



The effect of phenolic compounds contained in flour from green buckwheat, flaxseed, grape and dogwood seeds on the fermentation activity of yeast *S. cerevisiae*

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ABSTRACT

Background: Bakery products are one of the most widely consumed in the world, but they have very low nutritional value and a short shelf life. Unconventional flour can be used to address these issues. They contain vitamins, minerals, and polyphenols that can significantly increase the nutritional value of the final product. It is known that phenolic compounds have a beneficial effect on the human body, preventing various diseases, inactivating free radicals, and also suppressing the growth of pathogenic microorganisms that develop in bakery products. Despite these advantages, unconventional types of flour have a specific biochemical composition that can negatively affect the course of the technological process. One of the most important stages in the production of bakery products is fermentation. Earlier studies have shown that phenolic compounds can be adsorbed by yeast, but high concentrations significantly reduce the viability of yeast cells.

Objective: To study the influence of phenolic and flavonoid compounds contained in flour from green buckwheat, flax, grape, and dogwood seeds on the fermentative activity of *S. cerevisiae* yeast. To achieve this goal, it was necessary to determine the total amount of phenolic and flavonoid substances contained in flour from grape and dogwood seeds, green buckwheat, and flax flour, and to study the effect of flour and individual phenolic compounds on the fermentative activity of yeast. The most common polyphenolic compounds in all three types of flour were selected for the study – rutin, gallic acid, and tannin.

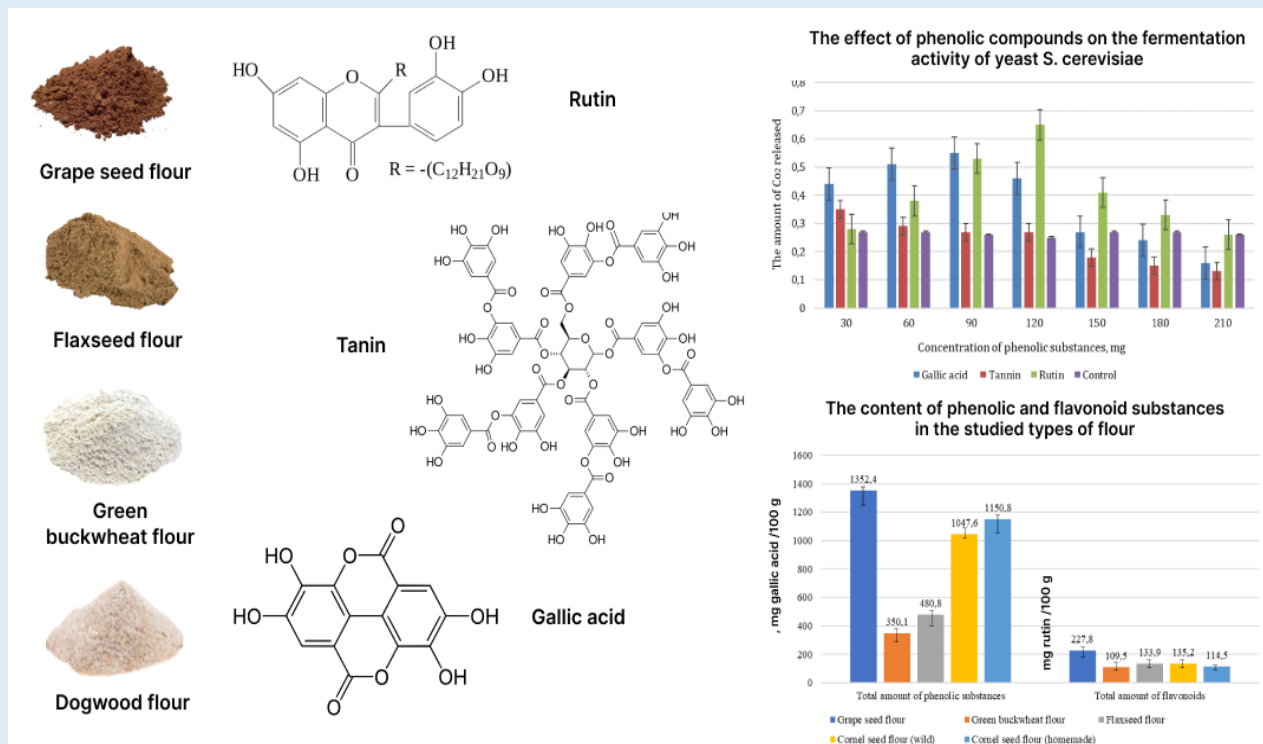
Methods: The fermentation activity of yeast was assessed by measuring carbon dioxide release during fermentation, with samples prepared using varying concentrations of flour from grape seeds, green buckwheat, and dogwood seeds. Ethanol

extraction from raw materials was conducted using 60% ethyl alcohol, followed by spectrophotometric determination of phenolic composition and flavonoid content. Rutin, tannin, and gallic acid were defined using specific titration and spectrophotometric methods.

