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The Effect of Exopolysaccharide Producer *Pediococcus Damnosus* 2.6 and Yoghurt Starter Cultures on Ethanol Content, Some Physicochemical and Sensory Properties of Oat Boza

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ABSTRACT

Cereal-based fermented beverages like boza are known functional and probiotic foods. Boza is manufactured by yeast and lactic acid bacteria fermentation of only ones or mixture of various cereals. In this research, the effects of sugar, different microorganisms and inoculum ratios on physicochemical, nutritional and sensory characteristics of oat based boza were investigated. For this purpose, oat based boza production was carried out by 3 different of inoculation rates (0, 3 and 5%) of 3 starter cultures (*Pediococcus damnosus* 2.6, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus)*. The usage aim of *Pediococcus damnosus* 2.6 and yoghurt starter cultures was for the production of exopolysaccharide and lactic acid. As a result of using mixed culture and rising inoculation rates stimulated fermentation activity in formulation of boza. Thus, the amount of total solid matter was reduced and increased the contents of ash, protein and mineral matter of oat boza samples. Although the lowest viscosity was determined in *Pediococcus damnosus* 2.6 inoculation, the most uniform texture was provided. Raw oat had unpleasant odor and flavor but fermentation enhanced sensorial properties of oats. The highest overall acceptance score was observed in oat boza with 3% of yoghurt starter cultures.

1. Introduction

In recent years, increasing attention of consumers to minimum-treated, chemical preservatives-free and natural foods have necessitated the development of alternative food preservation methods. Among these, the biological protection method, in which the lactic acid bacteria play an important role, is of great importance (Wood & Hodge, 1985). Cereal-based fermented beverages are known functional and probiotic foods because they have nutrimental and health promoting components such as nutritional elements, fibers and phytochemicals. In cereal based fermented product, fermentation enhance protein digestibility, nutritional bioavailability and organoleptic properties (Lorenzo, Emanuele, & Elke, 2016).

Boza, which is one of the fermented cereal products, is prepared with only ones or mixture of various cereals (maize, rice, barley, oats, wheat or millet). Semolina or flour of this cereals cooked by adding water. After that, sugar is added to the mixture and this slurry is subjected to fermentation by yeasts and lactic acid bacteria (Anonymous, 1992).

Oat is a fundamental cereal crop and is used commonly for the feeding of farm animals. However, in recent years, it is utilized in human diets due to its various natritional components that have beneficial effects to human health. Oat is richer nutritional source than other cereals in terms of protein quality, lipid, minerals, vitamins and phytochemicals contents (Arendt & Zannini, 2013). The oat contains starch approximately up to 60% as dry basis. The lipid content with a high content of unsaturated fatty acids of oat kernel is higher as two-five times than other cereal grains. They have high protein content ratio of 9-15% with a high lysine concentration. Oats are a grateful antioxidant resource (Zhu, 2017). The health promoting effects of oats associated with β-glucan contents (2-8%) that had the ability to reduce blood cholesterol and glucose levels (Skendi, Biliaderis, Lazaridou, &

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Izydorczyk, 2003). Oat products like oat flakes, oatmeal and oat milk are used to prepare human diets such as ready-to-eat breakfast cereals, baby foods, bread, cookies, and snacks (Bryngelsson, Dimberg, & Kamal-Eldin, 2002).

In this study, it was aimed to improve nutritional and functional properties of oats that was fermented by probiotic bacteria *Lactobacillus acidophilus*, and EPS forming bacteria *Pediococcus damnosus* 2.6. The effects of various microorganisms, different sugar and different inoculation rates used in boza production were investigated on the technological, chemical and sensory properties of the oat boza.

