

Rheological and some physicochemical properties of probiotic boza beverage fermented with *Lactobacillus casei* Shirota: application of principal component analysis for the characterisation

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RESEARCH ARTICLE

Abstract

Boza is a cereal-based fermented beverage. *Lactobacillus casei* Shirota, which rates among probiotic bacteria, is a member of starter culture combinations used for the production of industrial Yakult, a Japanese beverage. In this study, boza was produced using different starter culture combinations (*Weissella paramesenteroides*, *L. casei* Shirota and *Lactobacillus acidophilus* with two/three bacterial combinations) and principal component analysis was used for characterisation of their quality parameters. In the results, boza fermented with commercial boza (control) and with *L. acidophilus* + *L. casei* Shirota had the highest sensorial scores. Total lactic acid bacteria counts of the boza samples ranged from 9.09 to 10.48 log cfu/ml while pH was the lowest (4.08±0.02) in the boza produced with *L. acidophilus* + *L. casei* Shirota. Boza samples showed non-Newtonian pseudoplastic behaviours. Apparent viscosity values decreased with increasing shear rate. The first two principal components explain 80.71% of the total variation for measurements, with 54.90% of the total variance explained by the first principal component.

Keywords: boza, lactic acid bacteria, probiotic, principal component analysis, rheology

1. Introduction

Boza is a traditional cereal-based fermented food product made from millet, maize, wheat or rice semolina/flour. Boza, which was originated from Persians, has been known by Central Asians for centuries and introduced to Anatolia and Europe by mass migration (Arici and Daglioglu, 2002). Fermentation of boza is generally carried out naturally by lactic acid bacteria (LAB) and yeasts (Zorba *et al.*, 2003). Several studies have been conducted to determine the microflora responsible for the fermentation of boza. Hancioglu and Karapinar (1997) isolated eight strains of LAB of which the majority was heterofermentative (91%, 70 isolates) and the remainder was homofermentative (9%, 7 isolates) in addition to two strains of *Saccharomyces*. In Bulgarian boza samples, seven LAB strains belonged to *Lactobacillus* and *Leuconostoc* spp. were identified

while *Saccharomyces cerevisiae* was the dominant yeast spp. (Gotcheva *et al.*, 2000). *Candida* and *Geotrichum* strains were also determined. Topal and Yazicioglu (1986) detected *S. cerevisiae*, *Saccharomyces uvarum*, *Candida scottii*, *Trichosporon capitatum* from yeasts and *Pediococcus cerevisiae*, *Leuconostoc paramesenteroides*, *Lactobacillus plantarum* from LAB in the Turkish boza samples. Overall, it is evident from those studies that *Leuconostoc*, *Lactobacillus* and *Saccharomyces* spp. constitute the main microflora of boza.

Probiotics are defined as living microorganisms which benefit the health of consumers by maintaining or improving their intestinal microbial balance (Fuller, 1989). Probiotic effects of LAB have also been reported (Saarela *et al.*, 2000). On this basis, boza might be considered as a rich source of probiotic LAB (Todorov *et al.*, 2007). Studies

have shown that LAB present in boza such as *Lactobacillus plantarum*, *Lactobacillus pentosus*, *Lactobacillus rhamnosus* and *Lactobacillus paracasei* had the ability to produce bacteriocins (Todorov, 2010; Todorov and Dicks, 2005, 2006). Additionally, acidic media of boza and presence of bacteriocins prevent the growth of pathogens like *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* O157:H7 and *Salmonella* Typhimurium (Güven and Benlikaya, 2005; Hancıoğlu *et al.*, 1999). *Lactobacillus casei* Shirota, a probiotic spp. has been isolated from yakult, a traditional Japanese drink (Shah, 2007). *Lactobacillus acidophilus* has been considered as the predominant lactobacilli in the intestinal tract of healthy humans, and therefore it is commonly used in probiotic products (Arihara *et al.*, 1998). However, *L. casei* Shirota and *L. acidophilus* are not native members of traditional boza.

Principal component analysis (PCA) is a data analysis technique. A number of PCA applications in food science and technology have been presented in the literature (Decourcelle *et al.*, 2004; Destefanis *et al.*, 2000; Frau *et al.*, 1999; Thakur *et al.*, 2011). In PCA applications, the original variables are transformed into the new axes or principal components which are orthogonal. PCA decreases the numbers of variables and expresses the total variation in only a few principal components.

This study was conducted to determine the adaptability of two probiotic bacteria, *L. casei* Shirota and *L. acidophilus* in boza fermentation and to evaluate the descriptiveness of studied parameters of boza production in the boza quality by PCA technique.

2. Materials and methods

Materials

All the raw materials used for boza production, whole wheat, maize, rice flours, sugar and commercial boza were obtained from the local markets in Kayseri, Turkey. *Lb casei* Shirota was isolated from Yakult, a commercially available drink. *L. acidophilus* and *Weissella paramesenteroides* were provided from Kayseri Agricultural Control and Protection Management Center, Kayseri, Turkey. The strains were stored at -80 °C and each culture was prepared by inoculating cryopreserved cells in de Man Rogosa and Sharpe (MRS) Broth (Merck, Darmstadt, Germany) and incubating the cells for 24 h at 37 °C. Activation process was repeated twice.

Production of boza Wort

Boza wort was prepared according to the method of Zorba *et al.* (2003) with some modifications. Maize, whole wheat and rice flours were mixed in the ratio of 2:1:1, respectively. One litre of water was added to the flour mixture and boiled

on a moderate heating system with continuous stirring for 40 min. Since the mixture absorbs the water and some water evaporates, hot water (4 times of total flour weight) was added during the heating process. Then the mixture was cooled at 4 °C for 12 h and additional tap water was added (2-fold of the mixture weight) and 20% sugar (w/w) was added to the slurry and stirred for 1 min. Finally, the mixture was filtered using a sifter to remove the ungelatinised parts from the boza wort.

