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Oat, Buckwheat and Whole Brown Rice Flours as a Potential Prebiotic for *Lactobacillus acidophilus* (LA5), *Lactobacillus casei and Bifidobacterium animalis subsp. lactis* (BB-12)

Lactobacillus acidophilus (LA5), Lactobacillus casei ve Bifidobacterium animalis subsp. lactis (BB-12) için Potansiyel Prebiyotik Olarak Yulaf, Karabuğday ve Kepekli Pirinç Unları

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Araştırma Makalesi

Oat, Buckwheat and Whole Brown Rice Flours as a Potential Prebiotic for *Lactobacillus acidophilus* (LA5), *Lactobacillus casei and Bifidobacterium animalis subsp. lactis* (BB-12)

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Makale Bilgisi

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Abstract

The object of this study was to determine whether oat, buckwheat and whole brown rice flours have a prebiotic effect on *Lactobacillus casei, Lactobacillus acidophilus* (LA5), and *Bifidobacterium animalis* subsp *lactis* (Bifidobacterium BB-12). In the study, skimmed milk was inoculated with *the above-mentioned bacteria* fortified with oat, buckwheat and whole brown rice flours. According to the results, oat and buckwheat flours stimulated all bacteria investigated, whereas whole brown rice flour had no prebiotic effect for *L. casei.*

Anahtar Kelimeler

Lactobacillus acidophilus (LA)
Lactobacillus casei
Bifidobacterium BB-12
oat
buckwheat
whole brown rice flours

Lactobacillus acidophilus (LA5), *Lactobacillus casei ve Bifidobacterium animalis subsp. lactis* (BB-12) için Potansiyel Prebiyotik Olarak Yulaf, Karabuğday ve Tam Esmer Pirinç Unları

Öz

Bu çalışmanın amacı yulaf, karabuğday ve tam esmer pirinç unlarının *Lactobacillus casei, Lactobacillus acidophilus* (LA5*)* ve *Bifidobacterium animalis* subsp. *lactis* (Bifidobacterium BB-12) için prebiotik etkisi olup olmadığını belirlemektir. Çalışmada yulaf, karabuğday ve tam kahverengi pirinç unları ile zenginleştirilmiş yağsız süt yukarıda belirtilen bakterilerle aşılanmıştır. Sonuçlara göre yulaf ve karabuğday unları araştırılan tüm bakterileri uyarırken, tam esmer pirinç unu *L. casei* için prebiyotik etki göstermemiştir.

Keywords

Lactobacillus acidophilus (LA)
Lactobacillus casei
Bifidobacterium BB-12
Yulaf
Karabuğday
Tam kahvrerengi

1. INTRODUCTION (GİRİŞ)

Recently, the inclusion of probiotic organisms (especially *Lactobacillus acidophilus* and/or bifidobacteria) in fermented milks has greatly increased, owing to the health benefits attributed to them. The addition of probiotic bacteria is done for two reasons. The first is due to the specific health-promoting effects noted in the intestinal tract, the second is due to the sensory advantages and the increased variety of products that can be formulated with them [1,2] .

Lactobacillus acidophilus is a commercial probiotic strain that is widely utilized in the dairy industry to obtain high-quality fermentation products [3,4,5]. *Lactobacillus acidophilus* LA-5 has the ability to reduce

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the serum cholesterol level, balance and stabilize the enteric microbiota, stimulate an immune response, improve lactose digestion, and potentially kill cancer cells [6,7,8]. *Lactobacillus casei* is another probiotic bacteria which has excellent antibacterial properties [9,10]. When included in the diet, it can reduce alleviate lactose intolerance, gastrointestinal diseases, allergies, or cancer [11]. *Bifidobacterium animalis* subsp**.** ***l***actis (BB-12) is a Gram (+) bacterium that originates in the large intestine of most mammals. Depending on the strain consumed, various health benefits can be conferred, such as prevention of antibiotic-associated diarrhoea, relief from lactose intolerance, and treatment of inflammatory bowel disease, whereas other health claims pertain to the general well-being, such as management of obesity, immune stimulation and modulation and decrease of cholesterol level [12,13,14].