2. Materials and Methods

2.1. Materials

Hulled oat grain (*Avena sativa*), sugar (saccharose) and baker's yeast (*Saccharomyces cerevisiae*, press form) (Pakmaya) were purchased from local market, in Konya. *Lactobacillus acidophilus*, yoghurt bacteria (*Lactobacillus delbrueckii* ssp. *bulgaricus*, *Streptococcus thermophilus*) and *Pediococcus damnosus* 2.6 were obtained from Refik Saydam National Type Culture Collection Laboratory (Refik Saydam National Public Health Agency) in Ankara, Turkey, from Şekersüt Dairy Plant in Konya, Turkey and from the Lund University Biotechnology Laboratory of Sweden in Lund, Sweden, respectively.

2.2. Methods

2.2.1. Preparation of inoculum suspensions

For the activation of *L. acidophilus* strains were inoculated into 5 mL of sterile MRS broth (Merck KGaA, Darmstadt, Germany) and incubated at 42 ˚C for 24 h (pH 4.80 ± 0.2). The active culture was inoculated into UHT milk at a rate of 2% and incubated for the second time at 42 ˚C for 24 h (Mårtensson, Öste, & Holst, 2002).

Yoghurt bacteria (*S. thermophilus* + *L. delbrueckii* ssp. *bulgaricus* 1:1) were obtained from milk factory as an active form. The active cultures were inoculated into UHT milk at a rate of 2% and incubated at 42 ˚C for 24 h (pH 4.50 ± 0.2). After the second activation, this active culture was used in the oat boza production (Anonymous, 2005).

Pediococcus damnosus 2.6 strains were inoculated into 5 mL of sterile MRS broth (Merck KGaA, Darmstadt, Germany) and incubated at 30 ˚C for 18-20 h. This culture was inoculated into sterile MRS broth at a rate of 2% for the second activation. This activated culture were inoculated into 2% UHT milk and incubated 30 ˚C for 24 h and this active culture was used in the oat boza production (Mårtensson, Dueñas-Chasco, Irastorza, Öste, & Holst, 2003).

2.2.2 Production of oat boza samples

Oat boza was produced with some modification according to describe method by Hayta, Alpaslan, and Köse (2001). First of all, oat groats were ground in a hammer mill (Falling Number-3100 Laboratory Mill, Perten Instruments AB, Huddinge, Sweden) equipped with 1 mm opening screen to obtain whole-grain oatmeal as raw materials in boza production. After milling, the oatmeal was stored at -18 ˚C to stop the enzyme activity until used. Oatmeal was mixed with water (1:5 w/v) and slurry was boiled by continuous stirring for 1 hour. The oat mash was cooled at 4-6 ˚C for 7-8 hr. After cooling, the mash diluted with water at levels of 10% and it was blended homogeneously for obtained oat milk. The oat milk was filled in sterile conical flasks with (5%) or without saccharose. 1% of yeast and 0.5 % of *Lactobacillus acidophilus* were also added all boza formulation as constant ratio. The oat milk was inoculated with *Pediococcus damnosus* 2.6, yoghurt bacteria (*S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*1:1) or mixed culture (*Pediococcus damnosus* 2.6+ yoghurt bacteria 1:1) in 3 different of inoculation rates (0, 3 and 5%). Inoculated oat milk was incubated at 30 ˚C for nearly 6 h. Boza samples were analyzed at the end of the fermentation within the same day and were stored at +4 ˚C. Before sensory evaluation, 10% saccharose was added in boza samples for sweetening. Boza samples were identified as follows: inoculated with *Pediococcus domnasus 2.6* (Pd), inoculated with yoghurt culture (YC) and inoculated with *Pediococcus domnasus 2.6* + yoghurt culture (Pd+YC).

2.2.3. Chemical analyses of oatmeal and oat boza samples

Total solid matter (method 44-19), crude ash (method 08-03), protein (AACC 46-12) contents of oatmeal and oat boza samples were measured according to the AACC methods (AACC, 1990). Mineral matter content of the oatmeal was performed according to the method described by Skujins (1998).

