	0.977
S2	10	2.481 ± 0.39Aa	0.518 ± 0.04Bc	0.998	2.047 ± 0.21Aa	0.536 ± 0.02Ab	0.994	1.404 ± 0.15Ab	0.602 ± 0.03Ba	0.998
	20	1.235 ± 0.17Ba	0.604 ± 0.03Ab	0.998	1.689 ± 0.68Aa	0.549 ± 0.08Ac	0.986	1.033 ± 0.10Ba	0.635 ± 0.02Ba	0.983
	30	0.781 ± 0.03Cab	0.607 ± 0.01Ab	0.999	1.152 ± 0.34Aa	0.614 ± 0.07Ab	0.997	0.651 ± 0.07Cb	0.721 ± 0.02Aa	0.998

(a) K: Consistency coefficient, (b) n: flow behavior index, R^2 (c): Coefficient of determination, A–C: differences in uppercase letters indicate significant difference ($P < 0.05$) in a column for each sample (S1 or S2). a–c: differences in lowercase letters indicate significant difference ($P < 0.05$) in a row for each storage period.

fermented boza samples as 4.21 and 1.64 Pa s^n at 10 °C, respectively. Similarly, increasing temperature caused a decrease in consistency coefficient, which was calculated to be 3.14 and 1.02 Pa s^n for the same sample under the same conditions, respectively. In the present study, the consistency coefficient and apparent viscosity values were slightly lower than those reported by Hayta et al. (2001). Zorba et al. (2003) found that the consistency coefficient of fermented boza was 6.678 Pa s^n. However, the differences in consistency coefficient and apparent viscosity in different studies might have resulted from the starter cultures used since they may change the boza composition. Different production recipes and compositions of boza might also affect those values.

Table 6 shows the constants of the Arrhenius model commonly used to determine the temperature dependency of apparent viscosity and consistency coefficient. As can be seen from this table, those rheological parameters were temperature dependent. As is known, viscosity is dependent on the intermolecular distance and increasing temperature causes an increase in the molecular distance and so a decrease occurs in viscosity. The Arrhenius equation was proposed by many researchers for determination of the impact of temperature on the rheological parameters especially on the apparent viscosity (Özilgen and Bayındırlı 1992; Essa 2002; Bahlol 2005; Chin et al. 2009). In general, increasing storage period caused a decrease in the activation energy of boza samples, which were calculated from both apparent viscosity and consistency coefficient values. Activation energy values of S1 and S2 were 26.369 and 27.190 J mol^{-1} at the beginning of storage, while they decreased to 8.877 and 9.762 J mol^{-1} at the end of storage, respectively. Changes in activation energy with storage were statistically significant ($P < 0.05$). It is also clear from the table that the Arrhenius model was effective in describing the effect of temperature on both apparent viscosity and consistency coefficient with a high coefficient of determination (greater than 0.922). Steffe (1996) reported that increasing activation energy provided a rapid change in viscosity with temperature and vice versa.

The comparison between the measured and computed apparent viscosity by ANFIS and ANN is illustrated in Figure 5. The ANFIS model clearly exhibited a better relationship between estimated and measured apparent viscosity values than ANN. Apparent viscosity values estimated using ANFIS showed a close match with the measured ones. It has been well demonstrated that ANFIS showed better performance for the estimation of nonlinear parameters in food science research compared to ANN. Karaman et al. (2011) reported that ANFIS could be used effectively to construct a predictive model for the oxidation parameters of sunflower oil during storage. Yalçın et al. (2011) reported similar results. Figure 6 illustrates 3D surface plots that show the changes in estimated apparent viscosity values of boza samples depending on the storage period, sample type, and temperature, with the 3 input parameters used for the construction of nonlinear predictive models. As can be seen from the figure, apparent viscosity decreased with increase in temperature and increase in storage period caused a decrease in apparent viscosity.