Results: It was found that flour from grape seeds contains the highest amount of phenolic and flavonoid compounds compared to other types of flour, namely: 1352 milligrams of gallic acid/100 grams and 227 milligrams of rutin/100 grams respectively. The lowest amount was found in flour from green buckwheat – 350 milligrams of gallic acid/100 grams and 109 milligrams of rutin/100 grams respectively. The addition of the tested types of flour positively affects the fermentative activity of bread yeast, increasing the release of carbon dioxide by 1.5-2 times compared to the control sample. The highest amount of CO₂ was released when using flour from grape seeds. Studies of the phenolic and flavonoid composition showed that grape seeds contain the highest amount of gallic acid and tannin compared to other types of flour. Individual phenolic compounds have a positive effect on the process of carbon dioxide release. The highest amount of CO₂ was released when adding 120 mg of rutin per 1 gram of yeast, which is 2 times higher than the values relative to the control sample. It was noted that the introduction of this amount of rutin shortened the lag phase and increased the logarithmic phase. The fermentation process was most intense in the first 1.5-2 hours.

Conclusion: A promising direction is the use of flour from green buckwheat, grape, flax seeds, and dogwood seeds for baking. Since the introduction of phenolic substances does not have a negative effect on the fermentative activity of yeast, these types of flour can be a source of phenolic compounds that can significantly extend the shelf life of products. In addition, additional consumption of phenolic compounds in the human diet has a positive effect on health.

Keywords: dogwood seed flour, grape seed flour, green buckwheat flour, flaxseed flour, yeast fermentation activity, phenolic compounds



INTRODUCTION

Bakery products, a variety of food products based on grain, are widely consumed and sold due to their taste and texture. However, they typically contain high concentrations of simple carbohydrates and low concentrations of protein, vitamins, and minerals, as well as have a short shelf life [1]. One way to address these issues is to use alternative raw materials and ingredients with a more diverse chemical composition. These ingredients may include phenolic compounds, which have been shown to have beneficial effects on human health, preventing various diseases. Additionally, phenolic compounds have antibacterial properties that help prevent the growth of microorganisms that cause spoilage of bakery products [2]. Furthermore, consumers are growing increasingly aware of the significance of diet in human health leading to an increase in demand for functional foods [3].

However, phenolic compounds may have an ambiguous effect on the activity of bread-making yeast. It is known that some phenolic compounds are adsorbed by yeast cells, such as gallic acid, and do not have a negative impact on its activity at low concentrations [4-6]. All phenolic substances have different structures and degrees of toxicity that need to be studied with respect to the selected types of flour.

Grapes are one of the richest sources of phenolic compounds among fruits, and many of them are known for their therapeutic or health-promoting properties, making grapes an important fruit for human health [4]. About 64% of the total amount of free phenolic compounds is contained in the seeds, 30% in the skin, and 6% in the pulp. Grape seeds and skin are important sources of phytochemicals, such as gallic acid, catechin, epicatechin, rutin, and procyanidins. The most common compound is gallic acid, with an average content of 225,4 µg/g in grape seeds. These compounds are known for their antioxidant properties, as well as having cardiovascular, anti-inflammatory, and antiviral properties [8].

Furthermore, grape seeds contain flavonoids and anthocyanins, which have antioxidant activity in the body and are important for preventing oxidative damage to

tissues by reducing lipid oxidation or preventing and/or limiting the formation of free radicals [9].

Currently, the use of flaxseed in products is a subject of many studies. Flaxseed (*Linum usitatissimum*) is an oilseed rich in anti-inflammatory, gut-healthy bioactive compounds, including fermentable dietary fibers. It contains many essential fatty acids, especially polyunsaturated fatty acids, such as alpha-linolenic acid (ω-3-ALA), linoleic acid (ω-6), and oleic acid (ω-9). These fatty acids play an important role in reducing cardiovascular diseases, have anti-cancer properties, and improve brain development [10]. In addition, flaxseed contains many bioactive compounds, including dietary fibers, lignans (a class of phytoestrogens), high-quality proteins, vitamins, minerals, and phenolic compounds. The total content of flavonoids in flax seeds ranges from 0,66 µg/g (Sari-85) to 424,29 mg/100 g (Jihangbeili), and the total content of phenolic compounds ranges from 178,81 mg/100 g (Sari-85) to 243,73 mg/100 g (Jihangbeili) [11,12].