Starter culture addition and fermentation

The prepared raw boza wort was divided into five lots (500 ml each) in sterile volumetric flasks and selected bacterial combinations (2% v/v) were inoculated into each lot in the ratio of 1:1 or 1:1:1. Control sample was inoculated with commercial boza (2% v/v). Bacterial combinations were: (1) *L. acidophilus* + *W. paramesenteroides*; (2) *L. casei* Shirota + *W. paramesenteroides*; (3) *L. acidophilus* + *L. casei* Shirota + *W. paramesenteroides*; and (4) *L. acidophilus* + *Lb casei* Shirota. Fermentation process was carried out at 30 °C for 24 h.

Physicochemical analysis

pH values of the boza samples were determined during the fermentation at 0, 6, 12 and 24 h using a pH-meter (WTW-Inolab, Weilheim, Germany) at 25 °C. Brix values were measured after the fermentation. An automatic refractometer (Reichert AR 700; Reichert Technologies, Depew, NY, USA) was used for the determination of brix values at 20 °C. Dry matter content measurements of the boza samples were carried out according to AOAC methods (Cunniff, 1995).

Microbiological analysis

Total LAB counts of the boza samples were determined during the fermentation process (0, 6, 12 and 24 h). For this aim, 10 ml of each boza sample was transferred into 90 ml of sterile Ringer solution and appropriate tenfold dilutions were prepared. 0.1 ml of each test dilution was pour plated onto MRS agar petri plates and colonies were counted following the incubation at 37 °C for 48 h.

Rheological analysis

For the determination of rheological characteristics of the boza samples, a controlled stress rheometer (Thermo Scientific Haake RheoStress 1; Thermo Scientific, Karlsruhe, Germany) equipped with a temperature-control unit was used. For the analysis of the samples, 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 utilised. The measurements were performed over a shear rate range of 1-100 /s at a constant temperature (25 °C). For

this purpose, 0.85 ml of the boza sample was placed between the cone and plate. Total 30 data points were recorded in the shear rate range at 10 sec intervals during the shearing. After measurements, the shear stress and apparent viscosity values of the samples as a function of shear rate were determined using the Power law model (Equation 1). Rheowin Data Manager Software (version 2.96; Thermo Scientific) was used for the determination of coefficients of Power law:

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

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

To determine the relationship between apparent viscosity and shear rate, new expressions proposed by Falguera and Ibarz (2010) were used as follows:

$$\eta_a = \eta_\infty (\eta_0 - \eta_\infty) \exp(-k\dot{\gamma}) \quad (2)$$

$$\eta_a = \eta_\infty (\eta_0 - \eta_\infty) \cdot \dot{\gamma}^{-k} \quad (3)$$

where η_a is the apparent viscosity (Pa s), η_∞ is the equilibrium apparent viscosity (Pa s), η_0 static apparent viscosity (Pa s), $\dot{\gamma}$ is the shear rate (/s) and k (dimensionless) is a constant.

Sensorial analysis

Sensorial properties of the boza samples were tested by eight trained panellists who are members of the Food Engineering Department using a sensorial rating scale (1-9, bad-excellent). Panellists were selected in regard of their interest to boza. Sensorial analysis was carried out in a room with appropriate temperature in open sitting. Deionised water was served to the panellists to cleanse their palates prior to proceeding to the next sample. Before the sensory analysis, the panel members were trained and special information related to the boza and its main sensory characteristics were given and the definitions in the sensory score sheet were explained to the panellist group in the training. Panellists were asked to give their score to the samples according to the definition of each attribute. Colour, consistency, taste, sourness, mouthfeel, strange flavour, odour and overall acceptability of the boza samples were evaluated at the end of the fermentation by the panellists. Five boza samples were served to the panellists as 20 g per sample in glass at 10±1 °C.

Statistical analysis

Statistical Analysis System (SAS, Cary, NC, USA; 1988) was used for the one-way analysis of variance (ANOVA) that is carried out using the general linear model procedure. Duncan multiple range test was utilised for

the determination of differences among mean values with the significance level of 0.05. Principal component analysis (PCA) was carried out using XLSTAT (Addinsoft, 2004).

3. Results and discussion

Physicochemical properties

Dry matter content of the raw boza wort was 19.18%. Although a slight increase occurred in dry matter contents of all boza samples during fermentation (data were not presented), the increase levels were found statistically insignificant ($P>0.05$).

Boza can be produced using different raw materials such as wheat flour, bulgur, rice flour and also some other plants which are rich in starch (Arici and Daglioglu, 2002). Therefore, composition and physicochemical properties of boza may vary extensively. Uylaser *et al.* (1998) investigated dry matter contents of 17 retail Turkish boza samples and dry matter levels were found between 18.99% and 25.7%. Our findings of dry matter were compatible with previous studies.

pH change of the boza samples during the fermentation period is illustrated in Figure 1. As can be seen from the figure, initial pH value of raw boza wort was 6.50, and then the pH values decreased continuously with the increase of fermentation period. Commercial boza addition to the control boza sample caused the highest pH decrement compared to other samples and it exhibited the lowest pH values at all measurement points. pH value of the boza sample inoculated with *L. acidophilus* and *L. casei* Shirota (sample 4) was higher than those of other samples after 6 and 12 h of fermentation; however final pH of the sample was found to be lower than others (4.08) as a result of rapid decrease after 24 h. The highest pH value was observed in the boza sample that fermented with *L. acidophilus* and *W. paramesenteroides* after the fermentation (sample 1). Boza samples which were fermented with *L. casei* Shirota + *W. paramesenteroides* (sample 2) and with *L. acidophilus* + *L. casei* Shirota + *W. paramesenteroides* (sample 3) exhibited similar pH trends during the fermentation. Final pH values of the samples 2 and 3 were 4.21 and 4.19, respectively. At the end of the fermentation period, the pH values of the boza samples (samples 2, 3 and 4) containing *L. casei* Shirota strain were found to be with proximate levels.