In conclusion, boza contains a wide range of LAB and yeasts. However, traditional boza does not meet halal food requirements since yeast fermentation causes alcohol formation. In this study, alcohol-free probiotic boza was produced using *Lb. casei* Shirota. *Lb. casei* Shirota adapted

Table 6. Arrhenius model constants for effect of temperature on consistency coefficient and apparent viscosity of boza samples.

Effect of temperature on consistency coefficient									
	day 0			day 5			day 10		
	k_0 (Pa s^n) (a)	Ea (J mol^{-1}) (b)	R^2 (c)	k_0 (Pa s^n)	Ea (J mol^{-1})	R^2	k_0 (Pa s^n)	Ea (J mol^{-1})	R^2
S1	5×10^{-6}	30.244	0.948	4.8×10^{-4}	19.592	0.922	5.8×10^{-2}	8.577	0.996
S2	1.2×10^{-5}	28.572	0.944	6.4×10^{-4}	19.048	0.978	2.5×10^{-5}	25.781	0.991
Effect of temperature on apparent viscosity									
	day 0			day 5			day 10		
	η_0 (Pa s^n)	Ea (J mol^{-1})	R^2	η_0 (Pa s^n)	Ea (J mol^{-1})	R^2	η_0 (Pa s^n)	Ea (J mol^{-1})	R^2
S1	5×10^{-6}	26.369	0.999	4.9×10^{-3}	9.344	0.909	6.8×10^{-3}	8.877	0.968
S2	4×10^{-6}	27.190	0.998	1.7×10^{-3}	12.458	0.997	4.7×10^{-3}	9.762	0.983

(a) η_0 or k_0: Arrhenius model constant, (b) Ea: Activation energy, R^2 (c): Coefficient of determination

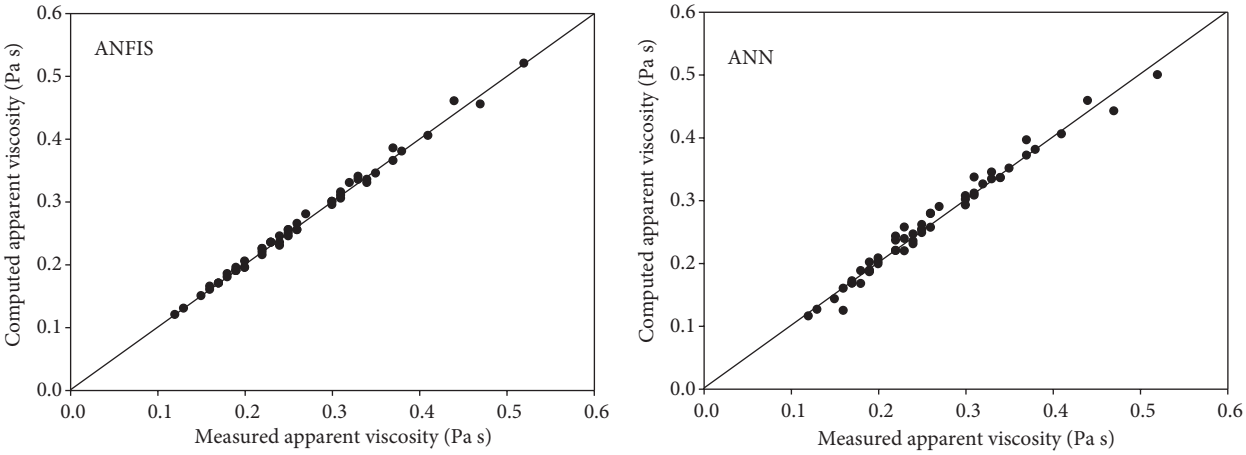


Figure 5. Measured and computed apparent viscosity calculated with ANFIS and ANN modeling techniques.