In recent years, buckwheat groats have been in high demand and have attracted the attention of scientists due to the presence of chemical compounds and high effectiveness as a functional food product. It contains biologically active compounds, such as phenolic acids and flavonoids (mainly rutin and quercetin), which have a rich antioxidant potential, exerting multiple positive effects on consumers' health. Rutin reduces high blood pressure and has antioxidant and lipid peroxidation effects. It also has hypolipidemic effects, reducing the absorption of cholesterol from the diet, leading to a decrease in cholesterol levels in the liver and blood plasma. The total content of flavonoids ranges from 100-1250 mg/100 g of dry weight, and rutin ranges from 0,2-14,5 mg/100 g [13,14].

Wild plants are also unique sources of biologically active compounds for enriching bakery products with essential nutrients [15,16]. For the republics of the North Caucasus, the processing of medlar and dogwood seeds is of interest as secondary raw materials for creating full-cycle technologies [17]. Scientific literature shows that vitamins, trace elements, and essential fatty oils found in dogwood seeds have a positive impact on human health.

Dogwood (*Cornus sanguinea* L.) belongs to the genus *Cornus* and the family Cornaceae Link. Species of the genus *Cornus* are rich in phenolic compounds, minerals, vitamins, especially vitamin C, tannins, and anthocyanins [15, 17].

Although the studied types of flour are valuable raw materials for bakery products, their use in this area is limited due to the negative impact on the organoleptic and rheological characteristics of the final product. Organoleptic and rheological properties of semi-finished products using non-traditional raw materials are actively studied by scientists. Previous research by the authors shows that the introduction of buckwheat flour reduces rheological parameters, increasing the hardness of the product and reducing elasticity [18]. Another study shows that the introduction of flaxseed flour negatively affects the gas retention capacity of the dough, specific volume, and increases the hardness of the product [19].

The authors explain the decrease in rheological properties due to the lack of proteins gliadin and glutenin, which are responsible for the formation of an elastic structure [18-20]. However, the investigated types of flour contain a complex of biochemical compounds different from wheat flour. Therefore, it is important to study their influence on the main stages of bread production. For example, one of the most important stages of production is fermentation [5]. It has been found that phenolic compounds influence the fermentative activity of yeast at high concentrations [21].

The purpose of fermentation is to aerate the dough, give it the necessary structural and mechanical properties, and accumulate substances that give taste, aroma, and color to the final product. Alcoholic fermentation is carried out by yeast, converting sugars into alcohol and carbon dioxide. Yeast ferment their own flour sugars, as well as maltose, which is formed in starch grains under the action of amylase present in the flour. The rate of fermentation depends on temperature, acidity, yeast quality, and increases with dosage, activity, as well as sufficient content of mono- and disaccharides, amino acids, and phosphoric acid salts for yeast nutrition. However, excess salt, sugar, and fat suppress gas formation in the dough due to increased osmotic pressure, which suppresses the vitality of yeast cells. It is

also known that phenolic compounds can suppress the vitality of yeast cells [5]. However, only a small number of such studies have been conducted. Therefore, the aim of this study is to investigate the influence of phenolic compounds found in flour from green buckwheat, flax seeds, grape seeds, and dogwood seeds on the fermentative activity of bread yeast.

MATERIALS AND METHODS

Materials: The following raw materials were used for the experiments:

1. green buckwheat flour (TU 9293-002-4317554-03), producer LLC "Garner";
2. flaxseed flour (Organization standard 53548590-020-2013), producer LLC "Khlebzeroproduct";
3. grape seed flour (TU 10.61.22-011-19683697-2020), producer LLC "Brunch life";
4. wheat baking flour of the highest grade (GOST 26574-2017), manufacturer of JSC "LKHP Kirova" (St. Petersburg), date of sampling: 08/27/2022, humidity 13.0%;
5. pressed baking yeast GOST R 54731;
6. drinking water GOST R 51232;
7. dogwood seed flour.

Cornel seed flour (CSF) was obtained in the laboratory conditions of the Department of the Grozny State Petroleum Technical University. M.D. Millionshchikov Academy. We used dogwood growing in the Chechen Republic, the village of Dubai-yurt, Shalinsky district, collection date 05.10.2022.

The dogwood seeds were thoroughly cleaned of pulp residues, dried at a temperature of 35-45 ° C, after cooling, the bones were ground on a disintegrator to a particle size of no more than 100 nm.

CSF is a loose, odorless cream-colored mass.

Fermentation activity of yeast: The fermentation activity of yeast was measured by the carbon dioxide released during the fermentation process. The sample flasks were weighed every 30 minutes. The samples were prepared with the addition of 1-, 5-, 10-, and 15% flour from grape seeds, green buckwheat, dogwood seeds (wild and

homemade), 1 g of yeast, 2 g of glucose and 100 cm³ of water. The experiment was carried out three times.