Brix values of boza samples are shown in Figure 2. Brix value of the raw boza wort was 17.03. Significant ($P<0.05$) changes were observed on the brix values during fermentation. Control and sample 4 (fermented with *L. acidophilus* and *L. casei* Shirota) exhibited significantly highest ($P<0.05$) brix values (17.39 and 17.82, respectively) while the unique decrease occurred in sample 1 (fermented with *L. acidophilus* and *W. paramesenteroides*).

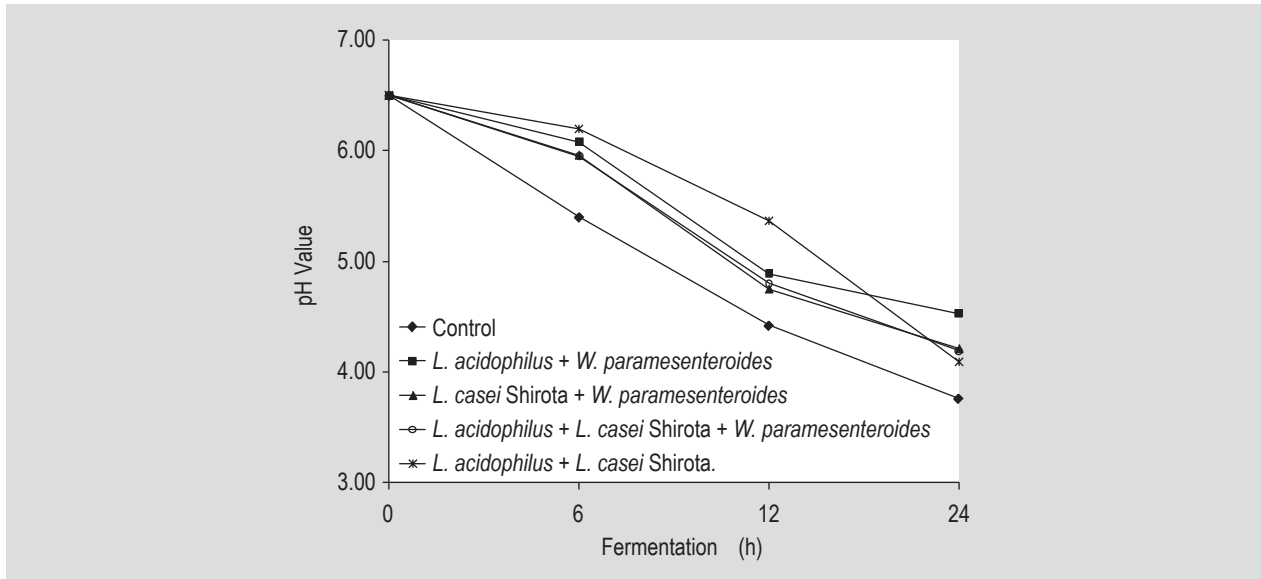


Figure 1. pH changes in the boza samples during fermentation with *Lactobacillus acidophilus*, *Lactobacillus casei* Shirota and *Weisella paramesenteroides*.

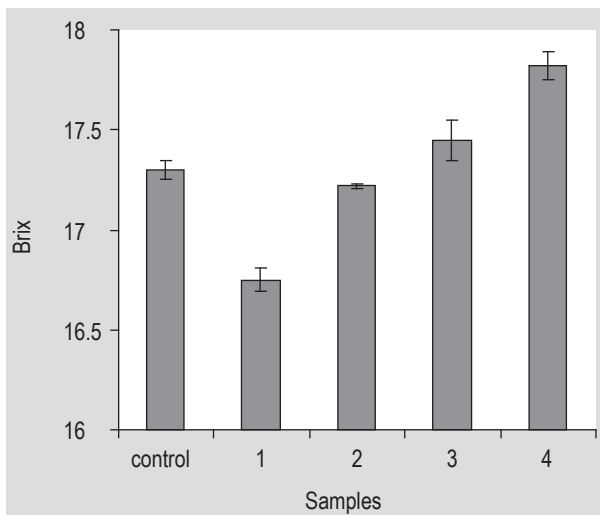


Figure 2. Brix values of the boza samples: (1) *Lactobacillus acidophilus* + *Weisella paramesenteroides*; (2) *Lactobacillus casei* Shirota + *W. paramesenteroides*; (3) *L. acidophilus* + *L. casei* Shirota + *W. paramesenteroides*; (4) *L. acidophilus* + *L. casei* Shirota.

Boza can be considered as acceptable for consumption when pH drops to about 3.5 (Gotcheva *et al.*, 2001). Hancioglu and Karapinar (1997) observed a decrease in pH to 3.48 from 6.13 during the fermentation. pH values ranging between 3.43 and 3.86 were determined in wheat, maize, rice and millet boza samples (Akpinar-Bayizit *et al.*, 2010). The pH values determined in this study were lower than 7.00. These results suggest that *L. acidophilus* and *L. casei* Shirota, the members of starter culture combinations of the boza samples, did not contribute to the pH development well.