Due to the sensitivity of *Lactobacillus acidophilus* LA-5, L. casei and *Bifidobacterium animalis* subsp. *l*actis (BB-12) against gastrointestinal system conditions and food processing, physical barrier or stimulating substances need to be added into food in order to maintain their viability and increase their tolerance to adverse conditions [2,10,15].

Prebiotics can be defined as non-digestible food ingredients that improve the host health via selectively stimulating the activity and/or growth of one or more bacterial strains in the colon. The main reason why many oligosaccharides are added to foods as prebiotics is that they provide preferential growth of probiotic organisms [16].

Today, the main prebiotic food components are fructooligosaccharides (FOS), inulin, oligofructose, glucooligosaccharides, galactooligosaccharides (GOS), trans galactooligosaccharides, isomaltooligosaccharides (IMO), isomaltooligosaccharides, polydextrose, pyro dextrin, raftiline, raffinose [17,18,19].

Fructose, whey protein concentrates, casein hydrolysates, papaya pulp and, tomato juice which stimulate *Lactobacillus acidophilus*, and tryptophan, cysteine, vitamins, acid hydrolysates, maltose and dextrin, which stimulate *Bifidobacteria,* are also considered as prebiotic [20].

One of the methods used to increase the number of live bacteria in probiotic foods, is to add a prebiotic ingredient to the food. For this purpose, the prebiotic effects of a lot of compounds are being investigated.

Oats *Avena* L. (Poaceae) is a cereal variety belonging to the *Gramineae* family of the Aveneae class and is a newer cultivated plant compared to wheat and barley. Although its origin is not known precisely, the most genetic diversity is seen in the Mediterranean, Middle East and Himalayan Regions. The average compositions of groats and oats were 9.6 % and 7.6 % moisture, 12.6 % and 13 % protein, 4.9 % and 7.7 % fat, 1.9 % and 1.7 % ash, 48.1 % and 31.6 % starch, 4.1 % and 3.8 % β- glucan, 17.6 % and 22.9 % total dietary fiber, 14.3 % and 17.6 % soluble fiber, respectively. Husked and oats contain an average of 500.4 and 520.4 mg kg-1 calcium, 47.1 and 200.5 mg kg-1 sodium, 26.5 and 32.1 mg kg-1 zinc, 7.8 and 9.5 mg kg-1 vitamin E, respectively [21].

Buckwheat, which shows both similarities and differences with grains and belongs to the pseudo-cereal (grain-like) group, is in the *Polygonaceae* family. The average composition of buckwheat ranges from moisture 9.6-13.8%, starch 55-75%, protein 10.0-12.5%, lipid 1.4-4.7%, ash 1.3-2.3%, fibre 7.0-10.7% and other components 15-20%. Containing high nutritional value protein and significant amounts of dietary fibre, vitamins (B1, B2 and E vitamins) antioxidants (Rutin and quercetin) and mineral substances, buckwheat has a high potential, especially for the functional food industry. The starch and fibre content of buckwheat is almost the same as grains and contains a high proportion of essential polyunsaturated fatty acids (such as linoleic acid). The buckwheat protein has high nutritional quality as it contains all essential amino acids (such as threonine, lysine, tryptophan) in its composition. However, tannins have a low digestibility due to their containment of phytic acid α-amylase and protease inhibitors [22,23,24,25].

Brown rice is grown in Southeast Asian countries such as Thailand, India, and China. It is called black rice because of its anthocyanin (cyanidin-3-glucoside) content. The composition of brown rice ranges from 10.64-13.0% moisture, 73.93-76.20% carbohydrates, 9.15-10.20% protein, 2.15-3.41% lipid, 1.0-1.80% ash and 3.83-4.32% fiber [26]. The rice bran layer has an intense content of pharmacological components such as anthocyanins, ɤ-oryzanol, flavonoid and phenolic components. Many studies have demonstrated that these components are anti-inflammatory and anticarcinogenic, can improve lipid profiles [27], and reduce oxidative stress [27], prevent diabetes and help combat heart disease [28]. It contains a higher concentration of amino acids than regular rice. Brown rice has high asparagine, apratic and glutamic contents, while cysteine and tryptophan amino acids are absent [29,30].