2.2.4. pH and titratable acidity of oat boza samples

pH measurements of the boza samples were performed by a digital type pH meter, WTW pH315 i/set model in compliance with TS 9778 (Anonymous, 1992). Potentiometric titration techniques were used for quantification of total titratable acidity of samples in lactic acid (Kentel, 2001).

2.2.5. Ethanol content of oat boza samples

The determination of ethanol in oatmeal boza samples was performed according to TS 1594 (Anonymous, 1998). Ethanol content was found in grams per 100 milliliters of the product.

2.2.6. Viscosity measurement of oat boza samples

A Brookfield viscometer which was equipped with a spindle 7 (Lab line, Model No 4535, Lab Line Instruments, Inc., Melrose Park, IL.,U.K.) was used for measurements of viscosity of samples at 4˚C at 20 rpm.

2.2.7. Sensory evaluation of oat boza

Sensory properties were determined oat boza samples by five panelists who were members of the academicians of the Department of Food Engineering, Selcuk University, Konya, Turkey. The oat boza samples were evaluated with regards to product acceptability using 5 point hedonic scale with 1-2 dislike, 3 acceptable, 4-5 like extremely.

2.2.8. Statistical analysis

Statistical analysis was performed with General Linear Model ANOVA by Minitab 7.1 (Minitab, 1991). The means which were statistically different from each other were compared using Tukey's test at $p < 0.01$.

3. Results and Discussion

3.1. Chemical properties of oatmeal

Some chemical results of oatmeal are shown in Table 1.

Table 1
Some chemical properties of oatmeal

Total solid matter (%)	91.56
Crude ash (%)[a]	1.98
Protein (%)[b]	15.48
Mineral matter content(mg/100g)	
Ca	1977.50
Fe	45.80
Zn	28.96
K	5331.73
Mg	1895.41
P	5075.90

[a] in dry basis
[b]Protein = N x 6.25

The protein and crude ash contents of the hulled oat kernel were determined to be 15.48% and 1.98%, respectively (Table 1). Oat has highest amount of protein among other cereals, its content ranges from 12 to 24% in oat kernel (Lasztity, 1999). In previous study, Kirk and Sawyer (1999) reported that oat grain had about 3.1% ash and 13% protein content. Wholegrain oat has high amounts of valuable components therefore it is a good cereal for nutrition of human.

3.2. Determination of changes in pH value of oat boza samples during fermentation

The pH values of samples ranged from 6.28 to 5.80 at the beginning of the fermentation and there were no statistical differences in initial pH values of boza samples ($P>0.05$). The pH values of boza samples showed a continuous reduction during fermentation ($P<0.01$). At the end of the fermentation, pH values of samples ranged between 5.67-4.76. Similar findings with pH values were reported by Rathore, Salmerón, and Pandiella (2012) who researched the effect of two probiotic strains on fermentation of single and mixed cereal substrates. In this previous study, it was indicated that the pH value was determined to be below 3.5 in mixed and single cereal media at the end of fermentation.

Whereas the highest pH value was determined in control without sugar, the lowest pH value was observed in 5% YC samples with sugar (Figure 1-2). The addition of sugar into the boza formulation was accelerated fermentation and supported the formation of lactic acid, which caused quickly reduction in pH values. Similarly, Hancioğlu and Karapinar (1997) and Gotcheva, Pandiella, Angelov, Roshkova, and Webb (2001) reported that pH of boza samples decreased from 6.13 to 3.48 and from 5.4 to 3.1 during the fermentation time, respectively.

3.3. Some chemical properties of oat boza samples

Total solid matter contents of oat boza are given in Table 2. The total solid matter content ranged from 16.11 to 18.68% and increased significantly ($p<0.01$) by addition of sugar into boza formulation. Moreover, the highest total solid matter content was determined in boza sample prepared with 5% Pd+YC inoculation and sugar addition. Besides, statistically no significant difference was found between the other inoculation rates and cultures types in boza samples with and without sugar.