Microbial profile

Changes on the microbial profile of the boza samples during the fermentation are shown in Table 1. Starter culture inoculation was made in such a manner that enable the initial population at the level of 10^5 - 10^6 cfu/ml. As is shown in Table 1, approximately 4 to 6 log cfu/ml increases occurred in the total microbial counts of the boza samples. Total LAB counts of the samples 2 and 3 reached to 10.48 log cfu/ml and 10.54 log cfu/ml where the numbers were significantly ($P < 0.05$) higher than those of the other samples. The lowest LAB population (9.09 log cfu/ml) was determined in the control boza sample after 24 h fermentation. However, the same boza sample had the lowest pH value. This suggests that bacteria and yeasts present in the commercial boza made the highest contribution to acidity of boza although they showed a slower multiplication compared to the combinations in other samples.

L. acidophilus is among the most commonly used species as probiotics. A number of probiotic products containing *L. acidophilus* have been developed worldwide (Shah, 2007). However, *L. casei* Shirota has been isolated from only yakult and there are limited studies highlighting the adaptability and survival of *L. casei* Shirota in fermented foods. Sagdic *et al.* (2012) investigated the survival of *L. casei* Shirota in ice cream mix and frozen ice cream during storage of 60 days. Magarinos *et al.* (2008) observed a logarithmic decrease of 8.41% in the *L. casei* Shirota count in a milk based dessert with cranberry sauce during the frozen storage for 21 days.

Probiotic properties of *L. acidophilus* and *L. casei* Shirota have been reported in the literature. Consumption of milk

Table 1. Microbial population of boza samples (log cfu/ml) during the fermentation¹.

Fermentation Time (h)	Control ²	Sample 1	Sample 2	Sample 3	Sample 4
0	4.92±0.15 ^{Cd}	6.12±0.14 ^{Ad}	4.85±0.17 ^{Cd}	5.85±0.17 ^{Ad}	5.37±0.17 ^{Bd}
6	7.81±0.00 ^{Bc}	7.95±0.01 ^{Ac}	7.10±0.01 ^{Cc}	7.78±0.13 ^{Bc}	6.81±0.02 ^{Dc}
12	8.37±0.09 ^{Bb}	8.82±0.08 ^{Ab}	8.16±0.12 ^{Bb}	8.72±0.02 ^{Ab}	7.63±0.17 ^{Cb}
24	9.09±0.10 ^{Da}	10.28±0.06 ^{Ba}	10.48±0.05 ^{Aa}	10.54±0.01 ^{Aa}	9.24±0.07 ^{Ca}

¹ 1 = *Lactobacillus acidophilus* + *Weissella paramesenteroides*; 2 = *Lactobacillus casei* Shirota + *W. paramesenteroides*; 3 = *L. acidophilus* + *L. casei* Shirota + *W. paramesenteroides*; 4 = *L. acidophilus* + *L. casei* Shirota.

² Control sample was inoculated with commercial boza.

^{A-D} Differences in uppercase letters indicate statistical difference in a row.

^{a-d} Differences in lowercase letters indicate statistical difference in a column.

fermented with *L. casei* Shirota is able to modulate the composition and metabolic activity of the intestinal flora (Spanhaak *et al.*, 1998) as well as stimulate the indigenous lactobacilli in the intestine (Ohashi *et al.*, 2001). Similarly, it has been reported that *L. casei* Shirota was able to inhibit the *in vivo* and *in vitro* growth of *Helicobacter pylori* (Cats *et al.*, 2003; Sgoruas *et al.*, 2004). Results of this study indicate that all bacterial combinations containing *L. acidophilus* and/or *L. casei* Shirota adapted in boza media well. Although *L. casei* Shirota and *L. acidophilus* are not native members of traditional boza, they reached to high numbers in all samples within the fermentation period.

Rheological properties

Figure 3 illustrates the flow behaviour curves of the boza samples. All boza samples showed non-Newtonian pseudoplastic flow behaviour while apparent viscosity

decreased depending on the increment in shear rate. As can be seen from the figure, the lowest apparent viscosity value was observed in the control sample. The apparent viscosity of the control sample at 50 /s (shear rate in mouth) was measured to be as 0.16 Pa s. Inoculation of the boza samples with LAB provided an increase in apparent viscosity and it is found in the range of 0.49-0.63 Pa s of the boza samples inoculated with LAB. Similarly, inoculation of LAB provided an increment in the consistency of the boza, and for that reason consistency coefficients were calculated to be high in fermented boza samples (Figure 4). The highest consistency coefficient (4.828 Pa sⁿ) was found for the sample 4 containing *L. acidophilus* and *L. casei* Shirota while the lowest (1.357 Pa sⁿ) was in the control. The increment in the consistency coefficient depending on the sample type, was statistically significant ($P<0.05$). The flow behaviour index of the sample was in the range of 0.476-0.509. Coefficient of determination for

