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International Journal of Food Engineering Research (IJFER) is peer-reviewed journal which provides a platform for publication of original scientific research and applied practice studies. Positioned as a vehicle for academics and practitioners to share field research, the journal aims to appeal to both researchers and academicians.

Aims and Scope

International Journal of Food Engineering Research (IJFER) is an international, peer-reviewed journal devoted to the publication of high quality original studies and reviews concerning a broad and comprehensive view of fundamental and applied research in food science&technology and their related subjects as nutrition, agriculture, food safety, food originated diseases and economic aspects.

IJFER is an international periodical published twice a year (April and October). The journal is published in both print and electronic format.

From The Editor

International Journal of Food Engineering Research (IJFER) has been publishing by Istanbul Aydın University Faculty of Engineering Department of Food Engineering since 2015. The journal covers wide ranges of area such as Food Processing, Food Preservation, Food Microbiology, Food Chemistry, Biotechnology, Nanotechnology, Novel Technologies, Food Safety, Food Security, Food Quality and their related subjects as nutrition, food and health, agriculture, economic aspects and sustainability in food production.

Food Engineering is getting more and more attention because it is directly related to human health. While the food and drinks we eat help to protect our health, on the other hand, improper conditions during the conversion of the raw material to the product, the use of poor quality raw materials, and the employees not working under hygienic conditions can cause the food harmful to health. Our aim in this journal is to include the recent research and reviews on food and beverages from field to fork. Articles submitted to the journal are accepted for publication after being reviewed by expert referees.

In the following years, the journal will include scientific activities such as symposiums, congresses, conferences and workshops held in the field of food science and technology, and information about the books published in this field. We hope that the journal will be a good resource for engineers, experts, researchers and students working in the food industry.

Prof. Dr. Z. Dilek Heperkan
Editor

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GLUTEN-FREE BREAD PRODUCTION FROM CORN AND DETERMINATION OF SENSORY PROPERTIES[1*]

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ABSTRACT

Bread is a product that is produced mainly from grains such as wheat, barley and oats and is consumed in many societies as a staple food. However, due to gluten causing small bowel disease called celiac, extensive research is being done on producing bread from gluten-free raw materials such as corn and rice. In this study, the sensory properties of breads produced using corn flour, and corn flour together with corn starch were investigated. The addition of corn starch to corn flour had a positive effect on both the rheological and sensory characteristics of bread and was more appreciated by panelists. Continuing the studies on gluten-free bread production using different gluten-free raw materials is important in terms of meeting the needs of celiac patients.

Keywords: *Celiac disease, Gluten-free bread, Cornbread, Corn starch, Quality characteristics*

INTRODUCTION

Bread is considered to be a staple food. When the historical development of bread is investigated, evidence showing that people were able to bake bread in special ovens in Mesopotamia in 4000 B.C. was found. Bread production using yeast was made in ancient Egypt in 1800 B.C. When a piece of bread dough was separated and stored in a cool place, it was observed that the dough was better when added to the new dough. This method, which is known as "sour yeast" and which is traditionally used in the production of fermented foods, has been used since ancient times **[1]**. In the documents belonging to the ancient Egyptians in 2600 B.C. it was reported that when yeast is added to the dough obtained from a mixture of wheat flour and water, more

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fluffy and soft bread is obtained. The production of bread from commercial bakeries observed after the 5th century. In Europe, bread making started much later (15th century) and bread was produced from rye and then from wheat.

In recent years, depending on consumer preferences, it is seen that classical wheat bread has gained more variety with the addition of other cereals such as rye and oats. It is observed that the demand for bread enriched with corn, potatoes or chickpeas gradually increases depending on the raw material supply. One of the important reasons for this variety in bread is that some people are extremely sensitive to grain proteins. Small bowel disease, which occurs due to gluten consumption in cereals such as wheat, rye, oats and barley, is called 'Celiac Disease' **[2]**. Celiac disease is an autoimmune enteropathy that occurs in genetically susceptible people. The incidence of the disease worldwide is 1%, and it is considered as one of the most common genetic diseases **[3]**. The disease was first diagnosed in 1888 by Samuel Gee. Clinical manifestations in celiac disease can be seen in the early stages of life or in the following years. The disease typically shows two symptoms, diarrhea and growth retardation **[4]**. Treatment of celiac disease can be done with a gluten-free diet. As a result of gluten deficiency in gluten-free bread production, unstable liquid dough formation is encountered instead of dough formation **[5,6]**. The bread produced from such a dough is of low quality and is fragile and without flavor **[6]**. In order to overcome these deficiencies, researches continue to increase bread quality similar to gluten bread, using mixtures supported by various starches (corn, potatoes, cassava and rice), fibers and hydrocolloids. Gluten-free raw materials such as sorghum, millet, buckwheat, corn, rice, chickpeas and other legumes, amaranth, quinoa, flaxseed, chestnut, carob, lupine, acorn are used in the production of gluten-free bread **[7-9]**. In this study, corn flour was replaced with wheat flour. In order to improve the technological properties of bread, gluten-free bread was produced using natural hydrocolloid and dietary fiber and the sensory properties of the bread were examined.

MATERIALS AND METHODS

In this study, bread was produced using corn flour, water, sugar, salt and corn starch. The organoleptic properties were examined 24 hours after the breads were baked in a domestic oven by a group of 20 people.

RESULTS AND DISCUSSION

Two different types of bread produced for gluten-free bread production. In the first group of bread, only corn flour was used and in the second group, corn starch was used in addition to corn flour. The amount of water on organoleptic properties of bread made with corn flour is shown in Table 1.

Table 1. The amount of water on organoleptic properties of bread made with corn flour

Properties of bread	**Corn flour**	
	Low amount of water	*High amount of water*
Crust formation on the outer surface	++	+
Inner part of bread		
Spongy	-	++
Stickiness	-	-
Tough and tight	-	-
Porosity	+	++
Chewiness	+	++
Voluminous	+	++
Flexibility	+	++
Brittleness	+	+

-: Not detected / weak

+: middle

++: good

The amount of water on the organoleptic properties of bread made with corn flour and corn starch is shown in Table 2.

