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Research Article / Araştırma Makalesi

Dietary Fiber Analysis of D-Allulose Added Cakes and Determination of Microbiological Changes During Storage

D-Alluloz İlaveli Keklerin Diyet Lifi Analizi ve Depolama Süresince Mikrobiyolojik Değişikliklerin Belirlenmesi

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Abstract: D-Allulose is a monosaccharide that is rarely found in foods and can be obtained commercially by enzymatic reactions and isolation from bacteria. It can not be absorbed because of its chemical composition's resistance to digestive enzymes. It is referred to as a "energy-free natural sweetener" for this reason. These findings has led to an increase in D-allulose usage in the food industry. In this study, the total dietary fiber of cakes with additional D-allulose was determined, as well as the microbiological changes that occurred during storage. Three distinct cakes were made for the trial as the control (sugar), 50% sugar plus 50% D-allulose, and 100% D-allulose. In the baked cakes, dietary fiber analysis was done on day 1 and microbiological analyses were done on days 0, 7, 14, and 21. On the first day of analysis, there was no difference between the groups in the total spor-forming bacteria counts of the cake groups, but on the 7th, 14th, and 21st days of analysis, there were statistically significant differences between the groups ($p<0.05$). There was no statistically significant difference between the AL100 group and the control and AL50 groups when the total thermophilic bacterial counts of the cake samples were compared according to the storage durations ($p>0.05$). The control group's total dietary fiber content were 5.93 g/100 g, and AL 100 group's content were 10.70 g/100 g. D-Allulose is regarded as a natural sweetener with significant antimicrobial properties and a high dietary fiber content.

Keywords: Cake, D-Allulose, Dietary fiber, Anti-microbial effect.

Öz: D-alluloz besinlerde nadir olarak bulunan ve ticari olarak enzimatik reaksiyonlar ve bakterilerden izolasyon ile elde edilebilen bir monosakkarittir. Kimyasal yapısına bağlı olarak sindirim enzimlerine direnç göstermekte ve emilememektedir. Bu nedenle enerji içermeyen doğal tatlandırıcı olarak isimlendirilmektedir. Bu etkisi nedeniyle D-allulozun besin endüstrisinde kullanımı giderek artmaktadır. Bu çalışmanın amacı D-alluloz ilave edilen keklerin toplam diyet lifinin belirlenmesi ve depolama süresince mikrobiyolojik değişikliklerin belirlenmesidir. Çalışmada kontrol (şeker), %50 şeker +%50 D-alluloz ve %100 D-alluloz olmak üzere 3 farklı kek üretilmiştir. Üretilen keklerde 1. gün diyet lifi analizi, 0, 7, 14 ve 21. günlerde mikrobiyolojik analizleri yapılmıştır. Kek gruplarının toplam sporlu bakteri sayımlarında analizin ilk gününde gruplar arası fark bulunmazken 7, 14 ve 21. gün analizlerinde gruplar arasında istatistiksel olarak anlamlı farklılıkar saptanmıştır ($p<0,05$). Kek örneklerinin depolama sürelerine göre toplam termofilik bakteri sayımlarına bakıldığında ise AL100 grubunda istatisitksel olarak anlamlı bir farklılık saptanmazken ($p>0,05$), kontrol ve AL50 grubunda istatistiksel olarak anlamlı farklılıklar bulunmaktadır. Kontrol grubunun toplam diyet lifi 5,93 g/100 g ve AL 100 grubunda 10,70 g/100 g'dır. D-Allulozun yüksek diyet lifi içeren ve antibakteriyel etkileri olan doğal bir tatlandırıcı olduğu düşünülmektedir.

Anahtar Kelimeler: Kek, D-Alluloz, Diyet lifi, Anti-mikrobiyal etki.

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Introduction

Cakes are baked products with a sweet flavor and a dense, soft texture that are appreciated by people all over the world. The primary factors that determine of the cakes' quality are the ingredients, wheat flour, eggs, sugar, fat or oil, and leavening agents, used in the recipe as well as the methods of combining and baking used to prepared (Dizlek, 2013). Sucrose (sugar) has a wide range of roles in baked goods, including those of sweeteners, softening and moisturizing, and browning agents. It is an essential ingredient that contributes to taste, flavor, and texture. Nevertheless, it has been demonstrated that reducing sugar in bakery products lowers the gelatinization temperature of starch and encourages the development of gluten proteins, which has a negative impact on the quality of cakes and inhibits the browning response (Kweon et al., 2009). Because sugar provides an important function in baked goods, sucrose substitutes are difficult to achieve identical attributes from. The qualitative properties of sugar alcohols or polyols, which are frequently used as sucrose substitutes in bread items, have been studied in a variety of ways (Mariotti et al., 2012).