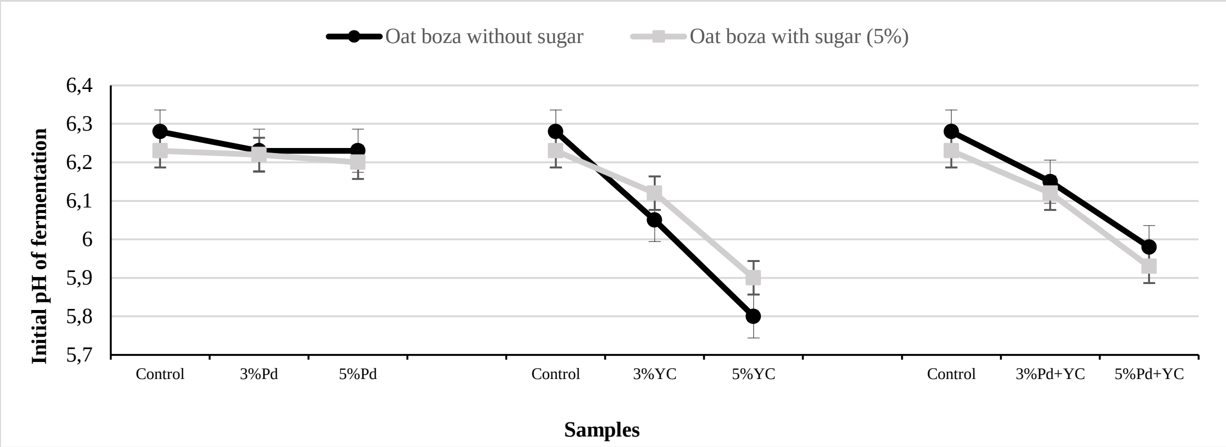

Figure 1
pH values of oat boza samples at the beginning of the fermentation

Pd: boza inoculated with *Pediococcus domnasus 2.6*; YC: boza inoculated with yoghurt culture (*S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*1:1); Pd+YC: boza inoculated with *Pediococcus domnasus 2.6* + yoghurt culture (1:1); 3-5%: inoculation rates of starter cultures