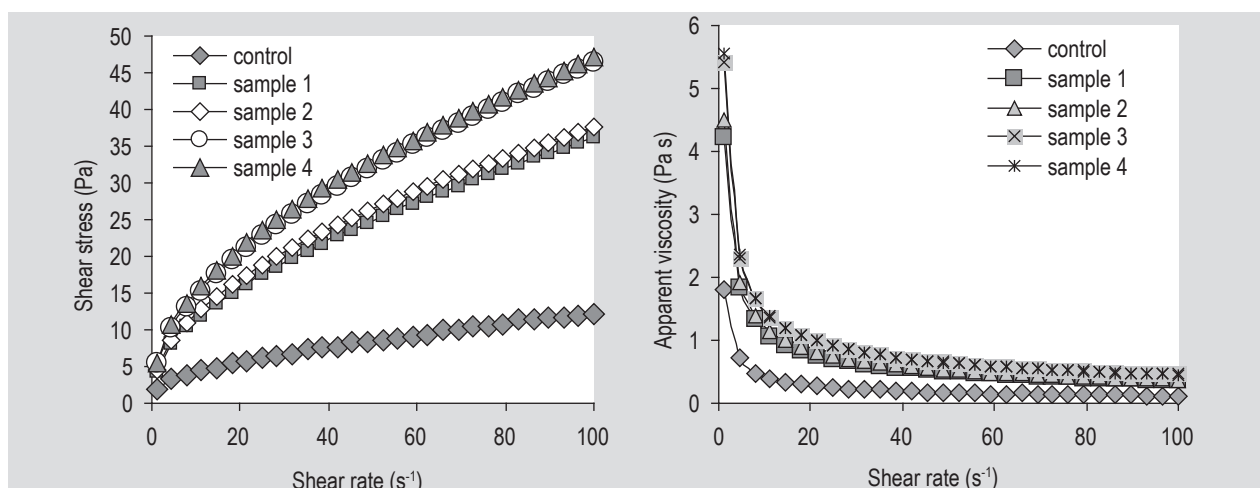


Figure 3. Flow rheograms of the boza samples at 25 °C. (1) *Lactobacillus acidophilus* + *Weissella paramesenteroides*; (2) *Lactobacillus casei* Shirota + *W. paramesenteroides*; (3) *L. acidophilus* + *L. casei* Shirota + *W. paramesenteroides*; (4) *L. acidophilus* + *L. casei* Shirota.

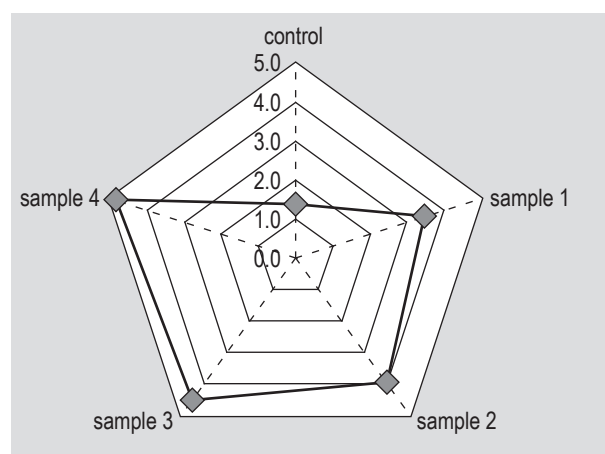


Figure 4. Consistency coefficient (Pa sⁿ) of the boza samples at 25 °C. (1) *Lactobacillus acidophilus* + *Weissella paramesenteroides*; (2) *Lactobacillus casei* Shirota + *W. paramesenteroides*; (3) *L. acidophilus* + *L. casei* Shirota + *W. paramesenteroides*; (4) *L. acidophilus* + *L. casei* Shirota.

the power law model was in the range of 0.996-0.999. To determine the effect of shear rate on apparent viscosity and to compare the boza samples, apparent viscosity variation of all boza samples with increased shear rate was fitted to the Equation 2 and Equation 3, new models proposed by Falguera and Ibarz (2010) as alternative to Power law. Table 2 shows the fitting results and model parameters for both Equation 2 and Equation 3. It is clear that the exponential type model described the effect of shear rate on apparent viscosity because it gave very high coefficient of determinations. Power law type model did not explain the effect because their coefficients of determinations were very low. As can be seen from the table, equilibrium apparent viscosity increased from control to the sample 4 as in the rheological parameters such as apparent viscosity and consistency coefficient. Similarly an increase was observed in static apparent viscosity. It can be concluded that the mathematical model that is exponential type can describe the flow behaviour with a high precision and accuracy. Figure 5 illustrates the experimental and predicted apparent viscosity versus shear rate for all boza samples. It is clear from the table that the models proposed by Falguera and Ibarz (2010) can be used with high accuracy.

Sensorial properties

Table 3 indicates the sensorial scores of the fermented boza samples. Scores higher than 7.00 indicate that the relevant property was considered as fairly acceptable. Although colour and consistency properties of the control sample received the lowest sensorial scores, it was the most approved sample in respect of the overall acceptability. Scores belonging to all sensorial properties of sample 4 were above 7.00 which indicate that all properties of the sample were accepted by the panellists. Also, according to Table 3,

Table 2. Parameters of fitting experimental apparent viscosity.

Sample ¹	Equation 2: $\eta_a = \eta_\infty + (\eta_0 - \eta_\infty) \exp(-k\dot{\gamma})$			
	η_∞ (Pa s)	η_0 (Pa s)	K	R ²
Control	0.176	2.225	0.250	0.985
1	0.502	4.968	0.218	0.982
2	0.535	5.344	0.224	0.980
3	0.655	6.439	0.227	0.980
4	0.649	6.626	0.228	0.980
	Equation 3: $\eta_a = \eta_\infty + (\eta_0 - \eta_\infty) \dot{\gamma}^{-k}$			
	η_∞ (Pa s)	η_0 (Pa s)	K	R ²
Control	0.579	0.572	0.100	0.581
1	1.485	1.468	0.100	0.611
2	1.583	1.566	0.100	0.614
3	1.904	1.884	0.100	0.611
4	1.938	1.916	0.100	0.612

¹ 1 = *Lactobacillus acidophilus* + *Weissella paramesenteroides*; 2 = *Lactobacillus casei* Shirota + *W. paramesenteroides*; 3 = *L. acidophilus* + *L. casei* Shirota + *W. paramesenteroides*; 4 = *L. acidophilus* + *L. casei* Shirota.

no significant difference ($P > 0.05$) was observed between the samples in respect of colour, taste and sourness properties. In the meanwhile, overall acceptability scores of the boza samples were higher than 7.00. In this respect, it may be suggested that all the boza samples were acceptable.