In Tables 1 and 2, the increase in the amount of water in both groups of bread made with corn flour and corn flour-corn starch has a positive effect on the quality of the bread. The small amount of water only affected the crust formation positively.

Table 2. The amount of water on the organoleptic properties of bread made with corn flour and corn starch

Properties of bread	**Corn flour and corn starch**	
	Low amount of water	*High amount of water*
Crust formation on the outer surface	++	+
Inner part of bread		
Spongy	-	++
Stickiness	-	-
Tough and tight	-	-
Pore formation	+	++
Chewiness	+	+
Voluminous	+	++
Flexibility	+	++
Brittleness	+	+

The definition of bread is given below according to the Turkish Food Codex Committee on Bread and Bread varieties **[10]**. Bread to wheat flour; water, salt, yeast (*Saccharomyces cerevisiae*) is defined as a product made by adding sugar, enzymes, malt flour as a source of enzymes, gluten and permitted additives and kneading, shaping, fermentation and cooking according to the technique of this mixture. Gluten-free bread is described in two sections **[11]**; Gluten content should not exceed 200 mg/kg in dry matter in foodstuffs defined as "gluten-reduced" and gluten content in foodstuffs

defined as "gluten-free" should not exceed 20 mg/kg in dry matter. Gluten-free foods that replace important basic foods such as flour or bread contain the same amount of vitamins and minerals as the foods they replace **[11].**

In cereal bread, gluten, the main source of protein, has many positive effects on dough and bread quality. When gluten is not used, various quality defects occurred. These deficiencies are eliminated in gluten-free bread production by using various hydrocolloids, emulsifiers and enzymes. In addition to structural deficiencies in gluten-free bread, it is also undesirable in sensory features. While the bread is crumbly, colorless and firm, the taste is smooth and flavorless. Some positive properties obtained after cooking change after a day or two. For example, the crumb, which is wet after baking and sticks together, the next day becomes dry, rough and crumbly **[6]**.

Corn is an important raw material that can be used in bread production as wheat. In our country, especially in the Black Sea region where corn production is common, bread is mostly produced from corn. Corn has low molecular weight protein. 60% of these proteins are made of zein **[12]**. Zein does not have a long polymeric structure like wheat gluten **[13]**. Corn flour contains 75-87% starch and 6-8% protein **[14]**. Corn contains bioactive compounds such as carotenoids, ferulic acid and anthocyanins with many therapeutic properties, as well. Corn seeds with blue, purple and red pigment are rich in anthocyanins with antioxidants and bioactive properties **[15, 16]**. In Table 3, evaluations made in terms of taste and flavor by panelists in bread made with corn flour and bread made with corn flour and corn starch are given.

Table 3. The points given by the panelists to the taste and flavor of bread

Panel-ists	**Bread (corn flour)**	**Bread (corn flour and corn starch)**
1	4	4
2	2	3
3	3	3
4	2	3
5	2	4
6	2	3
7	2	3
8	1	3
9	2	4
10	2	3
11	3	2
12	2	4
13	1	2
14	1	3
15	2	4
16	2	2
17	1	3
18	2	3
19	1	4
20	1	3
Total	**38**	**63**

In this table (Table 3), the breads are evaluated only in terms of taste and flavor.

CONCLUSION

The total score of bread made from corn flour and cornstarch is approximately 2 times higher than the score of bread made from corn flour alone. As a result, bread made with corn flour and corn starch is a product that can be consumed by celiac patients as an alternative to wheat and other gluten-containing breads. However, more studies are needed to improve both the sensory and structural features of cornbread.

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SOURDOUGH YEASTS IN BREAD MAKING: A REVIEW[1*]

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ABSTRACT

Sourdough yeast is used as a starter culture in preparation of dough and bread and is basically a flour mixture with metabolically active yeast and lactic acid bacteria (LAB). Sour yeasts are grouped under three groups worldwide depending on production technologies such as; Traditionally produced sour yeast (Type I), Industrially produced semi-fluid sour yeast (Type II) and Industrially produced dried sour yeast (Type III). Each species of sour yeast has its own unique LAB microflora. During sour yeast fermentation, as a result of microorganism activities, the acetic acid, lactic acid and ethyl alcohol bread in which heterofermentative LAB create a unique aroma. In addition, added sour yeast to the bread dough supports less elastic and smoother dough formation and delays the stale of the bread. The microflora of the sourdough may also change due to the process parameters such as temperature, pH as well as starter culture (grain, yoghurt, kefir, fruit and vegetable). In this study, research has been conducted on sourdough bread, which is known as the oldest biotechnological method but has become more popular and consumed with the awareness of consumers in recent years.

Keywords: *Sourdough, Sour yeast, Lactic acid bacteria (LAB), Classification of sourdough yeast*

INTRODUCTION

Bread, which is among the first foods processed by humanity, is one of the most consumed food products all around the world **[1].** In general, bread is considered a delicious and digestible food substance obtained by kneading the mixture formed by adding wheat flour, water, yeast and salt

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in certain proportions as the main element and cooking the resulting dough at a certain time and temperature after fermentation **[2].** Bread; although its content, shape and technique have changed. Many different techniques are used in its production, and one of them is the production of sourdough bread. In the production of sourdough bread, sourdough which directly affects the quality of bread is added to the dough of bread.