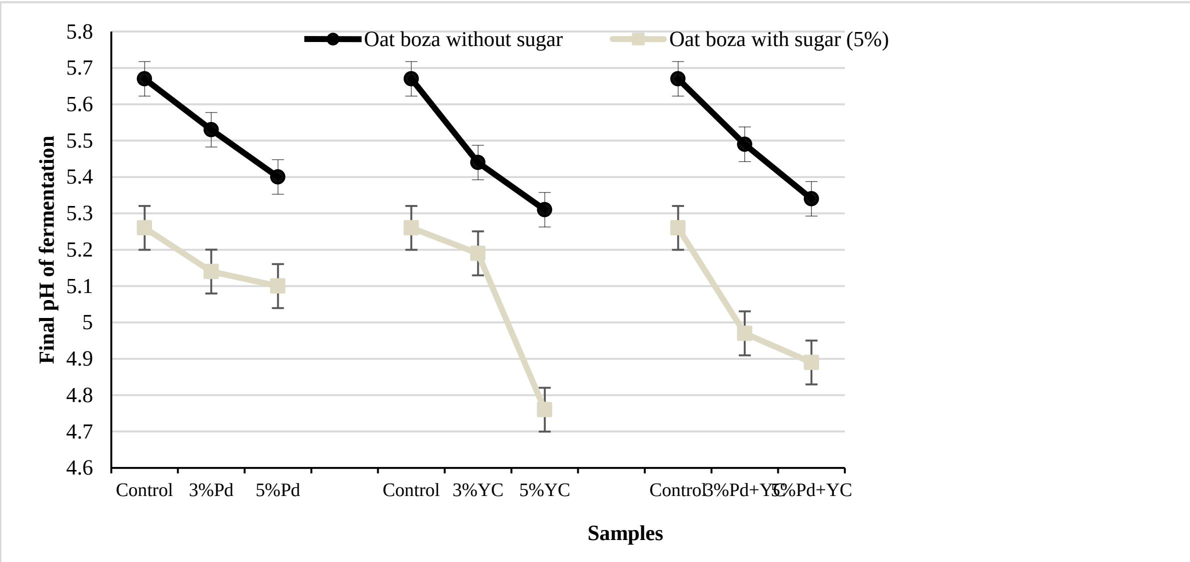

Figure 2
pH values of oat boza samples at the end of the fermentation

Pd: boza inoculated with *Pediococcus domnasus 2.6*; YC: boza inoculated with yoghurt culture (*S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*1:1); Pd+YC: boza inoculated with *Pediococcus domnasus 2.6* + yoghurt culture (1:1); 3-5%: inoculation rates of starter cultures

Crude ash content of oat boza are presented in Table 2. The highest crude ash value was determined in 5% Pd+YC sample to be 0.3944% (P<0.01). It was determined that adding sugar in boza formulation and increasing of amount of fermentation losses were proportionately decreased ash content of samples. These findings are in agreement with Aytekin (2001) who reported that as the sugar content increased in boza formulation, the ash contents of boza samples decreased. The protein contents of oat boza samples were determined to be between 3.32 and 3.81% (Table 2). While there were no statistical differences in protein contents between the boza samples which were inoculated different culture (P>0.05), the increasing inoculation rates and adding sugar were increased protein content of oat boza samples (P<0.01).

Table 2
Some chemical properties of oat boza

Sample	Titratable acidity (Lactic acid %)	Total solid matter (%)	Crude ash (%)[a]	Protein (%)[b]	Ethanol (g/100ml)
Control	0.28±0.01[d]	16.50±0.15[d]	0.3550±0.007[de]	3.62±0.04[bcd]	0.22± 0.01[bc]
3% Pd	0.30±0.07[cd]	16.44±0.15[d]	0.3682±0.005[cd]	3.68±0.04[abc]	0.13±0.03[de]
5% Pd	0.34±0.03[bcd]	16.21±0.07[d]	0.3934±0.006[ab]	3.70±0.04 [ab]	0.24±0.03[b]
3% YC	0.33±0.01[bcd]	16.55±0.21[d]	0.3722±0.006[bcd]	3.73±0.02[ab]	0.20±0.01[bcd]
5% YC	0.37±0.03[abcd]	16.11±0.11[d]	0.3874±0.002[abc]	3.81±0.01[a]	0.10± 0.01[e]
3% Pd+YC	0.33±0.04[bcd]	16.3±0.21[d]	0.3740±0.005[abcd]	3.63±0.04[bcd]	0.14±0.01[cde]
5% Pd+YC	0.34±0.04[bcd]	16.12±0.11[d]	0.3945±0.006[a]	3.70±0.04[ab]	0.17±0.02[bcde]
Control with sugar	0.38±0.01[abcd]	18.41±0.10[ab]	0.3250±0.007[fg]	3.32±0.04[f]	1.91± 0.01[a]
3% Pd with sugar	0.38±0.03[abcd]	17.90±0.04[c]	0.3241±0.004[g]	3.33±0.05[f]	1.86±0.041[a]
5% Pd with sugar	0.41±0.00[abc]	18.14±0.09[bc]	0.3422±0.004[efg]	3.54±0.04[cde]	1.86±0.01[a]
3% YC with sugar	0.39±0.06[abcd]	17.69±0.26[c]	0.3461±0.006[ef]	3.35±0.04[f]	1.89±0.02[a]
5% YC with sugar	0.43±0.04[abc]	17.95±0.06[bc]	0.3619±0.002[de]	3.51±0.03[de]	1.92±0.03[a]
3% Pd+YC with sugar	0.44±0.01[ab]	18.16±0.09[bc]	0.3525±0.006[de]	3.41±0.03[ef]	1.91±0.02[a]
5% Pd+YC with sugar	0.48±0.04[a]	18.68±0.11[a]	0.35845±0.006[de]	3.60±0.04[bcd]	1.92±0.02[a]

