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The effect of exopolysaccharide producer *Pediococcus damnosus* 2.6 and probiotic *Lactobacillus acidophilus* on ethanol content, some physico-chemical and sensory properties of oat boza

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Abstract

Exopolysaccharide producer *Pediococcus damnosus* 2.6, yoghurt culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) and mixed culture (*Pediococcus damnosus* 2.6+ yoghurt culture 1:1) at different inoculation levels (0, 3 and 5%), with sugar or not were used to produce oat boza. The effects of sugar, different microorganisms and inoculum ratios on physicochemical, nutritional and sensory characteristics of oat boza were investigated. To produce gas, in ratio of 1% of *Saccharomyces cerevisiae* and 0.5% of *Lactobacillus acidophilus* as a probiotic was also added all boza formulation as constant ratio. As a result of using mixed culture and rising inoculation rates in formulation of boza developed fermentation activity caused to reduce amount of total solid matter. Additionally, this status led to relatively increase ($p<0.01$) in the contents of ash, protein and mineral matter of samples. Although the lowest viscosity was determined in *Pediococcus damnosus* 2.6 inoculation, the most uniform texture was provided.

Keywords: Fermentation, Boza, Oat, Viscosity, Ethanol

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 [1]. Cereal-based fermented beverages are known functional and probiotic foods because they have nutrimental and health promoting components such as nutritional element, fibers and phytochemicals. In cereal based fermented product, fermentation enhance protein digestibility, nutritional bioavailability and organoleptic properties [2].

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). Groats and 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 [3].

Oat is a fundamental cereal crop and is used commonly for the feeding of farm animals. However, in recent years oat utilized in human diets due to it has various 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 content [4]. The oat contains starch approximately up to 60% of the dry weight. The lipid content with a high content of unsaturated fatty acids of oat kernel is two-five times as high as that of most other cereals. They have high protein content ratio of 9-15% with a high lysine concentration. Oats are a grateful antioxidant resource [5]. The health promoting effects of oats associated with β-glucan contents (2-8%) that had the ability to reduce blood cholesterol and glucose

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levels [6]. Oat products like rolled oats, oatmeal and oat milk are used to prepare human diets such as ready-to-eat breakfast cereals, baby foods, bread, cookies, and snacks [7].

In this study, it was aimed to improve nutritional and functional properties of oats that was fermented by *Lactobacillus acidophilus* to be a probiotic, *Pediococcus damnosus* 2.6 was able to produce exopolysaccharides, yoghurt bacteria were able to produce lactic acid and baker's yeast (*Saccharomyces cerevisiae*) was able to produce CO_2 and to increase their consumption. The effects of various microorganisms used in boza production were investigated on the technological, chemical and sensory properties of the oat boza.

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 2% of UHT milk and incubated for the second time at 42 ˚C for 24 h [8].

Yoghurt bacteria (*S. thermophilus* + *L. delbrueckii* ssp. *bulgaricus* 1:1) were obtained from milk factory as an active form. The active culture were inoculated into 2% UHT milk 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 [9].

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 were inoculated into 2% sterile MRS broth 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 [10].

2.2.2. Production of oat boza samples

Oat boza was produced with reference to Hayta, Alpaslan [11] with some modification. 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 in 5 times as much water as (w/v) the amount of their weights and slurry was boiled for 1 hour under continuous stirring.

This mash was kept at 4-6 ˚C for 7-8 h to be cool. 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 granulated sugar (saccharose). 1% of yeast (*Saccharomyces cerevisiae*) so as to produce gas and 0.5 % of *Lactobacillus acidophilus* as probiotic culture 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 tasting (performed sensory analysis), 10% granulated sugar (saccharose) was added in boza samples for sweetening.

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 [12]. Mineral matter content of the oatmeal were assessed by the method described by Skujins [13].

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 [3]. Potentiometric titration techniques were used for quantification of total titratable acidity of samples in lactic acid [14].

2.2.5. Ethanol content of oat boza samples

The determination of ethanol in oatmeal boza samples was performed according to TS 1594 [15]. 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 [16]. 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. The protein and crude ash contents of the hulless 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 the hulless oat kernel [17]. Kirk and Sawyer [18] 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.