Table 4 shows the correlation coefficients between the physicochemical, rheological, microbiological and sensorial properties of the boza samples. As can be seen from the table, most of the correlations were negative and insignificant statistically ($P > 0.05$). Increasing LAB counts resulted in decrement in taste scores because of the negative correlation. Similarly, overall acceptability decreased depending on the increase of LAB counts. As stated before, a positive correlation was found between the consistency as sensory parameter and consistency coefficient as rheological parameters as expected ($P < 0.05$). According to the results in Table 4, taste was the most considered sensorial property for the acceptability of the boza samples since a positive and significant ($P < 0.05$) correlation was determined between taste and overall acceptability.

The results of PCA for studied parameters are presented in Table 5. The first two principal components with eigenvalues greater than 1.0 (Kaiser criterion; Massart *et al.*, 1988) explain 80.71% of the total variation for measurements,

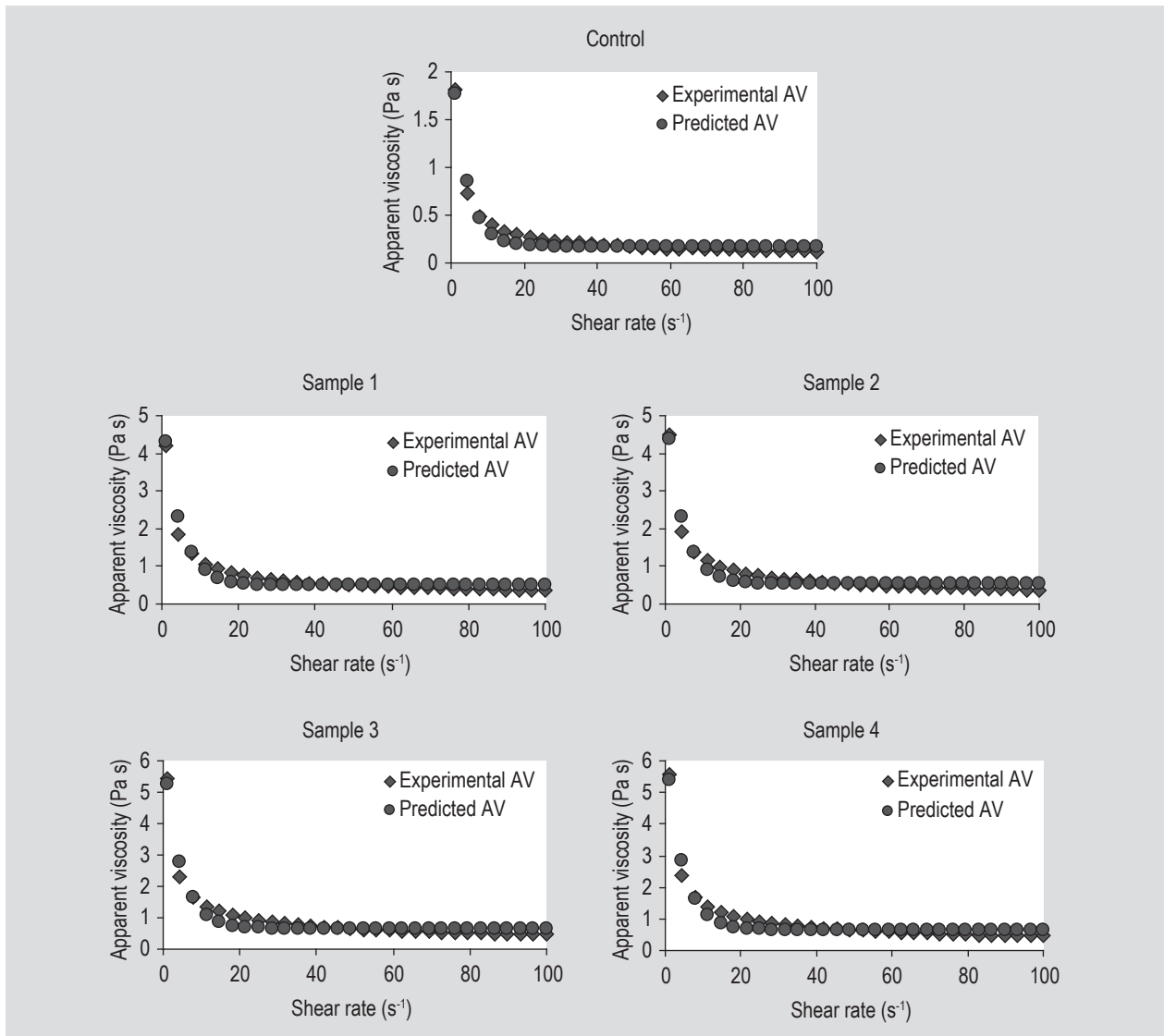


Figure 5. Experimental and predicted apparent viscosity values of the boza samples: (1) *Lactobacillus acidophilus* + *Weissella paramesenteroides*; (2) *Lactobacillus casei* Shirota + *W. paramesenteroides*; (3) *L. acidophilus* + *L. casei* Shirota + *W. paramesenteroides*; (4) *L. acidophilus* + *L. casei* Shirota. AV = apparent viscosity.

Table 3. Sensorial scores of the boza samples.