[a]in dry basis; [b]Protein = N x 6.25

Different letters indicate significantly different at P< 0.01

Pd: boza sample inoculated with *Pediococcus domnasus 2.6*; YC: boza inoculated with yoghurt culture (*S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*1:1); Pd+YC: boza inoculated with *Pediococcus domnasus 2.6* + yoghurt culture (1:1); 3-5%: inoculation rates of starter cultures

It could be revealed that increasing amount of inoculum and adding sugar supported fermentation of boza. Similar results were obtained in previous studies and an increase in amount of protein was observed in fermented products compared with non-fermented substrates (Hamad & Fields, 1979; Morcos, Hegazi, & El-Damhougy, 1973). Furthermore, Odunfa (1985) reported that increasing in the amount of protein during the fermentation could be resulted from proteinase activity of the fermentative microorganisms. The oat grain has higher protein content than other cereals and therefore it constitutes a good potential for protein source products (Pomeranz, 1975).

3.4. Titratable acidity of oat boza samples

Titratable acidity values of oat boza samples are shown Table 2. Titratable acidity values (as lactic acid) of boza were expressed to might be in between 0.2-0.5% according to TS 9778 standard of boza. Similar results were observed in oat boza samples and total acidity (in terms of lactic acid) ranged between 0.28-0.48%. While there was no statistically significant effect of culture type on titratable acidity values, adding sugar in oat boza samples before fermentation and inoculation rates of cultures had a significant effect on the amount of acidity of samples (P< 0.01). These results could be related to microbial activity of using cultures in fermentation of boza samples. Adding sugar (5%) in boza formulation could be supported growth of microorganisms. In parallel with our results, Üstün and Evren (1998) determined the acidity values of boza samples in between 0.242-0.448%. Besides, Salmerón, Thomas, and Pandiella (2015) reported that the oat beverages inoculated with *L. plantarum* and *L. acidophilus* had lactic acid at a concentration of 0.52 and 0.98 g/L, respectively. 0.3-0.5% lactic acid and carbon dioxide produced during fermentation gives aroma and refreshing feature in boza (Topal & Yazıcıoğlu, 1986).

3.5. Ethanol content of oat boza samples

The ethanol content of boza samples with (5%) or without sugar were determined in between 1.86-1.92 and 0.10-0.22 g/100mL, respectively (Table 2). Ethanol content increased significantly by increasing inoculation ratio of cultures in boza samples produced without the addition of sugar. On the other hand, increased inoculation rate of yoghurt bacteria decreased ethanol amount of the samples without sugar. In previous study, Kedia, Wang, Patel, and Pandiella (2007) determined that the ethanol production in mixed culture containing *Lactobacillus reuteri* and yeast was higher than the pure culture of yeast in a medium of 5% (w/v) malt suspension. Although, the highest ethanol content was determined in oat boza samples prepared with sugar addition, there were no statistical differences in ethanol contents of the samples with sugar. Adding sugar before fermentation supported metabolic activities of lactic acid bacteria and yeasts, so that the amount of ethanol in oat boza samples added sugar before fermentation was much higher than those without added sugar. This fact was also detected with an alcoholic odor and a bitter taste in boza samples with sugar. Salmerón et al. (2015) detected concentrations of the ethanol in fermented oat beverages, inoculated with *L. acidophilus, L. plantarum* or *L. reuteri* to be 0.67, 0.78 and 0.64 mg/L respectively. Likewise, Hancioğlu and Karapinar (1997) reported that alcohol content of boza samples increased from 0.02 to 0.79% during fermentation period (24h).