Sourdough yeast is utilized as a starter culture in preparation of dough and bread and is basically a flour mixture with metabolically active yeast and lactic acid bacteria (LAB). **[3].** After a while, the dough, which is left to its own state without the addition of yeast, changes, and gas bubbles form in it, the self-releasing dough softens and changes in smell. These changes in the dough are caused by flour and water, as well as microorganisms in the environment. This dough, which is spontaneously fermented and has a sour taste, is called 'sourdough' or 'sour yeast' **[4].**

Sourdough yeast contains homo- and heterofermentative LAB and yeasts in varying proportions and compositions. As a result of the symbiotic life maintained by yeasts and labs in the fermentation of sourdough, while yeasts and heterofermentative LAB produce significant amounts of CO_2, ethyl alcohol, acetic acid and other volatile compounds, as well as lactic acid, and are responsible for the blistering of the dough, homofermentative LABs ferment sugar to form lactic acid, affecting the elasticity, acidity and flavor of bread **[5, 6].**

The use of sourdough in bread making improves the volume, structure and sensory quality of bread, and extends the shelf life of bread physically and microbiologically **[7].**

The basis of sourdough is grain fermentation. Grain fermentation has significant potential because it improves nutritional quality and therefore the health effects of foods **[8].** Rye and wheat are the most commonly used cereals in making sourdough. Production of acid during yeast fermentation increases the enzyme activity such as amylases and proteases. Microbial and chemical changes in sour yeast vary depending on the type of flour, temperature, time, amount of water and type/amount of starter **[9].** From this point of view, sourdough is considered one of the main methods used to increase the sensory properties and shelf life of bread. Sour yeast fermentation can also alter the nutritional quality of bread in several ways, such as increasing mineral intake, improving the content of bioactive compounds, and delaying the digestibility of starch **[10].**

Carbohydrates found in grain products, minerals, nitrogen sources, lipids, free fatty acids, enzyme activities and endogenous factors such as temperature, dough yield

(DY), oxygen, process parameters such as fermentation time, the yeast microflora significantly affect the properties of bakery products and sourdough **[11].** Because the use of sourdough in bread making contains various parameters, it is necessary to ensure precise control. The most important of these parameters are fermentation temperature, pH value and selection of the appropriate starter culture. For this purpose, various starter cultures are used in the production of sourdough bread such as *Lactobacillus plantartum, L. brevis*, *L. reuteri*, *L. casei*, *Lactococcus*, *Candida* and *Enterococcus* often combined *with Saccharomyces cerevisiae*, also known as traditional 'bread yeast' **[12].**

Properties of Sour Yeasts

Sourdough is a traditional product that has been used for thousands of years to improve the nutritional value, sensory properties and shelf life of bread. These traditional products, called sourdough or sour yeast, are generally described as a mixture of fermented flour and water with labs and yeasts or with the addition of starter culture **[13, 14].**

Sourdough fermentation affects dough rheology in two ways: in the sourdough itself and in the sourdough bread dough. In the dough, fermentation reduces elasticity and viscosity, while adding sourdough to the bread dough provides less elastic and softer dough formation. The level of rheological changes in this dough and its effects on bread quality may vary depending on fermentation time and the ash content of the preferred flour **[15].**

During fermentation, biochemical changes occur due to microbial and natural enzymes in the protein and carbohydrate-based components contained in the flour. Due to the metabolic activities of the LAB in the environment during fermentation, many characteristics of sour yeast occur, such as lactic fermentation, synthesis of volatile compounds, proteolysis, production of mold and rope inhibitor agents **[11].**

A sourdough contains different number of yeast and LAB. Although the literature shows that a wide variety of LAB have been isolated from sour, there are a limited number of Lactobacillus species that can be highly adapted to sour yeast environment. In particular; *L. sanfranciscensis*, *L. plantarum*, *L. brevis*, *L. pontis*, *L. rossiae* species are regarded as key organisms in the production of sourdough **[16].** Several yeast species have also been isolated from sourdough, but among them only *Saccharomyces exiguus*, *Candida humilis* and *Candida krusei* species are thought to be fundamental in the fermentation process **[17].**

The ratio between water and flour in the dough is stated as dough yield (DY).

Dough yield value significantly affects dough consistency and flavor profile **[11].** Because the absorption capacity of different flour waters is different, dough of various consistency with the same dough yield can be obtained. Usually, traditional sourdoughs are hard dough, characterized by a value of approximately 150-160 DY. In contrast, a liquid sourdough pulp is characterized by a value of approximately 200 DY **[18].** A low DY value means a firmer and harder sourdough. Therefore, acetic acid production is higher than lactic acid production. Another factor that affects the DY of sourdough is the rate of acidification. As the DY value increases, it leads to a faster rate of acidification, as the diffusion of organic acids produced into the outsides will be better **[19].**

Temperature is considered one of the most important factors. It significantly affects the aroma of sour yeast and particularly the molar ratio between lactic acid and acetic acid **[20].** Accordingly, more acetic acid production occurs in a fermented hard pulp at 25-30°C, while more lactic acid production occurs in a fermented soft pulp at 35-37 °C. Optimum temperatures for the growth of sourdough LAB, depending on the strain, are between 30 and 40°C, and 25 to 27°C for yeasts **[18].** Factors such as the use of whole wheat flour, excess water content and high temperature increase the production of acid occurring in wheat yeast. **[11].**

Titratable total acidity (TTA) and pH value are important in the fermentation process. The acidity of sour pulp is measured as a function of pH and TTA values. The pH values of traditional sourdough are between 3.5 and 4.3, which is the ideal pH range for the growth of dominant sourdough microorganisms. **[21].** In general, lactic acid bacteria dominate this ecosystem due to their adaptation to the low pH of *Lactobacilli*. However, lactic acid bacteria such as *Lactococcus, Enterococcus, Pediococcus, Leuconostoc* and *Weissella* found in grain kernels and flour dominate sourdough yeasts, which is characterized by higher pH.