Since sucrose is the primary sweetener used on a worldwide basis, it is an essential nutrients in the food industry. However, because to its high glycemic index, people with diabetes should be careful to consume sucrose in moderate (less than 10% of daily energy intake) as excessive consumption might result in dental caries and diet-related diseases (WHO, 2015). Cakes are the baked good that has the most sugar among of all the rest of the baked goods. Therefore, it's crucial for health that this cake's sugar content be kept to a minimum or replaced with a low-calorie component.

The hydroxyl group position of C-3 is transformed in the conversion of D-fructose to D-allulose, commonly known as D-psicose, by the activity of the ketose 3-epimease enzyme (Izumori, 2006). D-Allulose has increased in value as a result of its high level of food safety, utility, and physicochemical similarities to sucrose. The US Food and Drug Administration recently classified D-allulose as "generally safe" and it is currently in the "relatively harmless" category, which denotes the lowest toxicity (Mu et al., 2012). Although D-allulose has a sweetness that is 70% as sweet as sucrose, it is categorized as an ultra-low-calorie sugar since it only offers 0.2 kcal/g of usable energy, or 5% of sucrose's energy. Also, D-allulose, unlike sucrose and D-fructose, has almost no effect on blood sugar levels due to its negligible glycemic index (Lamorte et al., 2019). Therefore, D-allulose is suitable for consumption by diabetic patients, making it a potentially ideal substitute for sucrose and is a nutritionally better option compared to other free sugars (such as D-fructose) (Belitz et al., 2004).

It is aimed to determine the effect of D-allulose, which will be added to the structure of the cake, which is a bakery product we will produce within the scope of the project, on the microbiological quality of the product, as well as the effect of D-allulose, which will be added to the structure of the cake, which is a bakery product that we will produce within the scope of the project, dietary fiber amount of the product. For these purposes, it is aimed to produce functional cakes from D-allulose added at different rates and to determine the shelf life of the cakes produced by microbiological analyzes to be made on different days.

Materials and Methods

Materials

D-Allulose was obtained from a D-allulose manufacturer. Wheat flour, sugar, eggs, milk, salt, margarine, vanilla and baking powder used in cake production were purchased from local markets in Burdur, Türkiye.

Preparation of Experimental Cakes

The ratios of D-allulose to be added to the cakes were determined on the basis of studies using D-allulose as a sugar substitute. 100% sucrose, 0% D-

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allulose (Control); Three types of cakes were produced: 50% sucrose, 50% D-allulose (AL50) and 0% sucrose, 100% D-allulose (AL100) (Table 1).

Table 1. Cake recipe to be used in the study

Components	Control	AL50	AL100
Wheat flour (g)	100	100	100
Sugar (g)	80/0	40/40	0/80
Egg (g)	60	60	60
Milk (mL)	65	65	65
Salt (g)	0.5	0.5	0.5
Baking powder (g)	4	4	4
Margarine (g)	60	60	60
Vanilla (g)	9	9	9

Appropriate baking temperature and determined in cake recipes, all components were kept at room temperature for 45 minutes before starting to work. In the study, firstly, wheat flour, baking powder and salt were mixed. In a separate bowl, it was whipped for 5 minutes at the 5th revolution with the help of an electric mixer until the sugar and oil were well mixed, and vanilla was added to it and whisking was continued for more minute. Eggs were added to this mixture and pre-mixed wheat flour, baking powder, salt mixture and milk were added to ensure that they were mixed well.

The doughs obtained were baked in the oven (Arçelik / 9620 Mi), which was previously set at 175°C. At the end of the baking time, the cakes were removed from the oven and allowed to cool for 1 hour at room temperature. They were then packaged until analyzed and stored at +4°C until the 21st day. Microbiological analyzes were performed on days 0, 7, 14 and 21 of production, total dietary fiber and soluble and insoluble dietary fiber analysis on first day.