The effect of individual phenolic compounds on the fermentation activity of yeast was carried out in the same way. Instead of flour, a pure phenolic compound was taken in concentrations of 30 mg, 60 mg, 90 mg, 120 mg, 150 mg, 180 mg, and 210 mg. The remaining components were introduced without quantitative changes.

Ethanol samples extraction: 1 g of crushed raw materials was placed in a 150 cm³ flask, and 30 cm³ of 60% ethyl alcohol was added. The extraction was carried out for 60 minutes at a temperature of 50 degrees Celsius. Extraction was performed twice, adding 60% ethyl alcohol to the remainder of 30 cm³. The solution was decanted, centrifuged, and cooled to room temperature.

Total number of phenolic compounds: The phenolic composition was determined using a spectrophotometer. 1 cm³ of the test sample, 20 cm³ of distilled water, and 10 cm³ of sodium carbonate solution (Na₂CO₃) are added to a 100 cm³ volumetric flask and then water up to 100 ml is added. The samples are left for 30 minutes, after which the optical density is measured at a wavelength of 670 nm. The experiment was carried out three times [10].

Total number of flavonoids: 1 g of flour was placed in a 150 cm³ flask, and 30 cm³ of 60 % ethyl alcohol was added and put in a water bath at a temperature of 50 degrees for 1 hour. Next, the resulting solution was decanted and filtered. 1 cm³ of the obtained extract was added to a measuring flask of 25 cm³, and 4 cm³ of a 5 % alcohol solution of aluminum chloride was added. In parallel, a comparison solution was prepared from 1 cm³ of the obtained extract. Both flasks were brought to the mark with 60 % ethyl alcohol. After 30 minutes, the optical density was measured at a wavelength of 395 nm [22]. The experiment was carried out three times.

Definition of rutin: 10 cm³ of extract was added to a 100 cm³ flask, and 10 cm³ of distilled water and 10 drops of indigocarmine were added. The resulting solution was

titrated with 0,05 n potassium permanganate solution until a stable yellow color was obtained. The experiment was carried out three times [23].

Definition of tannin: The amount of tannin was determined according to GOST 19885-74 by titration [22].

Definition of gallic acid: 1 cm³ of the extract was placed in a 25 cm³ volumetric flask and was brought to the mark with a borate buffer solution with a pH of 9,0. An aliquot of 1 cm³ was taken from the 25 cm³ solution and placed in a 10 cm³ volumetric flask and was brought to the mark with a buffer. The optical density of the resulting solution was measured three times relative to a borate buffer solution with a pH of 9,0 at wavelengths of 275±2 nm and 305±2 nm. The content of gallic acid in terms of absolutely dry raw materials (%) was determined by the Firordt method [25]:

$$C_{ga} = \frac{A_2^{\lambda_2} \cdot A^{\lambda_1} - A_2^{\lambda_1} \cdot A^{\lambda_2}}{(A_1^{\lambda_1} \cdot A_2^{\lambda_2} - A_1^{\lambda_2} \cdot A_2^{\lambda_1}) \cdot l'}$$

A₁^{λ₁} – the value of the specific absorption index of gallic acid at 275 nm;

A₁^{λ₂} – the value of the specific absorption index of gallic acid at 305 nm;

A₂^{λ₁} – the value of the specific absorption index of tannin at 275 nm;

A₂^{λ₂} – the value of the specific absorption index of tannin at 305 nm;

A^{λ₁} – the optical density of the extraction under study at a wavelength of 275±2 nm;

A^{λ₂} – the optical density of the extraction under study at a wavelength of 305±2 nm;

l – layer thickness, cm³.

RESULTS

The total amount of phenolic compounds in grape seed flour, green buckwheat flour, flaxseed flour, and two types of dogwood flour was determined during the experiment. The tests were carried out three times.

Table 1. The content of total phenolic substances in the studied types of flour

Name of the sample	Grape seed flour	Green buckwheat flour	Flaxseed flour	Cornel seed flour (wild)	Cornel seed flour (homemade)
Total amount of phenolic substances, mg gallic acid /100 g	1352,4 ± 2,6	350,1 ± 3,4	480,8 ± 2,3	1047,6 ± 2,1	1150,8 ± 2,8
Total amount of flavonoids, mg rutin /100 g	227,8 ± 3,4	109,5 ± 2,2	133,9 ± 4,1	135,2 ± 3,1	114,5 ± 3,6

According to the data obtained, the highest content of phenolic compounds was found in grape seed flour, while the lowest was found in green buckwheat flour, which corresponds to the values in the literature [18-20]. Small differences in the quantitative content of phenolic compounds may be justified by the geographical and climatic characteristics of the raw materials.

