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Investigation of the effect of kefir culture use on some microbiological and sensory properties in gluten-free bread production

Glutensiz ekmek üretiminde kefir kültürü kullanımının bazı mikrobiyolojik ve duyusal özelliklere etkisinin incelenmesi

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Abstract

This study was carried out to examine the effect of using kefir culture as baker's yeast on some sensory quality parameters of gluten-free bread and to offer a more consumable gluten-free bread option to individuals with celiac disease and gluten intolerance. *Lactococcus* sp. count, Total Bacteria Count and mold-yeast enumeration were made in gluten-free bread dough containing kefir culture (test bread) and control bread. The pH value of the the sample was measured with a pH meter. Sensory analyzes were performed on the test and control breads by 11 nutritionist panelists. The results were evaluated with the SPSS 23.0 program. As a result of the sensory analysis, the test bread scored significantly higher than the control bread in terms of volume, shape symmetry, texture, mouthfeel, odor, aroma, taste, general control and preferability ($p<0.001$). The *Lactococcus* sp. count was 3.3 log cfu/g, the total bacteria count was 4.25 log cfu/g, the mold-yeast count was 4.37 log cfu/g and the pH value was determined as 4.62 in the test bread. The use of kefir culture has made significant contributions to the sensory properties of gluten-free bread and has turned gluten-free bread into a more consumable and preferable form.

Özet

Bu çalışma, pişirme mayası olarak kefir kültürü kullanımının glutensiz ekmeğin bazı duyusal kalite parametrelerine etkisini incelemek ve çölyak hastaları ile gluten intoleransı bulunan bireylere daha tüketilebilir formda glutensiz ekmek seçeneği sunmak amacıyla yürütülmüştür. Kefir kültürü içeren glutensiz ekmek (çalışma ekmeği) hamurunda ve kontrol ekmeğinde *Lactococcus* sp., Toplam Aerobik Bakteri ve küf-maya tayini yapılmıştır. Çalışma ekmeğinin pH metre ile pH değeri belirlenmiştir. Çalışma ve kontrol ekmeklerine beslenme uzmanlarından oluşan 11 kişilik panelist tarafından duyusal analizler yapılmıştır. Sonuçlar SPSS 23.0 programı ile değerlendirilmiştir. Yapılan duyusal analizler sonucu çalışma ekmeği hacim, şekil simetrisi, tekstür, ağızda hissedilen yumuşaklık, koku, tat, aroma, satın alınabilirlik ve genel kontrol açısından kontrol ekmeğinden anlamlı derecede daha yüksek puan almıştır ($p<0,001$). Çalışma ekmeğinde laktokok sayısı 3,3 log kob/g, toplam bakteri sayısı 4,25 log kob/g, küf-maya sayısı ise 4,37 log kob/g olarak bulunmuştur. Ayrıca pH değeri 4,62 olarak saptanmıştır. Kefir mayası kullanımı glutensiz ekmeğin duyusal özelliklerine önemli katkılar sağlayarak glutensiz ekmeği daha tüketilebilir ve tercih edilebilir bir forma dönüştürmüştür.

INTRODUCTION

Celiac disease is defined as a chronic inflammatory disease of the small intestine that occurs with the consumption of foods containing gluten (1, 2). The primary treatment for celiac disease, which stems from gluten intolerance, is adherence to a strict gluten-free diet (3, 4). One of the most important challenges for celiac patients in adopting to a gluten-free diet is the quality and accessibility of gluten-free food. Especially in our country, the grain-based diet and the limited production and variety of gluten-free products restrict patients' diet options, and since most gluten-free products are imported, they are more expensive than gluten-containing foods (1). Gluten-free foods are required for celiac patients, as well as those with gluten sensitivity and individuals who avoid gluten due to lifestyle, to follow a gluten-free diet (5).

Gluten is a basic structural protein that provides viscoelasticity and good gas retention ability to the dough, which is an important factor in the bread making process, and is effective in the sensory properties and quality of bread (6-8). Additionally, gluten has a protective effect on starch particles and slows down the staling rate of food by absorbing excess water (9). The absence of gluten in the dough results in a paler color, lower volume and more liquid consistency. It prevents the cooked product from reaching the desired quality and cause it to stale quickly (10, 11).