L. sanfranciscensis shows a high maximum growth rate in sourdough fermentation under sub-acid conditions. But because the growth rate of *C. humilis* in the 3.5-5.5 pH range does not change significantly. Therefore the multipication factor of *L. sanfranciscensis* compared to *C. humilis*, *L. sanfranciscensis* is higher than *C. humilis*. **[18].**

The substrate used for sour yeast fermentation, especially flour, is a parameter that particularly affects sour yeast. It is important to know the ash content to determine the degree of flour and the extraction rate. Because the ratio of ash found in bran is approximately 20 times that of ash found in endospermran fraction is rich in mineral and micronutrient content,

which is quite important for LAB growth. The proportion of endospermin found in bran is higher in small seeds. In addition, ash increases the buffering capacity of the sourdough system, allowing it to achieve a higher TTA **[11].** *L. brevis*, *Saccharomyces cerevisiae* or a combination of these starter cultures increase the hardness of fermented sourdough breads due to a high fiber content of flour. **[22].** The fall number is an indicator for the enzymatic activity of flour. For microflora to grow, the free sugar ratio must be high. A low falling number leads to increased amylase activity **[11].**

Classification of sour yeasts

Sour yeasts are grouped under three groups worldwide depending on production technologies (Table 1);

- Type I sour yeast: Traditionally produced sour yeast
- Type II sour yeast: Industrially produced semi-fluid sour yeast
- Type III sour yeast: Industrially produced dried sour yeast **[23].**

Type 0 group is a dough in which sourdough fermentation is not performed, but commercial bread yeasts are used to provide fermentation. It is possible to distinguish between Type I, II and III groups, and each type of sour yeast has its own unique LAB microflora **[24].**

In the Type I group, sour yeasts are produced using traditional methods. In this technique, refreshing is performed daily to keep microorganisms active. During the refreshing process, a piece of yeast that has completed the fermentation process is taken and used for the next fermentation **[25].** In this way, the purpose of the microorganisms have high metabolic activity and gas formation by providing a good way for raising the dough.

When preparing sour yeasts in type i group, the dough is generally left to fermentation at 20-30°C for 3-48 hours. During the fermentation period, flour and water are regularly used to freshen the process. Type I sour yeast is predominant in the LAB, and the pH values of this dough are approximately 4 **[26].**

Sour yeasts in the type II group have emerged to meet industrial demands. Sour yeasts in this group are faster, more effective, controllable and semi-fluid. In addition, Type II sour yeasts have a higher temperature (T>30°C), longer fermentation time (48-168 hours) and higher water content than Type I sour yeasts. Type II sour yeasts are often used as acidifiers in doughs because they have a high acid content (pH <3,5) **[27].** In addition, microorganisms present in Type II sourdough show restricted metabolic activity, suggesting that in this case, they are in the stationary phase.

Type III sour yeasts are powdery sour yeasts dried by spray dryers or drum dryers. Sour yeasts in this group are mainly used in industrial furnaces as acidifiers and flavorants. The amount of acetic acid in the dry doughs of drying is changed depending on the drying technique. Because the temperature of acetic acid in the evaporation process is 118°C, dried sourdough contains less acetic acid than fresh one. Type II method is applied when sour dough with high acidity is requested **[26, 28].**

In sour yeasts obtained by Type II and Type III methods other than Type I, yeast is added from outside as a blistering agent, and this addition S. bread yeast, also known as cerevisiae **[26, 29].**

Table 1. Classification and characteristic microflora of sour yeasts **[21]**

Type I	Type II	Type III
Obligately heterofermentative	**Facultatively heterofermentative**	**Obligately homofermentative**
L. sanfranciscensis	*L. brevis*	*L. brevis*
L. brevis	*L. fermentum*	
L. buchneri	*L. frumenti*	
L. fermentum	*L. pontis*	
L. fructivorans	*L. panis*	
L. pontis	*L. reuteri*	
L. reuteri	*L. sanfranciscensis*	
W. cibaria	*W. confuse*	
Facultatively heteroferentative		**Facultativelyheterofermentative**
L. alimentarius		*L. plantarum*
L. casei		*P. pentosaceus*
L. paralimentarius		
L. plantarum		
Obligately homofermentative	**Obligately homofermentative**	
L. acidophilus	*L. acidophilus*	
L. delbrueckii	*L. delbrueckii*	
L. farciminis	*L. amylovorus*	
L. mindensis	*L. farciminis*	
L. amylovorus	*L. johnsonii*	
Yeasts		
C. humilis		
C. krusei		

The effect of using sourdough in bread making

To obtain sourdough, a mixture of water, wheat/rye flour must be fermented with LAB and yeasts. As a result of the metabolic activities of yeasts and LAB, sour yeast develops sensory characteristics such as a distinctive aroma, but also plays a significant role in improving the nutritional and health quality of fermented products **[30, 23].** However, sourdough fermentation delays the stale of the bread during storage and protects against bacterial degradation due to the bio-transformation of the flour components in the dough stage of the bread **[31].**

The greatest contribution of sour yeast to bread quality is that acidification and metabolite production are carried out by LAB. The resulting acidification prevents the advanced degradation of starch during cooking and therefore inhibits endogenous amylase. Acidification is considered a prerequisite for ensuring an acceptable bread volume because it increases the water binding and gas holding capacities of existing pentose. **[32].**

Different metabolites such as exopolysaccharides (EPS), organic acids and/or enzymes that positively affect the texture of bread are produced by the LAB. EPS also known as hydrocollites, are produced by bacteria belonging to various food classes. EPS has a significant effect on different properties, such as increasing the viscoelasticity of the dough and the volume of bread, reducing the hardness of the bread and extending the shelf life. **[33, 34].** However, on-site production of EPS during yeast fermentation forces simultaneous acidification due to the metabolic activity of the bacteria, in which case it can greatly reduce the positive effects of EPS **[35].** In particular, acetate and lactate have been observed to significantly affect to dough rheology such as bread volume and hardness and it also offsets the benefits of EPS. **[36].** EPS created by the LAB are divided into homopolysaccharide (HoPS) and heteropolysaccharide (HePS). HoPS are made up of a kind of monosaccharide, while HePS are made up of three or eight monosaccharides. Examples of industrial EPS production are dextran from *Leuconostoc mesenteroides* and xanhtan from *Xanthomonas campestris* **[37].** Dextran is a HoPS composed of glucosyl. Due to its hydrokloid properties, they can bind a large amount of water, improve dough stability and gas retention, and maintain the freshness of the final product for longer **[36, 38].**