3.6. Viscosity of oat boza samples

Viscosity values of oat boza are shown in Figure 3. The viscosity values of boza samples with (5%) or without sugar were measured in between 14200-18150 and 18200-23150 Pa.s., respectively. The highest viscosity value (21150 Pa.s.) was observed in oat boza samples without sugar inoculated with yoghurt culture, however the lowest viscosity value (13900 Pa.s.) was determined in the oat boza prepared with 5% Pd inoculation with sugar addition. The fact that the adding sugar promoted growing of microorganisms during fermentation could be considered as the reason for the decrease in viscosity in the oat boza with sugar (Peyer, Zannini, & Arendt, 2016). While inoculation rates in boza samples increased, viscosity values of samples decreased significantly ($P< 0.01$). The use of gelatinize starch and derivatives in the medium by microorganisms as a nutrients could be shown as a reason for decreasing the viscosity values. The present results are consistent with the findings of Lambo, Öste, and Nyman (2005) who determined lower viscosity values in oat concentrate fermented by lactobacilli than non-fermented substrates.

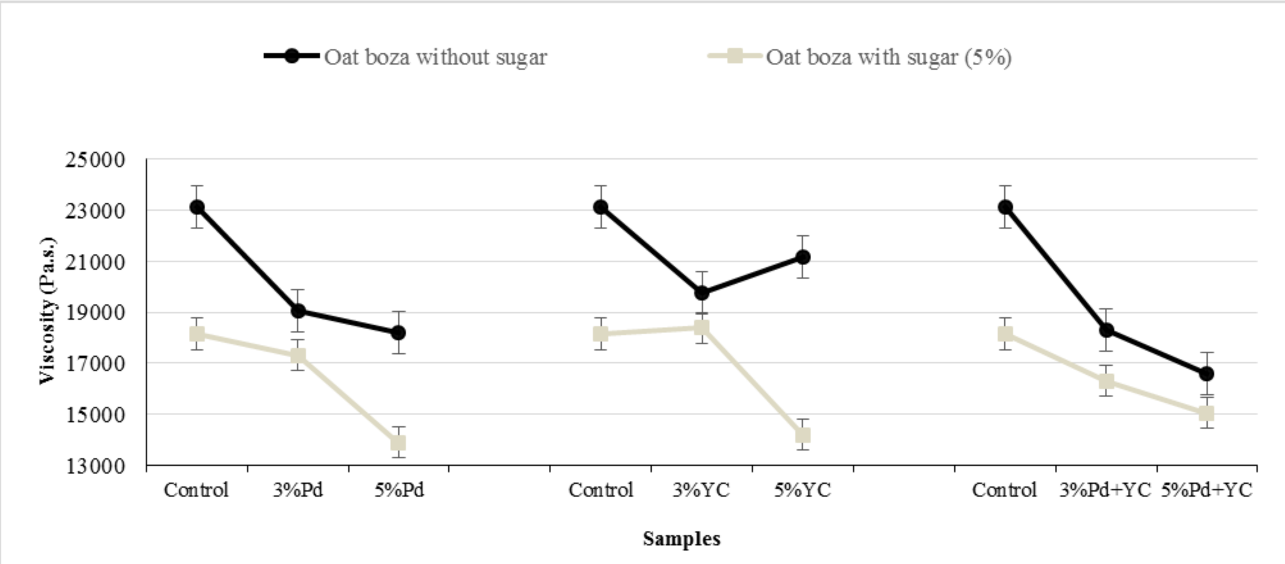

Figure 3
Viscosity values of oat boza samples

Pd: boza inoculated with *Pediococcus domnasus 2.6*; YC: boza inoculated with yoghurt culture (*S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*1:1); Pd+YC: boza inoculated with *Pediococcus domnasus 2.6* + yoghurt culture (1:1); 3-5%: inoculation rates of starter cultures