In recent years, many studies have been conducted on the positive effects of sourdough on bakery products and the addition of sourdough; a significant consensus has been reached that it positively affects breadcrumb structure and volume, nutritional value, taste and shelf life. In addition, it is thought that the quality characteristics of gluten-free bread leavened with sourdough will change positively (12, 13).

According to the Turkish Food Codex, kefir is defined as a fermented milk product in which kefir grains as a mixture of lactose-fermenting and non-lactose-fermenting yeast and bacteria that are responsible from fermentation, especially *Lactobacillus kefiri*, various strains of the *Leuconostoc*, *Lactococcus* and *Acetobacter* genera (14). Alcoholic and lactic fermentations are very important in the production of kefir, which is known for its functional and probiotic properties. Lactic acid, CO2, ethanol and other products resulting from fermentation are the components that directly affect the formation of aroma and taste (15). Kefir has many nutritional benefits due to its ease of digestion, regulation of stomach and intestinal flora, beneficial microorganisms, as well as its vitamin, mineral and protein content (16). This study aimed to investigate the effect of fermenting gluten-free bread with kefir culture on the use of kefir culture in gluten-free bread formulation and the sensory properties of bread and to investigate the presence of Lactococcus sp., a type of lactic acid bacteria, in kefir.

MATERIALS AND METHODS

Materials

In this study, gluten-free flour (contains corn starch and rice flour), salt, kefir culture (a well-known brand and has similar strain contents as in the study of Kok-Tas et al.) (17), sunflower oil and water were used to produce gluten-free bread. The salt, kefir culture and oil used in the study were obtained from markets in Istanbul. Gluten-free bread containing baker's yeast (control bread) was purchased in packaged form from a company in Istanbul. Gluten-free flour from the same company was used in the test bread.

Method

Production of Test Bread (Kefir Culture Containing Bread)

In the production of the test bread; 250 grams of gluten-free flour, 3 grams of salt, 2% kefir culture (5 grams) based on the weight of flour used, 15 grams of vegetable oil, 200 grams of warm water were placed in a mixing bowl and mixed until the dough became smooth. The dough was transferred to a pre-greased mold and flattened with a spatula. The mold with the dough was covered with a cloth and left to ferment in a closed cabinet at 24°C for 20 hours. It was then placed in the oven and left for final fermentation at 40°C for 1 hour. The oven temperature was set to 200°C and baked for 30 minutes (18).

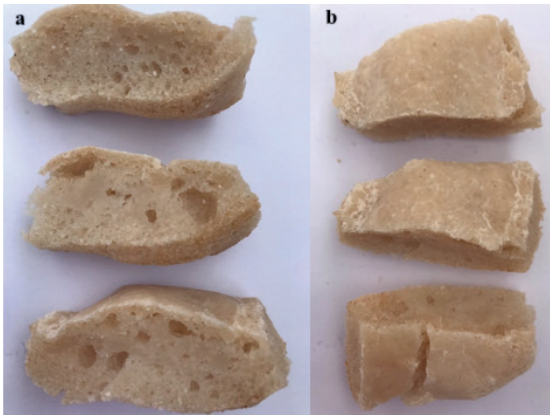

Figure 1a. Internal structure of test bread

Figure 1b. Crust appearance of test bread

Analyses

Microbiological analysis was conducted under aseptic sterile conditions. 10 grams from each of the purchased ready-to-eat bread (control bread) and gluten-free bread (test bread) samples were weighed, placed in a sterile stomacher bag and 90 mL of peptone water (Peptone water; Oxoid CM0009, ThermoFischer, UK) was added to dilute the sample 1/10. After homogenizing the samples, upper dilutions of ten were prepared with peptone water.

In order to count the *Lactococcus* sp. from the lactic acid bacteria in the gluten-free bread sample and gluten-free bread dough containing kefir culture, based on the method of Wang et al., tenfold serial dilution was prepared and inoculated on M17 (Merck Millipore, 63016, Germany) medium by the spread plate method (19). The cultured media were incubated at 37°C for 48 hours under anaerobic conditions (Anaerocult System, Merck 116275). At the end of the incubation, the colonies in the petri dishes were counted and examined with a microscope by gram staining.

In order to determine the Total Bacterial Count in gluten-free bread samples and gluten-free bread dough containing kefir culture, pre-homogenized samples were diluted to 10^{-3} and inoculated on PCA (Plate Count Agar, LabM LAB 149, UK) medium by the spread plate method. The cultured media were incubated at 37°C for 48 hours under aerobic conditions for total bacterial count after the study of Omurtag et al. (20). At the end of the incubation, the results were calculated by counting the petri dishes.