Microbiological analyzes

Total spor bacteria count: Plate Count Agar (PCA) was taken from a dilution of 1 mL and inoculated with the spread plate method, and after the petri were incubated for 48 hours at 28±1ºC, total spor bacteria counts were made.

Total thermophilic bacteria count: Plate Count Agar was taken from a dilution of 1 mL and inoculated with the spread plate method, and the total thermophilic bacteria formed after the petri were incubated at 45±1ºC for 48-72 hours were counted.

Coliform bacteria count: Violet Red Bile Agar was taken from a dilution of 1 mL and inoculated with the double layer pouring method, and the typical colonies formed after incubating the petri at 30±1ºC for 24 hours were counted.

Yeast and Molds count: 1 mL was taken from the dilutions and inoculated on Potato Dextrose Agar (PDA) medium by the cast plate method, and the colonies were counted by incubating the petri for 5 days at 22±1ºC.

Determination of the total amount of dietary fiber: The total, soluble and insoluble dietary fiber amounts of the cakes were determined according to the AOAC 991.43 method as a service purchase in the TÜBİTAK Marmara Research Center Food Laboratory.

Statistical analyzes

Data will be analyzed using SPSS (version 26) package software. One Way ANOVA test was applied to compare the differences between more than two groups. Tukey Post Hoc multiple comparison test was used to determine the difference between groups. $p<0.05$ was considered statistically significant. In order to determine the difference between days, ANOVA analysis was used in repeated measures, which is the equivalent of One Way ANOVA in dependent groups.

Results

In the study, 3 different cakes were produced using standard (control [100% sugar]), AL50 (50% D-allulose) and AL100 (100% D-allulose) and

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immediately after production and in the refrigerator (at +4°C) 7, 14 and after 21 days of storage, total spor-forming bacteria, total thermophilic bacteria (TTB), coliform group bacteria and yeast-mold counts are shown in Table 2. When the total spor-forming bacteria counts of the cake groups were examined, there was no difference between the groups on the first day of the analysis, but statistically significant differences were found between the groups in the analyzes of the 7th, 14th and 21st days ($p<0.05$). It was determined that this difference was due to the count difference between all groups on the 7th day, lower in the AL50 group on the 14th day, and higher bacterial count in the control group on the 21st day.

Table 2. Microbiological analyzes of cake samples

Parametres	Days	Cake groups (Mean±SD)		
		Control	AL50	AL100
Total Spor-Forming Bacteria	0	$5,77\pm0,1^{aA}$	$5,63\pm0,4^{aA}$	$5,81\pm0,2^{aA}$
	7	$6,8\pm0,7^{aB}$	$5,4\pm0,7^{bA}$	$5,77\pm0,7^{cA}$
	14	$6,48\pm0,1^{bB}$	$5,63\pm0,0^{aA}$	$6,63\pm0,4^{bB}$
	21	$7,51\pm0,2^{bC}$	$6,37\pm0,1^{aA}$	$6,30\pm0,2^{aB}$
Toplam Thermophilic Bacteria	0	$7,51\pm0,4^{aA}$	$6,63\pm0,3^{aA}$	$7,51\pm0,2^{aA}$
	7	$7,93\pm0,7^{aB}$	$6,88\pm0,7^{bA}$	$7,63\pm0,7^{cA}$
	14	$8,44\pm0,3^{aB}$	$8,57\pm0,2^{aB}$	$7,48\pm0,4^{aA}$
	21	$8,80\pm0,1^{bC}$	$8,92\pm0,0^{bB}$	$7,83\pm0,0^{aA}$
Coliform Bacteria	0	-	-	-
	7	-	-	-
	14	-	-	-
	21	-	-	-
Yeast-Mold	0	-	-	-
	7	-	-	-
	14	-	-	-
	21	-	-	-

*Statistical differences between groups are shown in lower case letters. Statistical differences between days are shown in capital letters.