Information on the content of phenolic compounds in dogwood seeds is insufficiently studied. Thus, based on our own experimental results, we can conclude that dogwood flour is a rich source of phenolic compounds, as it contains a large amount of common phenolic compounds compared to the studied types of flour.

The highest amount of flavonoid compounds is found in grape seeds, while the lowest is in green

buckwheat flour. The content of phenolic and flavonoid compounds also depends on the growing conditions and can vary widely [18].

However, adding high concentrations of phenolic compounds can negatively affect the processes occurring during dough fermentation. One of the important stages in the production of bakery products is fermentation, during which the structure, porosity, and aroma are formed. One of the factors influencing the intensity of the fermentation process is the fermentative activity of yeast. The higher the fermentative activity of yeast, the better the quality of bakery products.

The results of the influence of selected types of flour on the fermentative activity of bakery yeast are shown in Figure 1.

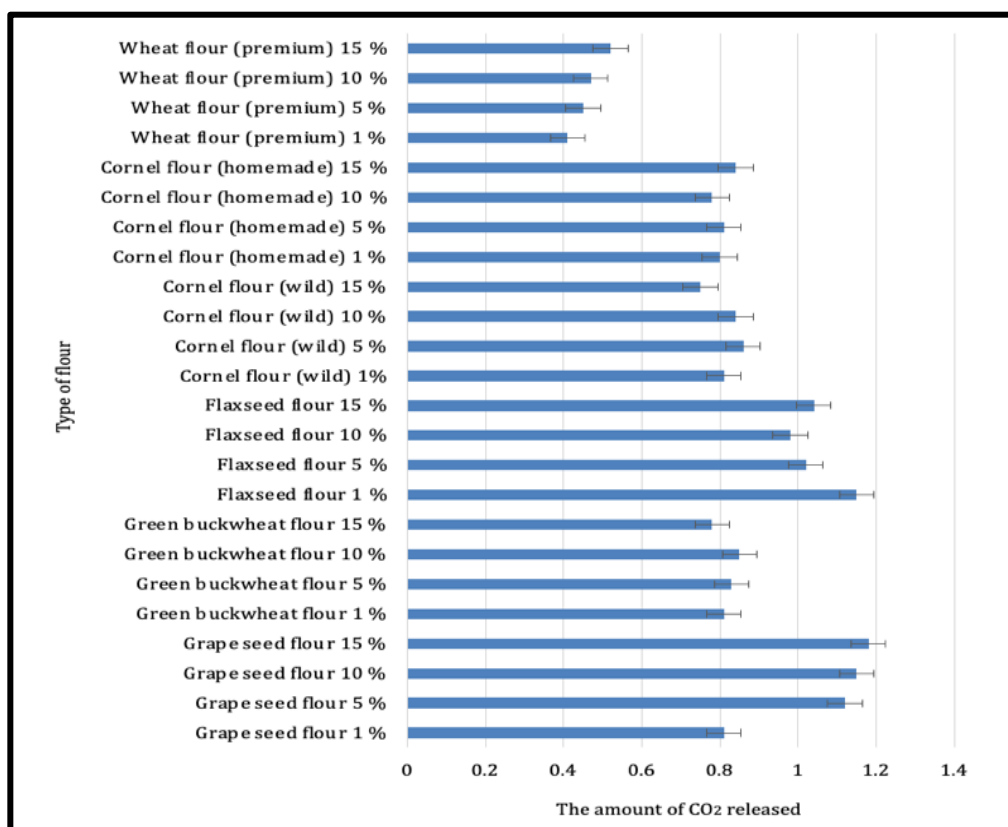


Figure 1. Fermentation activity of yeast *S. cerevisiae*

According to Figure 1, the introduction of the studied types of flour intensifies the fermentation process of bread yeast. This may be related to the additional introduction of sugars, mineral and vitamin components, as well as phenolic compounds, which can activate this process.

The most intensive process of carbon dioxide release occurs when adding 5-15% flour from grape seeds, which corresponds to a 2-fold increase in carbon dioxide release compared to the control sample. It is known that this flour contains a large amount of phenolic and flavonoid compounds, which allows us to make an assumption about the positive influence of these substances on the yeast cell activity.

Data obtained when adding dogwood, flaxseed, and buckwheat flour vary within the range of 0,7-0,86 g of CO₂ released. These values are 1.5 times higher than those measured when adding high-grade wheat flour.

Since flour is a complex system consisting of proteins, fats, carbohydrates, vitamins, minerals, and phenolic compounds, it is impossible to make a definitive conclusion about the positive influence of phenolic substances on the fermentative activity of bread yeast. Therefore, it is advisable to study the influence of some phenolic substances contained in all selected types of flour, namely flour from grape seeds, green buckwheat, flax seeds, and dogwood, on the fermentative activity of *S. cerevisiae* yeast. Based on the literature sources studied, common phenolic substances in the selected raw materials are: gallic acid, rutin, tannin [18-20].