Acetic acid, lactic acid and ethyl alcohol are formed in the environment as a result of the activity of microorganisms during the fermentation of sour yeast, which leads to the bread gaining a unique aroma. It has been shown in studies that organic acids

formed by heterofermentative LAB are more effective on aroma in the sourdough bread production. Lactic and acetic acids lead these compounds. Apart from these, minor acids such as propionic acid, isovaleric, n-butyric, α-methyl-n-valeric, valeric acid and so on are formed in very small amounts. Acetic acid ensures the formation of a strong aroma in bread, but also increases the effect of other aroma compounds **[6].**

Bread flavor varies depending on the raw materials used in general, the type of starter culture selected, the amount of sour yeast placed in it, fermentation time, fermentation temperature, pH, applied technology and cooking process **[39].** While the aroma of the bread interior is mainly due to enzymatic reactions during the fermentation process, the aroma of the bread crust is mainly due to thermal reactions during the cooking process **[40].**

Production of sour yeasts from different sources

In fermented food production, sources of microorganisms involved in fermentation may vary. The variety of LAB and yeast species that can be used as starter cultures in sour yeast batters is very wide. Given the known superiority of these microorganisms, the initial source of starter culture may have a major impact on the function of the initial organisms within a dough system **[41].**

The most important factors affecting the sourdough microbia are: process parameters such as water content and temperature; used grain flours microbiological, enzymatic, nutritional, and sensory properties; grain flour in the content of the interaction between LAB; other species of bacteria and yeast found in flour **[42, 27].**

When the microflora of natural sour yeast is formed, it is usually used in flour, the environment or things used as inoculum, such as fruit, yogurt, honey and etc **[17].**

Yogurt, kefir and/or kefir grain can be used as a starter in the production of sour yeast. In one study, sour yeast and sourdough breads were made using kefir grains developed from whey. As a result of the study, it was found that kefir acts on the pH and titration acidity (TTA) of sour yeast samples. However, commercial kefir sourdough bread made with yeast produced using the moisture retention of breads is compared with the capacity more than if a firmer texture, higher acidity (pH 4,9–5,5 and TTA 2.8–5.0 mg lactic acid/g) and have been identified they can maintain their freshness longer **[43].**

Sour yeast cultures used in bread production are mostly derived from flour or grain-derived foods. In addition, grapes, wet/dry apples, peaches, figs and such fruits with high sugar content are used. **[40].**

Fruits can be used to make sour yeast when in season. For example, breads prepared with sour yeasts from various fruits such as apples, grapes, pears, oranges have a unique taste and are preferred by consumers **[44].**

Fruits are an important part of a healthy diet. Due to its low energy content, high content of vitamins C and B complex, provitamin A, mineral substance and dietary fiber, it is considered a very important source for today's nutrition and human health.

Raw vegetables and fruits are subject to lactic acid fermentation when anaerobiose, water activity, salt concentration and suitable conditions such as temperature are provided **[45].** Lactic acid fermentation improves both the organoleptic and nutrient quality of fermented fruits and vegetables, as well as preserving the color pigments of such foods **[46].**

LAB forms a small part of the autochthonous (natural) microbiota (2.0-2.4 log CFU/g) of raw fruits and vegetables. The main LAB species isolated from self-fermenting vegetables and fruits are given in the Table 2. [**45].**

Table 2. LAB isolated from vegetables and fruits **[45]**

Lactic Acid Bacteria	**Source**
Lactobacillus plantarum	Tomato, Zucchini, Carrots, Cucumbers, Eggplant, Capers, Beetroot, Pineapple, Plum, Kiwi, Papaya, Fennel, Cherry, Cabbage
Lactobacillus pentosus	Capers, Papaya, Eggplant, Cucumber
Lactobacillus rossiae	Pineapple
Lactobacillus fermentum	Fresh Beans, Beets, Capers, Eggplant, Melon
Lactobacillus curvatus	Pepper
Lactobacillus brevis	Tomato, Capers, Eggplant, Cabbage, Cucumber, Melon
Lactobacillus paraplantarum	Cabbage, Capers
Leuconostoc mesenteroides subsp. mesenteroides	White Cabbage, Carrot, Pepper, Cucumber, Eggplant, Cherry, Lettuce
Weissella soli	Carrot
Weissella confusa/*Weissella cibaria*	Pepper, Tomato, BlackBerry, Papaya
Enterococcus faecalis/*Enterococcus faecium*	Fresh Beans, Tomatoes, Capers, Melons
Pediococcus pentosaceus	Fresh Beans, Tomatoes, Cucumbers, Capers, Cherries, Cabbage

In one study, it has been compared the fermentation activity of three sourdough breads containing sugarcane, apple and grape yeasts and concluded that yeast derived from grapefruit is the best **[47]**. In another study, the process of fermenting sourdough using apples and rye was compared and it was discovered that sour yeast prepared with apples produces more gas in total **[41].**

Studies have shown that strains of yeast and LAB derived from grapes, apples, peaches and other fruits with high sugar content differ from those associated with cereals. According to information from previous studies, it was observed that sour bread made with cultures whose source of production is fruit was less sour compared to bread made with cultures derived from cereals. **[48].**

CONCLUSION

Bread, which is constantly used from the past to the present and is not missing from our tables, can be produced by different methods. Sourdough yeast used in its production affects the physical and chemical properties of bread, so the consumer will choose which bread. As a result of the metabolic activities of LAB and yeasts, sourdough has different sensory characteristics, such as a distinctive pleasant aroma, as well as prolonging the shelf life of the product. The microbiota of sourdough yeast is home to many different organisms which are obtained from different foods such as cereals, milk and dairy products or even fruits and vegetables. In obtaining LAB, which are important for sour yeast microbiota, because they are less sour, especially compared to those obtained from grain, more fruit and vegetable sources can be used and studies can be done on this issue and new information can be added to the literature.