Oat flour (OF), buckwheat flour (BF) and whole brown rice flour (WBRF) are predicted to be potentially prebiotic due to above mentioned fibers in their structure. Thus in this study prebiotic effect of oat flour, buckwheat and whole wheat brown rice flour on *Lactobacillus casei, Lactobacillus acidophilus* (LA5) and *Bifidobacterium animalis* subsp. *lactis (Bifidobacterium* BB-12) grown in reconstituted skim milk was investigated.

2.MATERIALS AND METHODS

As materials *Lactobacillus acidophilus* (LA5), *Lactobacillus casei* and *Bifidobacterium* BB-12 (Chr. Hansen, Turkey), skim milk powder (Pınar Dairy, Türkiye), oat flour, buckwheat flour and whole wheat brown rice flour (Tito, Türkiye) were used. Man Ragosa Sharpe (MRS) agar sorbitol, lithium chloride, neomycin sulphate, MRS-broth, agar, nalidixic acid and paromomycin sulphate obtained from Sigma chemicals (İstanbul, Turkey). All other chemicals utilized were of analytical grade.

Thirtynine different reconstituted skim milks containing 12% dry matter were prepared. For propagation of *probiotic bacteria*, each sterile reconstituted skim milk was fortified with 0.25%, 0.50%, 1% and 2% OF, BF and WBRF. Milks were fermented at 37°C until pH reached to 4.7. Milks were inoculated by using probiotic bacteria at a rate of 0.05% (w/v). Bacterial counts were carried out after 8 h fermentation. The milks were kept in the refrigerator at 4°C until enumeration. The trial was conducted in triplicate.

A sample of 1 mL of fermented milks were diluted in sterile peptone water (0.1%) and plated over the differential and selective agars. *L. casei, L. acidophilus* and *Bifidobacterium* BB-12 were grown in MRS plus sorbitol [32,37], MRS-Broth [34] agar, and MRS-NNLP [35] agar, respectively by incubating the plates anaerobically at 37°C as intructed by the supplier. Anaerobic environments were created utilizing Anaerocult A sochets (Merck). The outcomes were stated as colony-forming units per gram (CFU g^{-1}) of the sample.

The pH values of the fermented milks were determined utilizing a digital pH meter. Statistical analysis was performed using SPSS Version 21.0 (SPSS Inc. Chicago, IL, USA). Duncan's Multiple Range Test was utilized to determine the statistically different groups [36].

3. RESULTS AND DISCUSSION

The type of probiotic bacteria and the type of vegetable flours significantly affected the incubation time ($p<0.05$). The shortest fermentation time was obtained with milk with *L. acidophilus* (~3.5 hours), followed by the milks inoculated with *Bifidobacterium* BB-12 (~6 h) and *L.casei* (~12 h), respectively. Use of *L acidophilus* and *Bifidobacterium* BB-12 have shortened the fermentation time by approximately 3.7-20% and 3.95-11.9%, respectively when the portion of OF, BF and WWBRF increased up to 2%. On the other hand, BF has shortened the fermentation time by 10% for milks fermented by *L. casei,* while the other flours had no effect. However, fermentation time for milks fermented by *L. casei* didn't change depending on the flour ratio. These results indicated that OF, BF, and WWBRF stimulated probiotic bacteria used in the experiment.