3.7. Sensory evaluation of oat boza samples

Sensory evaluation of oat boza that are presented in Figure 4. Sensory analysis was performed in oat boza samples that were 3% YC, 3% Pd and 3% Pd+YC without sugar addition before fermentation. Oat boza samples with adding sugar before fermentation presented bitter flavor and acid taste, so that these oat boza samples did not evaluated in terms of sensory characteristics. 3% Pd samples had the highest score in terms of mouthfeel ($p<0.05$). The odor scores observed for all boza samples were quite similar. However, the difference between color scores of 3% YC and 3% Pd+YC samples was statistically insignificant and their scores were higher than 3% Pd. The highest overall acceptability score was determined in the 3% YC samples. According to sensory evaluation score, 3% YC samples without sugar was the most preferred yoghurt samples for consumers.

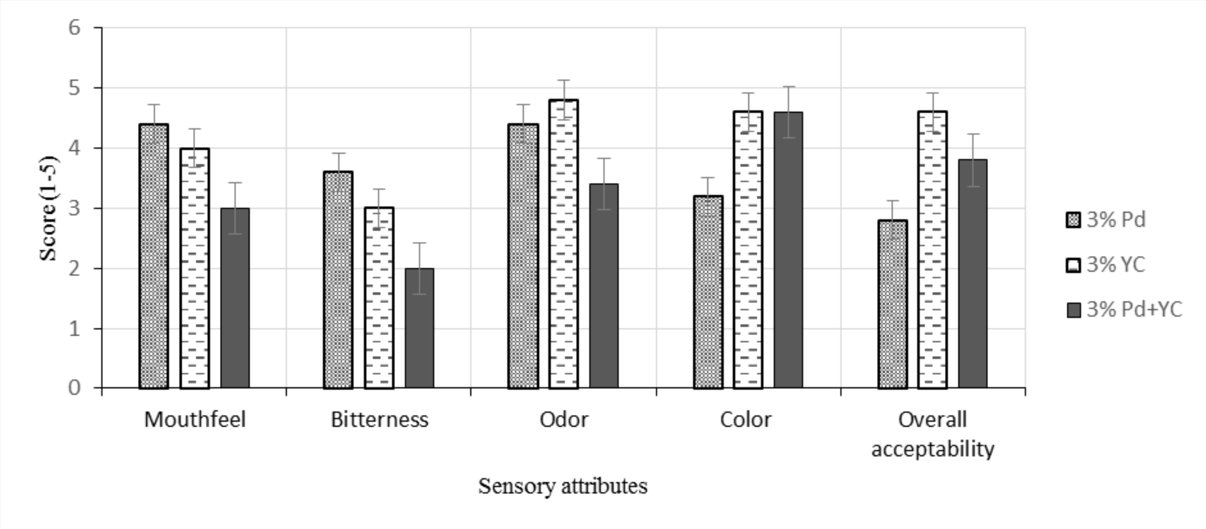

Figure 4
The sensory evaluation of oat boza samples

Pd: boza inoculated with *Pediococcus domnasus 2.6*; YC: boza inoculated with yoghurt culture (*S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*1:1); Pd+YC: boza inoculated with *Pediococcus domnasus 2.6* + yoghurt culture (1:1); 3-5%: inoculation rates of starter cultures

4. Conclusions

In this study, sugar addition into the boza before fermentation caused excessive alcohol production and bitter taste as a sensorial. *Pediococcus damnosus* 2.6, which is exopolysaccharide producer, obtained the highest mouthfeel score with smooth texture despite the low viscosity, but it did not give the desired palate in terms of overall acceptability. On the other hand, 3% YC samples gave optimal results and they got the highest overall acceptability scores by panelists. Raw oat has unpleasant odors and flavors, but fermentation improve the sensorial properties of oat product. In this context, fermentation of oat by lactic acid bacteria and yeast is thought to increase the consumption of oat as a human diet. Considering the scores of sensory and technological properties of produced oat boza samples, it can be proposed inoculation of yoghurt cultures at a rate of 3% without adding sugar for the best formulation of oat boza.

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