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PRODUCTION OF SUGAR ALCOHOLS WITH BIOTECHNOLOGICAL METHODS[1*]

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ABSTRACT

Sugar alcohols, which are increasingly used in industries worldwide, are sugar substitutes that are formed by the reduction of sugars. With its low calorie advantage, they have sweetness, such as non-carcinogenic, low glycemic index and non–insulin resistance, it strengthens the nutritional properties in food products and improves product properties in terms of technological properties. It also has positive contributions to health against the increase of diseases such as obesity and diabetes. Although these compounds are generally produced by catalytic hydrogenation of sugars in the industry, they are receiving increasing attention as they can be obtained on a microbial basis. Several interesting metabolic engineering studies were carried out in recent years to improve the ability of bacteria and yeast to overproduce xylitol, mannitol, and sorbitol. The aim of this review is to provide information about sugar alcohols and production using biotechnology.

Keywords: *Sugar alcohols, Xylitol, Mannitol, Sorbitol, Biotechnology*

INTRODUCTION

A class of polyols, sugar alcohols resemble sugars and strengthen the nutritional profile of food products due to their low calorie content, being non-carcinogenic and having low glycemic index **[1, 2].** Polyols, the sugar alcohols of polyhydric alcohols or polyalcohols **[3]**, are defined as saccharide derivatives with chemical exchange of an aldehyde or ketone group with a hydroxyl group and are classified according to the saccharide units found in the molecule **[4]**. Though sugar alcohols are found naturally in fruit and vegetables, most contain low amounts. Production of these compounds at industrial scale uses catalytic hydrogenation, though currently microbial-based processes have gained attention **[5].** Sugar alcohols have areas of use including medication

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applications to personal care products and as chemical intermediate agents, in addition to the food sector. They are encountered for food production from the confectionary industry to chocolate, from chewing gum, frozen dessert products, flour-based products, and diet products to drinks **[6-8**]. Most sugar alcohols in traditional industrial production are obtained from hydrogenation of sugars under high pressure and temperature conditions with nickel catalysts. Metabolic engineering has gained importance to increase the microbial production of the common sugar alcohols of xylitol, mannitol and sorbitol **[2].** After sugar alcohols are digested by the human body, they convert to fructose and do not immediately elevate blood sugar levels. In other words, sugar alcohols are absorbed slowly. Due to digestive and metabolic features, some sugar alcohols have calorie values varying from 0-3.2 kcal/g, and generally have low levels of glycemic index value. Sugar alcohols have lower degree of sweetness than saccharose, with the highest sweetness determined for xylitol and the lowest sweetness for lactitol **[9].** Table 1 gives some properties of sugar alcohols.

Table 1. Some properties of sugar alcohols **[10-14]**

Scientific name	Degree of Sweetness[a]	Glycemic index[b]	Areas of use in the food industry	Functional use
Lactitol	0.3-0.4	6	Chocolate, hard candies, caramel, fondant, chewing gum, confectionary, frozen desserts, ice creams, flour products, water products, biscuits	Sweetener, volumizer, texture agent
Xylitol	1.0	13	Sugar-free sweeteners, chewing gum, confectionary, chocolate, desserts, reduced-sugar jams and marmalade production, ice cream, yogurt, sweetener and humidifier in some flour products, sweetener in drinks	Sweetener, Humidifier
Sorbitol	0.5-0.7	9	Confectionary, Baked goods, low calorie foods, sugar-free sweetened chewing gum, diet drinks, ice cream, biscuits, peanut butter, jams	Sweetener, humidifier, texture, volumizer, stabilizer
Mannitol	0.5-0.7	0	Hard candies, cake products, dried fruit, chewing gum, chocolate, baked goods, ice creams	Sweetener, volumizer, anti-coagulant
Maltitol	0.9	35	Sugar-free rock candy, sugar, chewing gum, chocolate, ice cream	Sweetener, volumizer, humidifier, stabilizer
Isomalt	0.45-0.65	9	In sugar-free sugar production, hard candy, chocolate, coated foods, nutritional supplement, pastilles	Sweetener, volumizer, anti-thickening agent
Eritritol	0.8	0	Some types of cheese, milk powder, milk desserts, ice cream, breakfast cereals, processed meat products, products like egg desserts and sauces, confectionary, biscuits, sugar-free chewing gum, some drinks	Flavor enhancer, humidifier, sweetener, carrier, thickener, stabilizer

[a]Saccharose=1**[15, 16]**; [b]Glucose **[15, 17]** =100

Xylitol

Xylitol was the first sugar alcohol obtained from seaweed and yeasts. Found at very low levels in fruit and vegetables, xylitol was first obtained from mushrooms (*Psalliota campestris)***[18, 19]**. In 1891, it was extracted and produced as a syrup by Bertran Fischer Stabel and in later years pure xylitol was obtained. Stable and unstable forms of xylitol have been developed. Studies used *Penicillium chrysogenum* yeasts to reduce xylose to xylitol [**20**]. In industry xylose, a pentose not found in free form in plants, is converted to xylitol by hydrogenation. Xylitol comprising D-xylose units is combined with cellulose in xylan form [**21**]. Though xylose is present in nature, commercial production is difficult due to difficulties with separation from other carbohydrates like xylose. For this reason, obtaining xylose from plants and then performing the hydrogenation process represents the basis of xylitol production. Plant materials used with this aim include hardwoods like beech, oats and cottonseed husks, corn cobs, sugar beet pulp, straw and nutshells. These materials contain 20-30% dry weight of xylan and xylose material [**21**]. As a result of hydrolysis with acid from xylan, D-xylose and catalysts are hydrogenated to produce xylitol. Many microorganisms do not have the ability to use xylitol. Even the bread yeast of *Saccoramyces cerevisiae* cannot ferment xylitol. When xylitol is added to dough, just as fermentation does not occur, in situations involving other sugars the fermentation rate reduces [**22**]. A study by Gehrin et al. (1974) proved that xylitol does not undergo fermentation by oral microflora **[23]**. For this reason, it is very important in terms of oral health [**24**].