In order to perform mold-yeast counts on gluten-free bread samples and gluten-free bread dough containing kefir culture, the samples, which were previously homogenized and diluted to 10^{-2}, were plated on DRBC (Dichloran Rose-Bengal Chloramphenicol; Oxoid CM1148, ThermoFischer, UK) agar medium and incubated at 25°C, aerobically for 5 days according to the method in Da Silva et al. (21). At the end of the incubation, mold and yeast samples from the petri dishes were enumerated and the results were converted to log cfu/g.

The pH value of gluten-free bread dough fermented with kefir culture was determined by pH-meter (MILWAUKEE MW 102, United States). For this purpose, the pH meter probe was directly immersed in the dough and the reading was taken after the value was fixed.

Sensory Evaluation

Sensory evaluation of bread samples was carried out by a group of 11 nutritionists (9 women and 2 men) who did not smoke or have any food allergies. Then these panelists were trained and informed about the sensory evaluation. The breads were divided into equal pieces and coded with numbers on the production day. The taste and smell of the control and test bread were first examined with their eyes closed, then their eyes were opened and the external appearance characteristics of breads such as crust color and symmetry were evaluated. According to the sensory evaluation form which were prepared according to the protocol (22) panelists evaluated the breads in terms of volume, shape symmetry, crust color, crumb color, texture, mouthfeel, odor, aroma, taste, general control and preferability. A 5-point Likert scale (1, "dislike a lot," to 5, "like a lot") was applied.

Statistical Analysis

Statistical evaluation was made with SPSS (Statistical Package for the Social Sciences) 23.0 program to determine the differences for the results of sensory parameters. Comparative analyzes were made according to groups and Student-t tests were used to analyze the data, and the results were evaluated at the $p<0.05$ significance level within the 95% confidence interval. Microbiological analyzes were calculated using the Microsoft Excel program in accordance with the following formula (23):

Number/mL = (Number of colonies x Dilution factor) / Volume transferred from the dilution tube to the petri dish (mL)

Dilution factor = 1 / Dilution ratio

RESULTS AND DISCUSSION

The evaluation scores of the panelists according to the characteristics of the breads are given in Table 1. According to the table, a significant difference was found between the control bread and the test bread in the evaluations of the bread samples in terms of volume, shape symmetry, crust color, crumb color, texture, mouthfeel, smell, aroma, taste, general control and preferability ($p <0.001$).

Gluten-free breads were evaluated in terms of volume, shape symmetry, crust color, crumb color, texture, mouthfeel, odor, aroma,

Table 1. Sensory properties of test and control breads

	Test Bread Mean±SD	Control Bread Mean±SD	p
Volume	4.09±0.94	3.27±1.10	**<0.001**
Shape Symmetry	4.00±1.00	3.00±1.09	**<0.001**
Crust Color	3.09±1.04	4.45±0.52	**<0.001**
Crumb Color	3.72±1.00	4.45±0.68	**<0.001**
Texture	3.63±1.28	3.00±1.34	**<0.001**
Mouthfeel	4.09±0.94	3.45±1.03	**<0.001**
Odor	3.81±0.98	3.45±0.93	**<0.001**
Aroma	3.90±0.83	3.09±0.70	**<0.001**
Taste	4.45±0.68	3.00±1.09	**<0.001**
General Control	3.90±0.70	2.45±0.82	**<0.001**
Preferability	3.81±0.75	2.54±1.21	**<0.001**

taste, general control and preferability; it was observed that the test bread scored statistically significantly higher than the control bread in all parameters except crust color and crumb color. Accordingly, the kefir culture used in our study created significant sensory differences and it was observed that the bread was at an acceptable level in terms of all evaluated properties. In addition, it is thought that having the panelists make sensory evaluation of gluten-free bread sold in the market together with the test bread was effective in understanding the effect of kefir culture used in the test bread on sensory quality parameters.