When the bacterial counts depending on the shelf life of the cake groups were examined, it was determined that there was a statistical difference depending on the day in the control group and this difference was more pronounced on the 21st day. While there was no statistical significance with storage in the AL50 group, statistically significant differences were found in the AL100 group on the 14th and 21st days. While there was no statistically significant difference in total thermophilic bacteria counts on days 0 and 14 of the analysis ($p>0.05$), significant differences were found on days 7 and 21 ($p<0.05$). On the 7th day, it was determined that the reason for this difference was higher bacterial counts in the control group, and on the 21st day, it was due to the lower bacterial counts in the AL100 group compared to the AL50 and control group. When the total thermophilic bacteria counts of the cake samples were examined according to the storage times, there was no statistically significant difference in the AL100 group ($p>0.05$), while there were statistically significant differences in the control and AL50 groups. While this difference was more pronounced on the 21st day of storage

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in the control group, it was found to be associated with an increased bacterial count from the 14th day in the AL50 group. During the preservation of the cake groups, coliform bacteria and yeast-molds were not found in all groups. Total soluble and insoluble dietary fiber of the control group was 5.93 g/100 g, soluble dietary fiber was <0.65 g/100 g, and insoluble dietary fiber was 5.93 g/100 g. In the AL 100 group, these values were determined as 10.70 g/100 g, <0.65 g/100 g and 10.70 g/100 g, respectively (Table 3). Total soluble and insoluble dietary fiber amounts of AL 50 group were not determined.

Table 3. Total dietary fiber values of cake samples

	Total dietary fiber	Soluble dietary fiber	Insoluble dietary fiber
Control	5.93 g/100 g	<0.65 g/100 g	5.93 g/100 g
AL100	10.70 g/100 g	<0.65 g/100 g	10.70 g/100 g

Discussion

Microbiological Analysis

Typically, the primary factor limiting the shelf life of baked prodcuts is microbiological deterioration. Microbial growth disruption results in economic loss for both producers and consumers. Numerous specific factors, including packaging, hygienic production procedures, storage conditions, and product turnover, might contribute to these losses. Yeast is a concern, particularly in baked goods. Osmophilic yeast contamination of items is typically brought on by dirty tools and equipment. As a result, osmophilic yeast contamination is reduced to a minimum when excellent production processes are followed (Goranova et al., 2020). Mold formation in bakery products is a serious problem that causes economic losses. Mold spores are generally destroyed by baking in fresh bread and other baked goods (Malkki and Rauha, 2000). Therefore, for the product to mold, it must be contaminated from the air, oven surfaces, equipment, food processors or raw materials during post-cooking cooling, slicing or packaging operations. This means that all spoilage problems caused by molds must occur after cooking (Knight and Menlove, 2006). In warmer weather and more humid storage conditions, the number of mold spores is higher in summer than in winter, due to airborne contamination. In addition, moisture condensation on the surface of a product due to packaging before it is completely cooled can cause mold growth (Saranraj and Geetha, 2012).

Lactic acid bacteria can prevent yeast-mold formation in bakery products due to their antifungal activities. It is also known that many members of lactic acid bacteria produce bacteriocins. The antibacterial effect was defined by antibiotic and antibiotic-like substances such as acidophilin and lactocidin produced by *Lactobacillus acidophilus*, lactolin produced by *Lactobacillus plantarum* or *nisin* produced by *Lactococcus lactis*. Depending on the type of bacteriocin, they can inhibit food-borne pathogenic bacteria such as *Staphylococcus aureus*, *Listeria* spp., *Bacillus cereus*, *Clostridium perfringens* (Dinçer et al., 2010). It is known that D-allulose promotes the growth of lactic acid bacteria in foods (Kimoto-Nira et al., 2017). Therefore, in this study, it is thought that the possible reason why the total mesophilic and total thermophilic bacteria counts related to shelf life were lower in the cake groups containing D-allulose compared to the control group. Alshehry (2019) investigated the anti-microbial effects of cupcakes with natural antioxidant supplementation during storage (days 0, 7, 15 and 21) and compared the total bacteria and yeast-mildew supplement in beet cupcakes during storage compared to control cupcakes made from 72% extracted wheat flour. showed