Area of Use in Food Production

As xylitol does not have sufficient viscous structure for food production, it is generally not used alone as a sweetener. Xylitol may be used instead of glucose with gum Arabic in chewing gum. Xylitol can also assist in controlling kneading processes [**25**]. Xylitol, the 5-carbon sugar alcohol, is obtained by reduction of xylose. In recent times, microbial production of xylitol has attracted attention **[1]**. Xylitol production has annual 340 million dollar market share **[26].** When examined from a microbial aspect, yeasts, especially *Candida* species, appear to be the best xylitol producer. Xylitol production by yeast is obtained by reduction of xylose to xylitol with the xylose reductase (XR) enzyme or by oxidation of xylitol to xylulose with NAD^+ and xylitol dehydrogenase (XDH). After converting to glyceraldehyde 3 phosphate and pyruvate in order via the pentose phosphate route, xylose 5 phosphate converts by reduction to ethanol or enters the tricarboxylic cycle. As NAD is produced again in anaerobic conditions, the xylitol produced in the first step is continuously consumed and mainly ethanol production is observed. Under fully aerobic conditions, NADH accumulation and as a result inhibition

of the XDH enzyme dependent on NAD produces xylitol at high rates **[1].** For microbial-based natural xylitol production from yeasts, *Candida sp.* has gained importance in recent times **[27, 28]**. A study by Kwon SG et al. **[29]** produced 260 g/L zylose with an osmophilic strain of *Candida tropicalis* isolated from honey and produced 234 g/l xylitol in 48 hours as a result of fermentation within a glucose feed batch **[29].** Ko et al. **[*30*]** studied xylitol production with the disrupted mutant XYL2 xylitol dehydrogenase gene (XDH) of *Candida tropicalis* and obtained xylitol with oxygen transfer and the effect of glycerol. It was determined that 0.98 mol xylitol (mol xylose^{-1}) was obtained in aerobic conditions with 20 g glycerol from mutant D-xylose [**30**]. A study by Jin et al. [**31**] completed xylitol production in aerobic and limited oxygen culture environments by alternative metabolic routes with a *Pichia stipitis* (FLP-YS30 XYl3) mutant strain and determined the highest production was in anaerobic conditions [**31**]. As *Saccharomyces cerevisiae* does not contain natural xylose special carriers **[32],** xylose production is not effective. A study completed production with xylose recombinant species of *S. cerevisiae* **[33].** Apart from yeasts, it was determined that xylitol can be produced from bacteria. Xylitol may be produced from *Corynebacterium* and *Enterobacter* species containing enzymes reducing xylose to xylitol. However, bacteria are not good xylitol producers due to low amounts of xylitol production [**34**]. Due to this situation, studies were completed for bacteria to acquire the features that yeast has for xylitol production. A study by Cirino et al. [**35**] about this completed xylitol production from *E. coli* which does not have the ability to produce xylitol but has the ability to assimilate both hexose and pentose sugar. Positive results were obtained from studies about *E. coli* strains that may act as biocatalyst for conversion of biological mass to valuable products like glucose and xylose **[2, 36]**. It was determined that the key enzyme for the central metabolism of *E. coli* for xylitol production was an NADPH source. All xylitol dehydrogenases contained in *Candida boidinii* (CbXR enzyme), *Candida tenuis* (CtXR enzyme), *P. stipitis* derived xylose reductases (PsXR enzyme), *S. cerivisiae* (ScXR enzyme), *Glucono bacteroxydans* (GoXDH enzyme) and *P. stipitis* (PsXDH enzyme) microorganisms are functional to a variety of degrees in *E. coli.* Among these enzymes, the highest xylitol concentrations were produced with shake flask cultures with over expression of CbXR dependent on NADPH **[35**]. A similar study was performed by Yukimato et al**. [37]**. High xylitol production was performed with *E. coli* expression of the xylitol producer of D-xylose permease (xylE) with the addition of an XR chromosome linked to NAPH from *Kluyveromyces lactis* (XYL1)**[37]**. A study by Nyyssola et al. (2005) produced xylitol from xylose with XR expression from *P. sitipitis* (XYL1) with recombinant *Lactococcus lactis* [**38**]. A study by Povelainen and Miasnikov [**39**] reported completion of xylitol production

from glucose with *Basillus subtilis*. Xylitol was produced with nearly 23% yield with expression of the *B. subtilis* strain producing the XPDH enzymes for d-ribulose and d-xylulose contained in *Lactobacillus rhamnosus* and *Clostridium difficile* [**39**].

Some molds

Penicilium aspergillus and *Neurospora rhizopus* molds produce xylitol. However, xylitol production from molds is low, and xylitol production from molds is limited due to their metabolism of xylitol [**34**].

Mannitol

Mannitol, with a pleasant taste, is a carbohydrate alcohol like xylitol and sorbitol. It is the most abundant polyol found in nature, found in microorganisms such as bacteria, yeast, as well as in various plants such as pumpkin, celery, olive, onion and mistletoe. It is frequently used in pharmaceuticals and some nutritional tablets as it maintains its stable structure in humid environments and does not experience color loss at high temperatures. Just as it is dentally friendly, it is only absorbed in the small intestine and the section that cannot be absorbed by colonic bacteria is metabolized like indigestible carbohydrates **[40-42]**. Mannitol, which stands out with its antioxidant effect, is assumed to be an unmetabolized sweetener. For this reason, mannitol may be used in foods with health-promoting effects (functional foods). Studies determined that mannitol, which may be directly produced by LAB, can be applied in food production of fermented food products. While large amounts of mannitol production occur from fructose by heterofermentative LAB, only small amounts of mannitol production occur with homofermentative LAB **[43]**.