In a study, it was found that the bread baked with 3% kefir had better flavor, and consumers showed a preference for kefir-leavened bread in sensory evaluations (24). In another study, the bread produced using sourdough with kefir had higher scores in sensory evaluations (25). Filipčev et al. (2007) stated that adding kefir grains to bread dough contributed to the bread's milder taste, better aroma, increased shelf life, better crumb elasticity, and structure. Furthermore, panelists preferred the kefir-based sourdough breads than others (26). However, in a different study using kefir in sourdough, it was reported that there was no significant difference between the samples in terms of odor and structure, while an increase in bread volume was observed (18). In addition to these studies in the literature, as a result of sensory evaluations, test bread in our study was liked more than control bread. Chawla and Nagal reported low nutritional value, crumbling, short shelf life, lack of flavor, unpleasant mouthfeel and low bread volume as the main defects seen in gluten-free breads (20). In this context, it is thought that the gluten-free bread we produced using kefir culture is both better and acceptable in terms of sensory parameters and better quality.

Lactococcus sp., total bacteria and mold-yeast count results of test and control breads are given in Table 2.

Ataç et al. determined lactic acid bacteria count as 8.49 log cfu/g and yeast count as 6.23 log cfu/g in a gluten-free bread sample obtained with kefir yeast (27). In another study, lactic acid bacteria count of a produced sourdough was 7.96 ± 0.08 log cfu/g and yeast count were 5.32 ± 0.25 log cfu/g (28). Meroth et al. found the number of lactic acid bacteria between 1.2-1.6 x 109 cfu/g and the number of yeasts between 2.7-5.0 x 107 cfu/g in naturally developed perennial rice sourdough, while the number of lactic acid bacteria between 1.2-8.2 x 108 cfu/g and the number of yeasts between 1.7 x 105-5.4 x 107 cfu/g in rice sourdough developed using commercial starter culture (29). As a result of our study, the number of *Lactococcus* in gluten-free bread leavened with kefir culture was found to be 3.3 log cfu/g, the total number of bacteria was 4.25 log cfu/g, and the mold-yeast count was 4.37 log cfu/g. It has been reported that the number of alive *Lactococcus* should be 8-9 log cfu/g and the number of yeasts should be 6-7 log cfu/g to obtain a good bakery product (12, 30). Accordingly, it is seen that the number of *Lactococcus*, as a sort of lactic acid bacteria, and mold-yeast contained in the dough we used in the study is low. As a result of this situation, it is thought that the contribution of sourdough to bread quality can be investigated more extensively by conducting additional microbiological analyses.

In this study, the pH of gluten-free bread dough fermented with kefir culture was measured as 4.62. Dagnas reported that pH is an important factor for mold growth in bakery products. Low pH reduces the activity of microorganisms and affects the product stability positively (31). It is also reported that pH is an important factor in phytic acid degradation and directly affects the

Table 2. Results of microbiological analysis of test and control breads

	Test Bread		Control Bread	
Type of Microorganisms	**n**	**log cfu/g**	**n**	**log cfu/g**
***Lactococcus* sp.**	22	3.3	3	2.47
Total Bacteria	180	4.25	4	2.59
Mold-Yeast	240	4.37	–	–

quality. Increasing the amount of sourdough decreases the amount of phytic acid (32). Özülkü reported that the pH value of sourdough obtained from durum wheat decreased from 6.24 to 3.28 as a result of fermentation (28), while Ataç et al. measured the pH value as 4.87 (27). Even if the leavening technique applied in the studies remains the same, pH changes according to the number of cultures used and the suitable environment for leavening. The use of pH meter and the waiting time of the pH meter in the dough are also important parameters. Although such studies have been carried out, there is no clear range for pH because there are not enough studies, but some studies suggest that it should be around 4.50 to 6.0 (33-36).

CONCLUSION

In this study, kefir culture was shown to be able to ferment gluten-free dough and to provide leavening activity without the need for an additional baker's yeast for gluten-free bread production. The most important contribution of kefir culture to the quality of gluten-free bread was to improve the sensory properties of the bread and to transform gluten-free bread into a more consumable and preferable form. As a result of usage of kefir culture, *Lactococcus* was higher in test bread, which can be further analyzed regard to their biochemical content like bacteriocin like beneficiary substances.

Author Contributions

Study design: AHİ, BİOK, Data obtaining: BİOK, HB, HY, Data analyze: AHİ, BİOK, Drafting the manuscript: AHİ, HB, HY, Critical review: AHİ, BİOK, Final approval: AHİ, BİOK, HB, HY.

Conflict of Interest

All authors declared that they have no conflict of interest.

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