During the study, the amount of the above-mentioned substances in the studied types of flour was determined. The quantitative values of the selected substances are presented in Table 2.

Table 2. The quantitative content of gallic acid, rutin and tannin in the studied types of flour

Name	Grape seed flour	Green buckwheat flour	Flaxseed flour	Cornel seed flour (homemade)	Cornel seed flour (wild)
Gallic acid, mg/100 g	18,48 ± 1,65	9,84 ± 2,10	3,85 ± 1,80	3,20 ± 0,89	4,95 ± 1,21
Rutin, mg/100 g	3,62 ± 1,35	4,47 ± 1,50	2,61 ± 0,80	1,25 ± 0,44	1,92 ± 0,60
Tannin, mg/100 g	0,31 ± 0,08	0,04 ± 0,01	Traces	0,12 ± 0,06	0,18 ± 0,1

According to the data obtained, it can be seen that grape seed flour contains the highest amount of gallic acid and tannin. Similar results were previously obtained by Lenka Sochorova and others [19]. Small differences in values may be justified by the fact that flour is produced from different varieties, differences in growing conditions, and geographical location. Similarly, high

values of gallic acid and rutin were obtained during the study of buckwheat flour. Meral and others obtained similar results [21].

The lowest content of the substances studied was found in flaxseed and dogwood seed flour.

Figure 2 shows the influence of gallic acid, rutin, and tannin in different concentrations on the activity of carbon dioxide release.

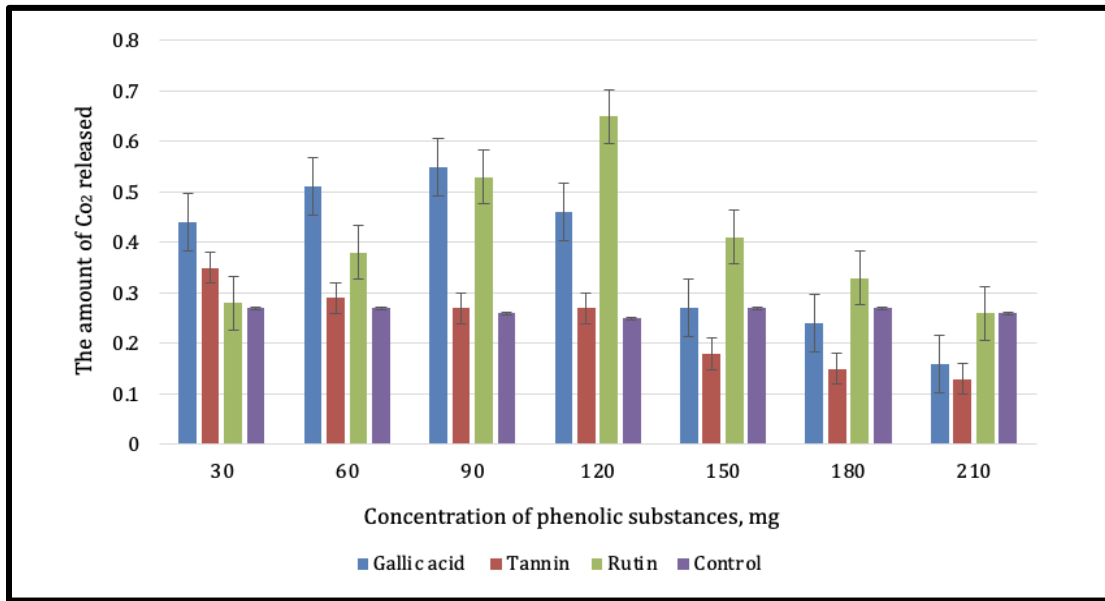


Figure 2. The effect of gallic acid, rutin, and tannin on the activity of carbon dioxide release

According to Figure 2, it can be observed that yeast produces carbon dioxide most intensively when 120 milligrams of rutin are added per 1 gram of yeast. With further increase in rutin concentration, the activity of yeast cells decreases, which can lead to deterioration of the rheological properties of the product, such as porosity. The addition of gallic acid also results in an increase in the enzymatic activity of yeast cells, although less pronounced. The highest release of carbon dioxide was observed when 90 milligrams of gallic acid were added to one gram of yeast. As the concentration increases, there is a gradual decrease in the content of

carbon dioxide. The obtained results can be explained by the absorption of phenolic compounds by yeast cells. Adebayo et al. also noted in their work the ability of yeast to absorb phenolic compounds [6].