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

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 progressively decreased during fermentation. At the end of the fermentation, pH values of samples were observed in between 5.67-4.76. ($P< 0.05$). Whereas the highest pH value was determined in control (0%) without sugar, the lowest pH value was observed in YC5% with sugar (Fig.1-2).

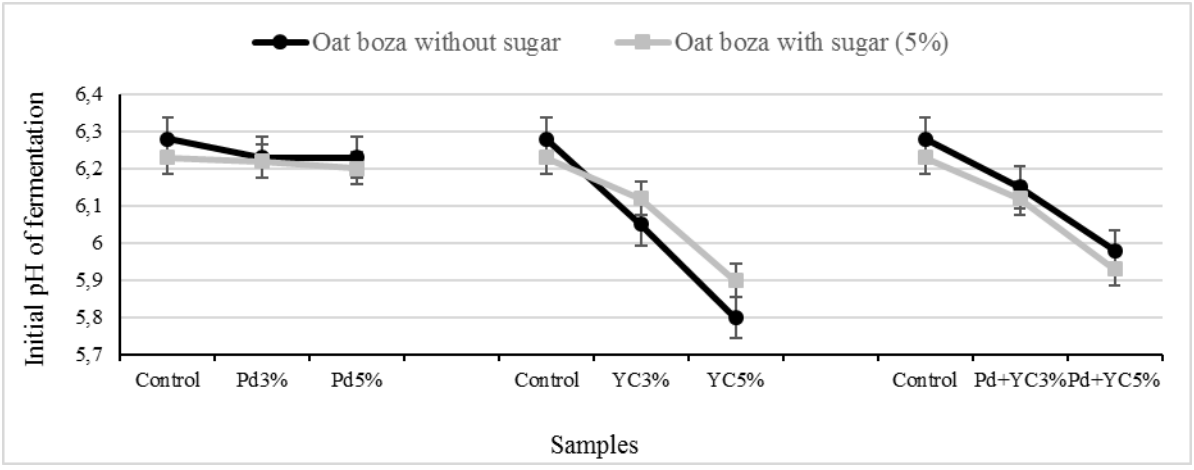

Figure 1. pH values of oat boza samples at the beginning of the fermentation

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

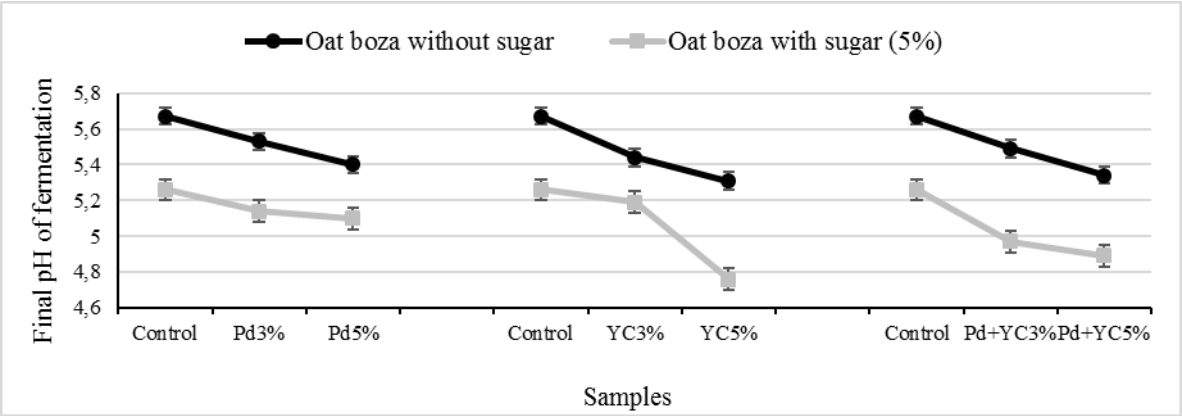

Figure 2. pH values of oat boza samples at the end of the fermentation

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

Adding sugar was speeded up fermentation of boza and it supported to be formed lactic acid that provided to reduce quickly in pH values. Similarly, Hancioğlu and Karapinar [19] and Gotcheva, Pandiella [20] reported that pH values 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 amounts of total solid matter in samples were determined to be between 156.11 and 18.68%. There was significant effect of sugar addition before fermentation on total solid matter contents of oat boza samples ($p< 0.01$). Total solid matter contents of oat boza added sugar before fermentation were found to be higher than samples without sugar before fermentation. However, it was found that culture types and inoculation rates used in boza production were not effective on the total solid matter values of oat boza samples.