Boza sample ¹	Colour	Consistency	Taste	Sourness	Mouthfeel	Strange flavour and odour	Overall acceptability
Control ²	6.00±0.00 ^B	6.00±0.00 ^C	8.00±0.00 ^A	7.75±0.46 ^A	7.37±0.52 ^{AB}	7.25±0.46 ^A	8.00±0.00 ^A
1	7.75±0.46 ^A	6.12±0.35 ^C	7.37±0.74 ^A	7.62±0.52 ^A	7.25±0.46 ^{AB}	7.25±0.46 ^A	7.25±0.46 ^B
2	7.62±0.52 ^A	7.50±0.53 ^A	7.25±0.71 ^A	8.00±0.00 ^A	7.00±0.00 ^{AB}	7.50±0.53 ^A	7.00±0.00 ^B
3	8.00±0.00 ^A	7.00±0.00 ^B	7.12±0.83 ^A	8.12±0.83 ^A	6.75±0.89 ^B	7.25±0.46 ^A	7.37±0.52 ^B
4	7.87±0.35 ^A	7.12±0.35 ^A	8.00±0.00 ^A	8.50±0.53 ^A	7.50±0.53 ^A	7.12±0.83 ^A	7.75±0.46 ^A

¹ 1 = *Lactobacillus acidophilus* + *Weissella paramesenteroides*; 2 = *Lactobacillus casei* Shirota + *W. paramesenteroides*; 3 = *L. acidophilus* + *L. casei* Shirota + *W. paramesenteroides*; 4 = *L. acidophilus* + *L. casei* Shirota.

² Control sample was inoculated with commercial boza.

^{A-D} Differences in uppercase letters indicate statistical difference in a column.

Table 4. Correlation matrix of physicochemical, microbiological and rheological characteristics of the boza samples.

Parameters	Brix	MC ₂₄	K	n	Colour	Consistency	Taste	Sourness	Mouthfeel	Strange flavour and odour	Overall acceptability
Brix	1	-0.813	-0.516	-0.860	-0.737	-0.234	0.784	0.075	0.282	-0.251	0.872
MC ₂₄ ¹	-0.813	1	0.445	0.788	0.651	0.385	-0.997	-0.087	-0.786	0.337	-0.935
K ²	-0.516	0.445	1	0.458	0.955	0.769	-0.377	0.808	-0.232	-0.363	-0.492
n ³	-0.860	0.788	0.458	1	0.680	-0.001	-0.753	-0.104	-0.402	-0.113	-0.664
Colour	-0.737	0.651	0.955	0.680	1	0.668	-0.588	0.599	-0.329	-0.227	-0.681
Consistency	-0.234	0.385	0.769	-0.001	0.668	1	-0.358	0.750	-0.410	0.091	-0.514
Taste	0.784	-0.997	-0.377	-0.753	-0.588	-0.358	1	0.146	0.808	-0.396	0.930
Sourness	0.075	-0.087	0.808	-0.104	0.599	0.750	0.146	1	0.013	-0.548	0.027
Mouthfeel	0.282	-0.786	-0.232	-0.402	-0.329	-0.410	0.808	0.013	1	-0.238	0.612
Strange flavour and odour	-0.251	0.337	-0.363	-0.113	-0.227	0.091	-0.396	-0.548	-0.238	1	-0.508
Overall acceptability	0.872	-0.935	-0.492	-0.664	-0.681	-0.514	0.930	0.027	0.612	-0.508	1

In bold significant values (except diagonal) at the level of significance $\alpha=0.050$ (two-tailed test).

¹ MC₂₄ = microbial count after 24 h of fermentation in boza.

² K = consistency coefficient.

³ n = flow behaviour index.

Table 5. Results from the principal component analysis for the first two principal components.

	PC1	PC2
Eigenvalue	6.039	2.839
% variance	54.899	25.808
Cumulative %	54.899	80.707

with 54.90% of the total variance explained by the first principal component (PC1). The first PC summarises most of the variations better in the original data matrix while the second explains the rest of the information better. Therefore, eleven physicochemical, microbiological, rheological and sensorial variables were reduced to two PCs with 19.29% variation loss. Factor loadings for PCs and their contribution percentages in the PCs were tabulated in Table 6. In the first PC, microbial count, brix, taste and overall acceptability showed the highest proportions while the

Table 6. Principal component (PC) factor loadings of boza samples.

	PC1	Contribution of PC1 (%)	PC2	Contribution of PC2 (%)
Brix	-0.858	12.193	0.190	1.269
MC ₂₄ ¹	0.945	14.798	-0.295	3.063
K ²	0.703	8.185	0.704	17.468
n ³	0.779	10.047	-0.163	0.932
Colour	0.856	12.132	0.476	7.990
Consistency	0.581	5.588	0.548	10.585
Taste	-0.919	13.975	0.359	4.546
Sourness	0.202	0.677	0.963	32.633
Mouthfeel	-0.663	7.269	0.233	1.906
Strange flavour and odour	0.186	0.570	-0.704	17.439
Overall acceptability	-0.938	14.564	0.248	2.169

¹ MC₂₄ = microbial count after 24 h of fermentation in boza.

² K = consistency coefficient.

³ n = flow behaviour index.

sourness and strange flavour had the lowest value. For the second PC, sourness and strange flavour showed the highest contributions in second PC while the other parameters had the lowest value. Figure 6 illustrates that the loading vectors plot the variables that has important contribution. The variables such as brix, taste, overall acceptability are placed far from the origin of the PC1 and close to each other. Therefore, it can be suggested that they are the most important parameters for the first PC1 and it can be characterised by these parameters. Similarly, consistency coefficient and flow behaviour index are placed far from the origin of the PC1 in the positive zone. Consistency coefficient and flow behaviour index are also important for PC1. However, important parameters for the PC2 are sourness and strange flavour/odour because they are placed away from the origin of the PC2.

The boza samples analysed are plotted as a function of PC1 and PC2 in Figure 7. As can be seen from the figure, the boza samples are allocated in different positions from the right to the left side of the PC1 axis. Only the control sample and sample 4 are allocated in the left of PC1 axes. The boza samples that inoculated with similar bacterial cultures are allocated through the same axes because they have similar characteristics. It is clear that the samples except sample 4 and the control boza sample are represented by PC1. In Figure 7, the biplot of sample and parameters show that samples and their relationship between rheological, microbiological, sensorial and physicochemical parameters.