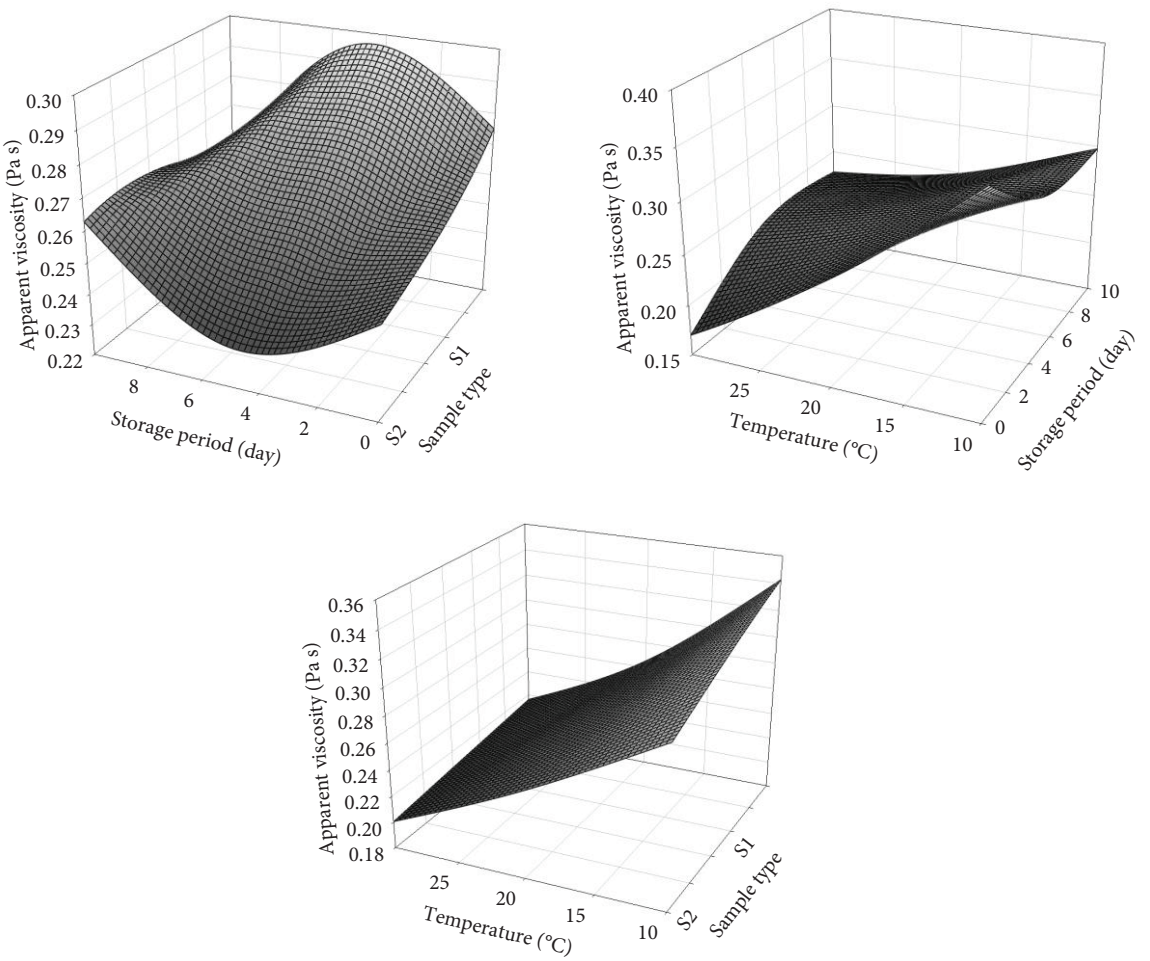


Figure 6. Change in computed apparent viscosity versus storage period, measurement temperature, and sample type.

and survived in boza well during storage. In addition, the general acceptability of the boza samples produced was high and they showed acceptable quality characteristics for daily consumption. However, extending the storage period is not recommended since it caused some decreases in sensorial scores. Boza showed non-Newtonian pseudoplastic flow behavior where apparent viscosity decreased with increasing shear rate. Storage caused a significant decrease in apparent viscosity, which is an important parameter in terms of sensorial acceptability. Nonlinear predictive models were constructed using ANFIS and ANN. The best fit was observed in ANFIS models with a greater coefficient of determination and lower root mean square error. Therefore, ANFIS can be used efficiently for the estimation of change in apparent viscosity during storage on an industrial scale.

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