Table 1. Fermentation time to reach pH 4.7 for milks fermented by L. acidophilus (LA5), L. casei and Bifidobacterium BB-12 supplemented with Oat Flour, Buckwheat Flour and Whole Brown Rice Flour*

Bacteria	Flour rate (%)	Oat flour (OF)	Buckwheat flour (BF)	Whole wheat brown rice flour (WWBRF)
		Fermentation time (min)	**Fermentation time (min)**	**Fermentation time (min)**
***L. acidohilus* (LA5)**	0	270 ± 2^{a1}	271 ± 1^{a1}	270 ± 2^{a1}
	0.25	260 ± 3^{a2}	257 ± 3^{a2}	264 ± 3^{a2}
	0.5	240 ± 1^{a3}	238 ± 4^{a3}	246 ± 1^{a3}
	1	210 ± 2^{a4}	210 ± 1^{a4}	205 ± 2^{a4}
	2	220 ± 3^{a4}	219 ± 2^{a4}	224 ± 2^{a4}
L. casei	0	720 ± 4^{a1}	719 ± 4^{a1}	720 ± 1^{a1}
	0.25	721 ± 5^{a1}	647 ± 3^{b2}	719 ± 3^{a1}
	0.5	720 ± 3^{a1}	644 ± 5^{b2}	720 ± 2^{a1}
	1	718 ± 2^{a1}	650 ± 3^{b2}	720 ± 1^{a1}
	2	719 ± 3^{a1}	645 ± 2^{b2}	721 ± 3^{a1}
***Bifidobacterium* BB-12**	0	480 ± 2^{a1}	481 ± 3^{a1}	479 ± 2^{a1}
	0.25	460 ± 3^{a2}	462 ± 2^{a2}	450 ± 1^{a2}
	0.5	445 ± 2^{a3}	447 ± 3^{a3}	431 ± 3^{a23}
	1	432 ± 1^{a4}	431 ± 1^{a4}	422 ± 2^{a3}
	2	450 ± 4^{a3}	449 ± 2^{a3}	443 ± 3^{a2}

* Different letters and numbers indicate significant differences among the samples, depending on flour type and level, respectively ($p<0.01$).

Table 2 shows the variations in *L. acidophilus* (LA5), *L. casei* and *Bifidobacterium* BB-12 numbers in reconstituted skim milk with different vegetable flours. The viable numbers of *L. acidophilus* (LA5), *L. casei* and *Bifidobacterium* BB-12 were determined to be between 8.54-10.10, 9.19-10.49 and 7.31-8.21 log CFU g^{-1}, respectively.

OF had a prebiotic effect for all probiotic bacteria investigated ($p<0.01$). The highest prebiotic effect was obtained with 2 % of fortified milk for *L. acidophilus* (LA5) and *L.casei*, and 1% for *Bifidobacterium* BB-12. It is thought that the high percentage of β-glucan and dietary fibres, vitamins and mineral substances in the composition have a positive effect on the growth of bacteria used in the research. The capability of probiotic organisms to break down and utilize β-glucan has been reported in previous studies [33,35,37,38]. Additionally, the number of *L. acidophilus* (LA5) *L.casei*, and *Bifidobacterium* BB-12 significantly was affected by OF level ($p<0.01$). The numbers of *L. acidophilus* (LA5), *L.casei* and *Bifidobacterium* BB-12 increased up to 1% OF and then began to decrease due to the possible decrease in water activity of milk. [39] reported that OF has a high-water absorption capacity due to their pregelatinized starch, gluten, and β-glucan. [35] reported that as the β-glucan ratio added to apricot fermented milk drinks increased, the number of probiotic bacteria increased.