Crude ash values of oat boza are presented in Table 2. The highest crude ash value was determined in Pd+YC 5% 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 [21] 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 significantly different ($p< 0.01$) (Table 2). While there were no statistically significant 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 quantity of protein of oat boza samples ($p< 0.01$). It could be said that increasing amount of inoculum and adding sugar supported fermentation of boza. An increase in amount of protein was observed in fermented products compared with non-fermented substrates [22, 23]. Odunfa [24] 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 [25].

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.

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}
Pd3%	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}
Pd5%	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}
YC3%	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}
YC5%	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}
Pd+YC3%	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}
Pd+YC5%	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}
Pd3% 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}
Pd5% 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}
YC3% 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}
YC5% 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}
Pd+YC3% 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}
Pd+YC5% 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
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; Different letters indicate significantly different at p< 0.01

Adding sugar (5%) in boza formulation could be supported growth of microorganisms. Similar results were seen in previous studies. Üstün and Evren [26] determined the acidity values of boza samples in between 0.242-0.448 %. Salmerón, Thomas [27] 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 [28].

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). No statistically differences were observed in ethanol contents between the boza samples which were inoculated different culture (p> 0.05), however the increasing inoculation rates and adding sugar in boza formulation were increased amount of ethanol (p< 0.01). Sugar that was added as a nutrient for lactic acid bacteria and yeasts in media supported metabolic activities of microbial flora. It was found 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 a pungent odor and a bitter taste in boza samples with sugar. Salmerón, Thomas [27] 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. Hancioğlu and Karapinar [19] reported that alcohol content of boza samples increased from 0.02 to 0.79% during fermentation period (24h). Boza was a non-alcoholic fermented beverage that had 0.5% (v/v) ethanol content at the end of fermentation [20].

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. It could be said that the differences between viscosity values were statistically significant in sugar addition, culture type and inoculation rate applications (p< 0.01).

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. 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% *Pediococcus damnosus* 2.6 inoculation with sugar addition. While inoculation rates in boza samples rose, viscosity values of samples decreased significantly (p< 0.01). The use of microorganisms as a nutrients of gelatinize starch and derivatives in the medium could be shown as a reason for decreasing the viscosity values while the amount of inoculated culture increased in formulation. Similarly, Lambo, Öste [29] determined lower viscosity values in oat concentrate fermented by lactobacilli than non-fermented substrates.

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 YC3%, Pd3% and Pd+YC3% without sugar addition before fermentation because bitter flavor and acid taste was detected in oat boza samples with sugar addition before fermentation. Pd3% samples had the highest score in terms of mouthfeel ($p<0.05$). While there was no difference between the overall acceptability and color scores of YC3% and Pd+YC3% samples, they received appreciation more than Pd3% samples.