However, large amounts of these compounds can cause osmotic stress in the cell and negatively affect its viability, as evidenced by the decrease in carbon dioxide release activity when relatively high concentrations of rutin, gallic acid, and tannin are added. Phenolic substances not only affect the total amount of carbon dioxide, but also the phases of cellular activity.

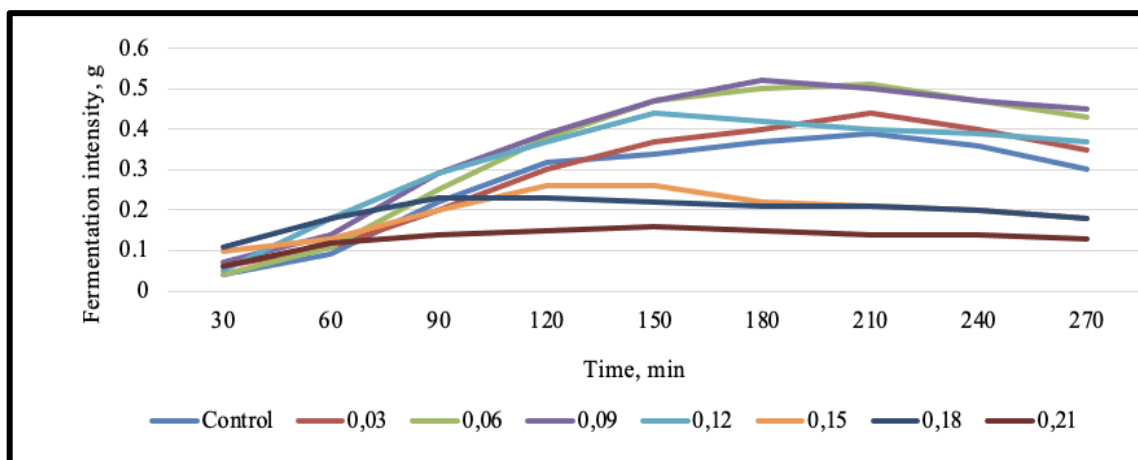


Figure 3. The effect of gallic acid on the fermentation activity of yeast *S. cerevisiae*

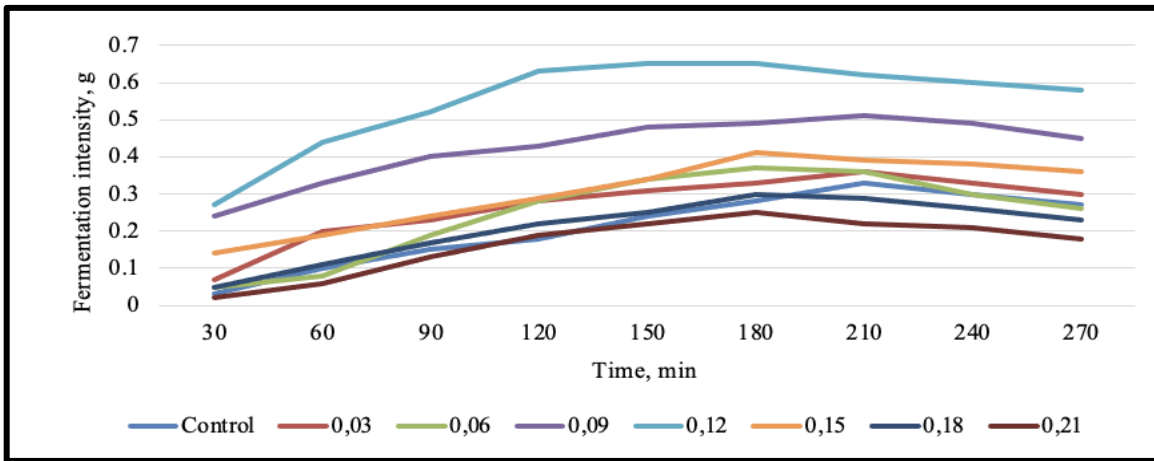


Figure 4. The effect of rutin on the fermentation activity of yeast *S. cerevisiae*