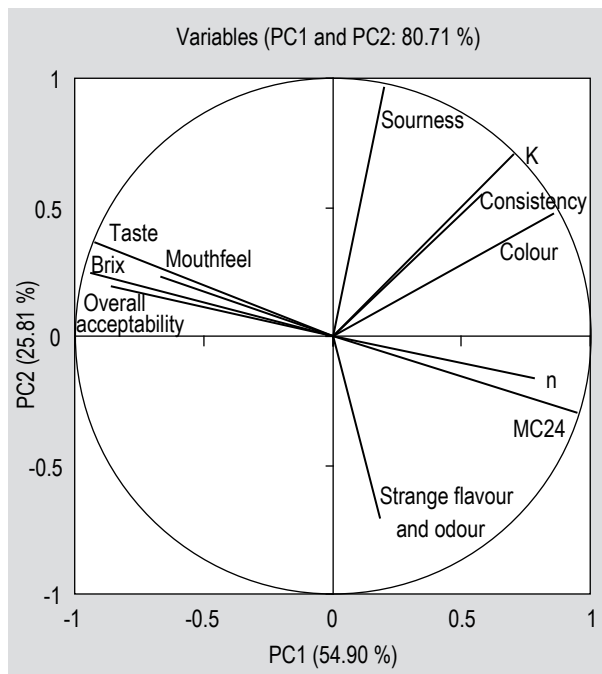


Figure 6. Plot of the first two principal component loading vectors. MC₂₄ = microbial count after 24 h of fermentation in boza; K = consistency coefficient; n = flow behaviour index.

As can be seen from the biplot figure, samples 1, 2 and 3 can be characterised using the microbial count at the end of the fermentation, flow behaviour index, consistency and consistency coefficient. These parameters are predominant characteristics of samples 1, 2 and 3 that are allocated close to each other (Figure 7).

Two basic PCs were obtained by the PCA, the first one containing microbial count, brix, taste and overall acceptability and the second one sourness and strange flavour. In general, similar results will be obtained from the parameters that belong to same group and this is very

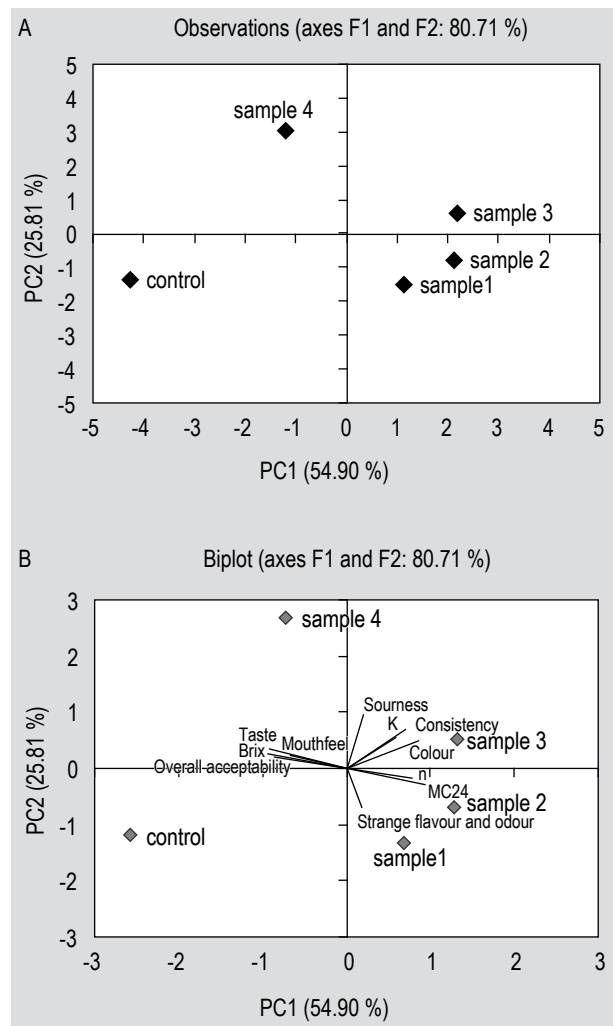


Figure 7. (A) Plot of the first two principal component score vectors and (B) biplot representing both samples and rheological, microbiological and physicochemical characteristics of the boza samples: (1) *Lactobacillus acidophilus* + *Weisella paramesenteroides*; (2) *Lactobacillus casei* Shirota + *W. paramesenteroides*; (3) *L. acidophilus* + *L. casei* Shirota + *W. paramesenteroides*; (4) *L. acidophilus* + *L. casei* Shirota. MC₂₄ = microbial count after 24 h of fermentation in boza; K = consistency coefficient; n = flow behaviour index.

useful technique to identify the different samples using fewer variables.

4. Conclusions

In this study, we investigated the sensorial, physicochemical, rheological and microbiological properties of boza produced with different bacterial culture combinations composed of *L. acidophilus*, *L. casei* Shirota and *W. paramesenteroides*. *L. acidophilus* and *L. casei* Shirota adapted and multiplied in boza media well. All the boza samples were found to be organoleptically acceptable and exhibited good quality characteristics for the consumption in the daily life. The samples showed non-Newtonian pseudoplastic flow behaviour in which apparent viscosity decreased with increase of shear rate. Two groups of studied parameters were created with PCA. Those parameters from the same group exhibited similar results and therefore, it can be suggested that characterisation of the boza samples in industrial scale can be done using only a few parameters (brix, taste and overall acceptability).

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