Table 2. Viable counts of L. casei, L. acidophilus (LA5) and Bifidobacterium BB-12 in reconstituted skim milk with Oat Flour, Buckwheat Flour and Whole Brown Rice Flour (log kob g-1)

Bacteria	Flour rate (%)	Oat flour (OF)	Buckwheat flour (BF)	Whole wheat brown rice flour (WWBRF)
***L. acidohilus* (LA5)**	0	8.54±0.06^{a1}	8.55±0.06^{a1}	8.54±0.06^{a1}
	0.25	9.00±0.01^{a2}	9.09±0.04^{a2}	8.97±0.01^{a2}
	0.5	9.45±0.03^{a3}	9.50±0.04^{a3}	9.03±0.04^{a3}
	1	10.11±0.08^{a4}	10.10±0.06^{a4}	9.72±0.00^{a4}
	2	9.82±0.04^{a4}	9.84±0.00^{a4}	9.64±0.02^{a4}
L. casei	0	9.22±0.02^{a1}	9.22±0.01^{a1}	9.23±0.01^{a1}
	0.25	9.19±0.04^{a1}	10.10±0.05^{b2}	9.24±0.01^{a1}
	0.5	9.25±0.02^{a1}	10.45±0.01^{b2}	9.19±0.07^{a1}
	1	9.82±0.01^{a2}	10.49±0.01^{b2}	9.23±0.01^{a1}
	2	9.49±0.03^{a1}	10.21±0.01^{b2}	9.16±0.04^{a1}
***Bifidobacterium* BB-12**	0	7.31±0.04^{a1}	7.31±0.02^{a1}	7.31±0.02^{a1}
	0.25	7.82±0.01^{a2}	7.88±0.04^{a2}	7.92±0.02^{a2}
	0.5	7.97±0.03^{a3}	7.96±0.03^{a23}	8.07±0.03^{a23}
	1	8.00±0.01^{a23}	8.00±0.01^{a3}	8.21±0.09^{a3}
	2	7.87±0.04^{a23}	7.95±0.01^{a23}	8.17±0.01^{a23}

* Different letters and numbers indicate significant differences among the samples, depending on flour type and level, respectively (p<0.01).

BF also had a prebiotic effect for all probiotic bacteria investigated (p<0.01). The higher inulin and pea fibre added, the more *Bifidobacterium* BB-12 enumerated up to 1% BF and then a decrease was observed. This result is thought to be related to the fact that the fermentable carbohydrates, proteins and degradation products of proteins, nucleic acids, unsaturated fatty acids and mineral substances such as magnesium, iron and manganese in BF added in low proportions stimulate the growth of bacteria. It could be speculated that the growth of probiotic bacteria may be adversely affected due to the increase in the anti-nutritional components such as phytic acid and saponins in its composition and the binding of water by the fibers in its structure as the rate of BF added to milk increases. It has been found that a diet rich in buckwheat promotes the activity and growth of Bifidobacteria and Lactobacilli in the large intestine [40].

WWBRF stimulated *L. acidophilus* (LA5) and *Bifidobacterium* BB-12 (p<0.01), while it had no prebiotic effect for *L. casei* (p>0.05). Zhu et al. (2018) reported that WWBRF had a prebiotic effect for *L.acidophilus and Bifidobacteria*, as their-glucosidase enzyme can use anthocyanins by breaking down. The viable counts of *L. acidophilus* (LA5) and *Bifidobacterium* BB-12 increased up to 1% WWBRF and then began to decrease. This could be related to the decrease in the water activity of the environment with the increasing flour ratio and/or the increase in the concentration of inhibitor substances (such as polyphenol, trypsin and alpha-amylase inhibitors) in its composition.

4. CONCLUSION

According to the results, the type of bacteria, the type of vegetable flour and level of flour significanltly affected fermentation time. The shortest fermentation time was obtained with milk inoculated with *L. acidophilus* (~3.5 hours), the longest fermentation time was obtained with *L.casei* (~12 h). Addition of OF, BF and WWBRF shortened fermentation time compared to the control samples. As the flour ratio increased up to 1%, the fermentation time was shortened.

Results showed that the results OF and BF flour stimulated all bacteria investigated, whereas whole wheat brown rice flour had no prebiotic effect for *L. casei.* On the other hand, the use of these flours at a rate of 2% had negatively affected bacterial counts. These flours can be added to functional food without any significant changes in the production steps. However, to design new functional foods supplied with these vegetable flours it is necessary to determine the sensory properties of the foods too.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of the paper.

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