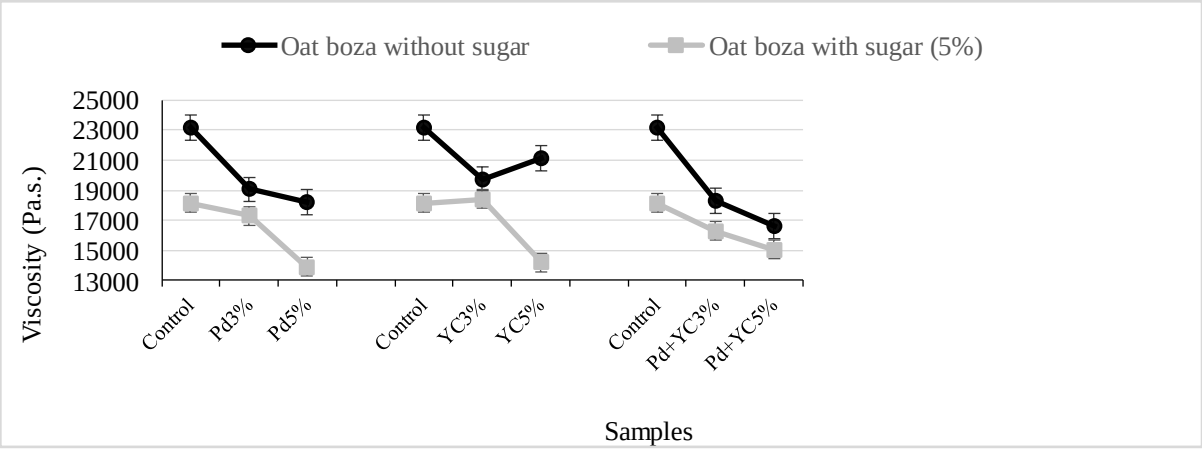

Figure 3. Viscosity values of oat boza samples

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

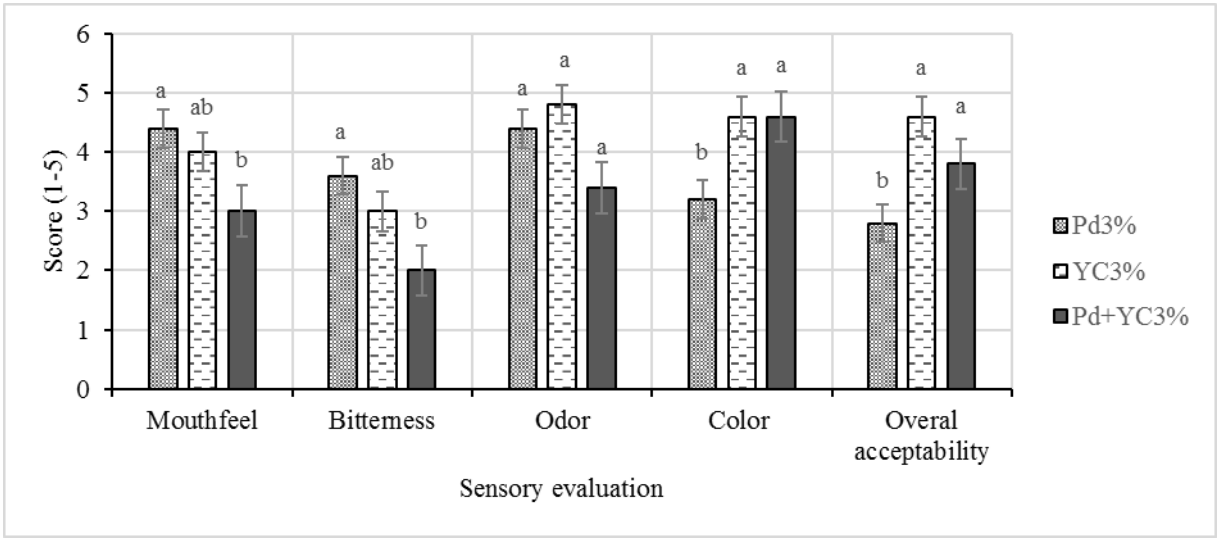

Figure 4. The sensory evaluation of oat boza samples

Pd3%: boza inoculated with 3% *Pediococcus domnasus 2.6*; YC3%: boza inoculated with 3%yoghurt culture (*S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*1:1); Pd+YC3%: boza inoculated with 3% *Pediococcus domnasus 2.6* + yoghurt culture (1:1); Different letters indicate significantly different at $p< 0.01$

4. Conclusions

Sugar additive before fermentation as a cause of excessive alcohol production and bitter taste gave negative results. As a result, added sugar before fermentation for supporting exopolysaccharide production did not provide taste and composition of traditional boza and it was understood that sugar additive was not necessary in boza production. *Pediococcus damnosus* 2.6, which was tested with exopolysaccharide production capability, 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. YC3% samples gave optimal results and they won the highest recognition by panelists in terms of overall acceptability scores.

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.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

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