Area of Use in Food Production

Different from sorbitol, mannitol is used as powder with the aim of preventing adhesion of chewing gum to the production machinery in chewing gum production as it is not hygroscopic [**16**]. Mannitol has volumizing properties and may be used as sweetener [**44**]. It has a high share of the global market for Mannitol [**45**]. Mannitol and sorbitol formation occurs with catalytic reduction of a glucose and fructose mixture and these are separated by selective crystallization to gain mannitol. As chemical production of mannitol has low products and costs, interest in production from glucose or fructose with selective fermentation has increased [**45**]. Mannitol production by LAB and other microorganisms which can be used in food offers several significant advantages. The first of these advantages is that microorganisms and products which can be used in food can be directly applied to food products without any limitation. Another advantage is that some lactic acid bacteria are proposed to be beneficial to the gastrointestinal system [**43**]. Mannitol production by these bacteria has positive health effects. A study by Kiviharjuet al. [**46**] used lactic acid bacteria, yeast and molds for natural mannitol production and obtained high

degree titers of mannitol with manipulation. Lactic acid bacteria are important in industrial terms for food and medication production. Mannitol production may be completed by heterofermentative lactic acid bacteria and homofermentative lactic acid bacteria. Heterofermentative lactic acid bacteria, mannitol from fructose, it is produced by catalyzed mannitol dehydrogenase (MDH) and by NADH [**43**]. The *Lactobacillus intermedius* strain NRR-B-3693 has commercial potential for mannitol production from fructose syrup [**47**]. Glucose is used as NADH source for mannitol production by heterofermentative LAB [**48**]. In homofermentative situations, after conversion of fructose-6-phosphate (F6P) to mannitol-1-phosphate dehydrogenase (Mtl1PDH) and mannitol-1-phosphate (Mtl1P) linked to NADH, it occurs by phosphorylation of Mtl1P **[49, 50]**. As a result of fermentation, by-products like lactic acid and acetic acid are obtained **[51, 52].** A study by Von Weyman et al. [**48**] used *Leuconostoc mesenteroides* and obtained 93-97% rates of mannitol from fructose [**53**]. However, as the process is linked to sugar consumption, mannitol may be produced with 61-62% yield per sugar consumed as a result of additional nutritional requirements [**54**]. In the study by Reshamwalaet al.[**54**] originally mannitol synthesis was completed by mannitol-1 phosphate expression from *Eimeria tenella* (M1 Pase)from an *E. coli* strain to transform D-glucose into D-mannitol. Kaup et al. [**55**] used *E. coli* to convert fructose to mannitol. Biotransformation developed in the whole cell by founding an oxidation/reduction cycle within recombinant *E. coli* to convert D-fructose into D-mannitol. Additionally, another study defined the structure of recombinant *E. coli* for conversion of glucose to mannitol without adding extracellular enzymes and completed mannitol production with 87% yield with carbon flow from fructose 6-phosphate for mannitol biosynthesis [**56**].

Sorbitol

Found abundantly in nature, sorbitol **[13, 57]** is found in many fruits with and without seeds like apple, prune, cherry and grapes **[6].** Sorbitol is used as sweetener in diabetic products as it does not cause an increase in blood glucose [**13**]. Globally, the market is constantly increasing, with 25% of sorbitol production being used for vitamin C synthesis **[5, 6, 58].** Sorbitol has hygroscopic structure. It may absorb and release moisture very slowly under varying humidity conditions. Safety was supported by many scientific studies, so JECFA defined the acceptable daily intake (ADI) amount for sorbitol as 'undetermined' and for this reason there is no limit to its use **[59]**. The sorbitol base can be used by *Lactobacillus* species, just as it can be used as a carbon source by intestinal *Bifidobacteria* in humans so it is qualified as a prebiotic **[60, 61].**

Area of Use in Food Production

Sorbitol is used in the dessert, beverage industry with its non-calorie sweetening feature, and because it gives a sweet cold and

pleasant taste effect **[62, 63]**. Sorbitol is used in the dessert, beverage industry with its non-calorie sweetening feature, and because it gives a cold and pleasant taste effect. It is also used as an important precursor in the production of vitamin C, sorbose and surfactants. **[6, 64, 65].** While in the industry sorbitol can be obtained from glucose and sucrose via catalytic hydrogenation, some yeast and bacterial strains, particularly *Zymomonas mobilis* and *Candida boidini*, appear to be potential producers of sorbitol **[16].** It has been biotechnologically derived from glucose and fructose by *Z. mobilis* bacteria **[6, 66].** In the fermentative food class, dehydrogenases from the LAB of *Lactobacillus plantarum* and *Lactobacillus casei* have the ability to be used as different electron receivers for NAD^+ regeneration, making this a valuable field for polyol production via metabolic engineering **[43].** Ladero et al. **[65]** by reversing the catabolic route **[40]** of sorbitol from production from F6P of a strain of by expression from a mutant strain of *Lb. plantarum* (sorbitol-6-phosphate dehydrogenase (Stl6PDH) gene), sorbitol production was synthesized **[66].** A similar study by Yebra et al. **[67]r¦r¿** constructed the gene coding stlgph containing remnant chromosomal lactase with a recombinant strain of *Lb. casei* producing sorbitol. They produced 0.024 moles of sorbitol from 1 mole of glucose from the parent strain lactose **[67].**

CONCLUSION

Demands for sugar alcohols are increasing every day. Biotechnologically, microbial production of xylitol, mannitol and sorbitol has gained importance in recent years. In vitro production of synthetic sugar alcohols are costly. Due to the costly reproduction of enzyme preparations and cofactors in enzyme-based production of sugar alcohols, biotechnological approaches for production of these compounds have gained importance. For this reason, production of these compounds using all cells in raw sugar stocks has become attractive. At the same time, microorganisms which do not produce sugar alcohol offer production possibilities with biotechnological methods. Yeasts like *Candida, Pichia,* and *Saccharomyces cerevisiae*, lactic acid bacteria and *E. coli* have gained an important place in sugar alcohol production.

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ELİNİZDE BULUNAN KİTAPLARDAN, **BAŞKALARININDA YARARLANMASINI İSTER MİSİNİZ?**

KÜTÜPHANEMİZDE BULUNAN **KİTAPLARDAN FAYDALANABİLİR, OKUYABİLİR VE ÖDÜNÇ ALABİLECEĞİNİZİ** BİLİYOR MUSUNUZ?

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