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that the number was inhibited. D-allulose is thought to be a natural sweetener that increases antioxidant capacity and inhibits yeast and mold formation in the product it is used in. In the microbiological analysis of Goranova et al., (2020) sponge cake cakes, coliform group, yeast-mold and total mesophilic aerobic group were not found on the first day of production. Gill et al. (2020) investigated microbiological analyzes in bakery products and found the total number of mesophilic aerobic bacteria in cake samples to be $5.7/10^4$ cfu/L. Coliform bacteria and yeast-mold were not detected. In another study, it was determined that the total aerobic bacteria counts in cakes produced from buckwheat flour were $2.9*10^2$, $4.7*10^3$ and $9.4*10^5$ cfu/g at 0, 7 and 10 days, respectively. In addition, total coliform bacteria and yeast-mold results were shown to be within normal values (Farzana et al., 2021). The effect of shelf life was not investigated in this study. According to the Turkish Food Codex Microbiological Criteria Regulation (2013) of the Ministry of Agriculture and Forestry, plain cakes, plain biscuits, plain crackers, etc. The upper limit for coliform bacteria in coated, filled and/or flavored biscuits, cakes and crackers and wafers is 10^2 cfu. It is seen that the results of coliform bacteria obtained from this project are suitable for Turkish Food Codex Microbiological criteria.

Determination of the total amount of dietary fiber

Dietary fibers are defined as the edible parts of plants that cannot be digested in the small intestine, but are fully or partially digested in the large intestine, according to the AACC International (International Society of American Cereal Chemists) (Anonymous, 2001). Soluble and insoluble dietary fibers in the diet are important in terms of many nutrition-related errors such as obesity, diabetes, hypertension, cardiovascular diseases, especially the gastrointestinal system, and should be included in the diet in sufficient quantities. It is reported that healthy adult individuals should take at least 25-35 g/day or 10-13 g/1000 kcal dietary fiber per day to prevent diseases. Legumes, whole grain foods, oilseeds, vegetables and fruits are rich sources of dietary fiber. In addition, foods with increased functionality are also used as a source of dietary fiber. In this study, in which we developed 3 different cake samples using D-Allulose and sugar, the dietary fiber values of the groups are shown in Table 3. When the experimental cake groups consumed 100 g (approximately 2 portion), the control group cakes met 23.7% of the total daily recommended dietary fiber (25-35 g/day), while the cakes produced with 100% D-allulose provided 42.8% of the total dietary fiber. meets. No studies have been found in the literature showing the effect of D-allulose on dietary fiber in bakery products. Rugji et al. (2022) reported that D-allulose has a prebiotic effect in whey beverages. We demonstrated the prebiotic effects of D-allulose in a previous traditional review (Özgür et al., 2022). The contribution of D-allulose to the total dietary fiber in the diet has been clearly shown in this study. This project is the first study to show the effect of D-alllulose on dietary fiber. In the literature, there are studies showing the effects of different components on the physico-chemical properties of cakes. In a study by Goswami et al. (2015) on muffin muffins, it was determined that muffin muffins with millet added more dietary fiber compared to the control group. In a study in which the total dietary fiber of the cakes with blueberry was determined, it was reported that the cakes produced with blueberry added at different rates (8%, 16% and 24%) could meet 13.3%, 17.1 or 19.6%, respectively (Işık and Urgancı, 2017). In a study in which dietary fiber was determined in cakes produced using different fiber sources, it was reported that the highest dietary fiber was found in the sample containing 10% palm kernel flour (Hamzaçebi, 2017). Ataman and Gül (2020) investigated the effect of cracked chickpea flour on muffin quality in a study where the total dietary fiber content, which was determined as 4.65% in the control sample, was 5.05%, 5%, respectively in muffin cakes with 10%, 20% and 30% cracked chickpea flour added. 65 and 6.43%. In line with these results, it is thought that the cakes produced from D-allulose have the

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potential to be a healthy snack especially for obese and diabetic individuals with their high dietary fiber contribution that does not contain sugar.

As a result, it is believed that cakes with D-allulose contained and controls are produced in hygienic conditions and protected against microbial deteriorating while in storage. Depending on the shelf life, D-allulose can inhibit both total thermophilic and total mesophilic bacteria. D-allulose is therefore expected to also have antibacterial properties. D-Allulose contributes to dietary fiber because digestive enzymes do not alter most of it before it reaches the large intestine. Because of its prebiotic impact, it is believed that it can be an important alternative sweetener for diseases related to nutrition. Future research should support these effects with human studies. Additionaly, even though cakes with D-allulose are a healthy snack with low energy, high dietary fiber and antibacterial effects, they should be consumed by paying attention to portion control in both healthy adults and individuals with diabetes.

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