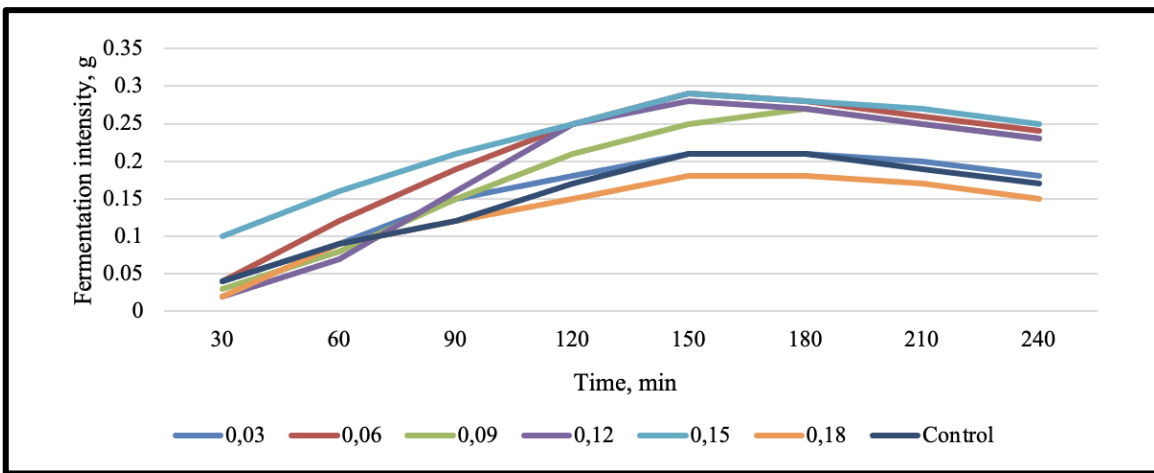


Figure 5. The effect of tannin on the fermentation activity of yeast *S. cerevisiae*

As can be seen from the graphs, the introduction of phenolic substances can alter the duration of the cell's life cycle. It was noted that when rutin was introduced in quantities of 90 mg, 120 mg, and 150 mg, the lag phase noticeably shortened, and the logarithmic phase became more intense. When 150 mg of rutin was introduced, the majority of carbon dioxide was released within the first 1,5 hours, and fermentation noticeably decreased in the subsequent hours of fermentation.

With higher concentrations of rutin, 180 mg and 210 mg, the intensity of fermentation decreased by 1,5-2 times, and the lag phase noticeably increased. With lower concentrations of rutin, 30 mg and 60 mg, no significant changes were observed compared to the control sample.

The process proceeds less intensively when gallic acid is used. The majority of carbon dioxide is released

when 120 mg, 150 mg, and 210 mg of gallic acid are added at the beginning of fermentation, followed by a gradual decrease. The addition of 120 mg of tannin also contributed to a slight reduction in the lag phase, while the other concentrations did not bring about significant changes.

DISCUSSION

The result of the experiment was the study of the influence of major phenolic substances found in flour from grape seeds, dogwood seeds, and flax seeds, as well as green buckwheat, on the enzymatic activity of bread baking yeast. The analysis of the data revealed a positive effect from the introduction of these types of flour. Compared to high-grade wheat flour, the addition of the tested types of flour increased the yeast fermentation

activity by 1.5-2 times. Since flour is a rich source of antioxidants, we studied the total phenolic and flavonoid composition of the individual types of flour. According to our data, the highest amount of phenols and flavonoids is found in flour from grape seeds, which is consistent with previous studies by other authors. Flaxseed flour contains the lowest amount of phenolic compounds, while green buckwheat flour contains the least flavonoids. However, these values exceed those relative to high-grade wheat flour. Therefore, we evaluated the impact of specific phenolic compounds on CO₂ production by yeast cells.

According to the literature, the main compounds found in the studied types of flour are gallic acid, rutin, and tannin. Relatively high concentrations of these substances were found during the experiment. The highest amount of gallic acid and tannin is found in flour from grape seeds. High levels of these compounds are also found in green buckwheat.

Furthermore, the influence of gallic acid, rutin, and tannin on the fermentation of *S. cerevisiae* yeast was studied. It was found that rutin has the most favorable effect on yeast fermentation, increasing carbon dioxide release by up to 2 times compared to the control. It was also noted that high concentrations of rutin (120 and 150 mg) shorten the lag phase and make fermentation more intense in the first two hours.

An important direction for future research is to study how phenolic compounds affect the rheological properties of the test semifinished product and the final product, as well as their bioavailability and absorption in the end product. This will not only extend the shelf life of bakery products, but also make them more functional.

CONCLUSION

The results of the research are important for the food industry and the scientific community, especially in the field of food products and healthy eating. The use of non-traditional flours, such as grape, flaxseed, buckwheat,

and dogwood, will extend the shelf life of bakery products, enriching them with necessary nutrients and diversifying the bakery industry. However, compounds found in these flours may negatively affect the quality of the final product, reducing consumer attractiveness. Therefore, it is important to study the influence of individual components at key stages of production and adjust technical production processes. In conclusion, the prospects for using non-traditional types of flour are very promising and require further research to fully utilize their potential and promote healthy eating and rational resource use.

List of abbreviations: LDL, low-density lipoprotein

Competing interests: The authors have not declared any competing interests.

Authors' contributions: All authors contributed to this study.

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