

Effect of complex plant supplement on shelf life of wheat bread

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Abstract

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Introduction. The purpose of the work was to determine the effect of the complex plant supplement on the preservation of freshness of bread.

Materials and methods. Complex plant supplement consisted of sunflower lecithin, rice flour, rice protein concentrate and dry lamium leaves which were added to recipe of wheat bread. Brittleness, firmness, water activity, amount of water absorbed by the crumb, deformation of the bread crumb, content of total polyphenols, phenolic acids, and flavonoids, as well as antioxidant activity and oxidative stability of bread were studied.

Results and discussion. It was proved that the crumb of wheat bread added with a complex plant supplement was less susceptible to deformation during 48 hours of storage and faster regained form. The brittleness of the developed product decreased compared to the control due to change in hydrophilic properties – water absorption capacity of the crumb. Studies of the hydrophilic properties of bread crumb showed that the developed bread had a higher water absorption capacity and lost its hydrophilic properties more slowly. The water activity index of bread with additives was lower compared to the control. After 48 hours of storage, the increase in the firmness of bread with added complex plant supplement was lower compared to the control, which indicates the ability to preserve the textural properties of bread. Determination of moisture bond forms in the crumb of bread showed that after 48 hours of storage the free moisture in the control bread was removed at a lower temperature – 92°C, while for the developed sample – at 105°C. It means that more energy is needed to remove free moisture from the developed bread. It was proved that the addition of rice flour, rice protein concentrate, sunflower lecithin and dry lamium leaves had a stable antioxidant effect in relation to the processes of free radical oxidation of lipids. An increase in the content of phenolic compounds, phenolic acids and flavonoids was found in breads after baking, and their decrease in the developed products during the storage process was more rapid, compared to the control.

Conclusions. Therefore, the introduction of the complex plant supplement – rice flour, rice protein concentrate, sunflower lecithin and dry lamium leaves extended the shelf life of wheat bread and increased its antioxidant activity.

Introduction

Bread belongs to products with a short shelf life. The term of its freshness depends on many factors, one of which is the properties of the raw materials included in its composition.

Despite the general tendency to increase number of products with a high content of dietary fiber in the human diet, there are people who suffer from inflammatory bowel diseases and they are not recommended to use these functional ingredients (Forbes et al., 2017; Zallot et al., 2013). In this aspect, rice-processing products attract attention, in particular rice flour, which contains 5 – 6 times less amount of dietary fibers than wheat flour (Kaur et al., 2022).

An increase in firmness of the crumb of wheat bread with an increase in the dosage of rice flour (to substitute 0-50% wheat flour) was observed. It was explained by different structure and smaller size of rice starch granules (0 – 400 μm) compared to wheat starch as well as greater susceptibility of rice flour starch to amylolysis (Rathore and Pandey, 2023). Similar results were obtained in other studies (Khoshgozaran-Abras et al., 2014). It was found that replacement 5 – 10% wheat flour with rice flour led to increase of firmness of the crumb by 8.3 – 11.8%.

The effect of replacing 30% of wheat flour with rice flour from three different varieties of rice on the preservation of freshness of bread was determined. It was found that replacing 30% wheat flour with waxy rice flour reduced the firmness of bread over three days due to higher amylose content in its starch compared to wheat flour (Purna et al., 2011). Addition of other types of rice flour increased firmness compared to bread from wheat flour. Aoki et al. (2012) established similar dependencies.

Bread has a soft, porous texture that can be deformed. The control bread crumb almost restored the structure after deformation and bread with rice flour with lower amylose content had a lower recovery and a stickier texture. Blending rice flour with wheat flour reinforced resistance to deformation because of an increase in crumb extensibility (Sasaki et al., 2014).

When rice flour was added to wheat flour, there was a redistribution of moisture bond forms in bread, the amount of weak-bounded moisture increased, so the structure became more compact, which caused decrease in the amount of energy needed to remove moisture, and therefore, staleness (Chen et al., 2022).

Rice by-products are mostly used in the technology of gluten-free rice bread. In addition, bread mostly has a low biological value and requires correction of the protein component in order to increase the content and applicability of protein by the body. Rice and wheat flour have different fractional composition of proteins and starch (Shevchenko and Litvynchuk, 2022), which affects the structural and mechanical indicators of bread, water absorption capacity of the crumb, crumbliness, and therefore the preservation of freshness by bread.

The mixture of rice flour and starch was replaced by 5 and 10% of rice protein, and the indicators that characterize the staleness of bread were determined. A decrease in crust moisture by 10.6-14.1% (Pico et al., 2019) and water activity by 9.6% (García-Segovia et al., 2020) was found in bread in case of replacing 5 and 10% mixture of rice flour and starch with rice protein.

The preservation of freshness in bread is also depends on the content of raw materials with antioxidant properties due to their ability to reduce free radical oxidation of lipids (Wang et al., 2023), in particular due to the introduction of raw materials with a powerful antioxidant complex into the bread recipe. Antioxidant activity and concentration of total soluble phenolic compounds was evaluated in rice grains with light brown, red and black pericarp colors. Concentrations of total soluble phenolic compounds were 7 to 15 times higher in the

grains with red and black pericarp colors compared to grains with a light brown pericarp color (Walter et al., 2013).

Taking into account the need for development of recipes for bakery products with high biological and nutritional value, as well as extending the shelf life of products, the aim of the present study was to determine the effect of complex plant supplement – rice flour, rice protein concentrate, sunflower lecithin and dry lamium leaves on the freshness of bread.

Materials and methods

Materials

Bread was prepared from main components – premium wheat flour, 1.5% of salt - and 3% of pressed baker's yeast. Complex plant supplement consisted of 3% sunflower lecithin, 5% rice flour instead of wheat flour, 4% rice protein concentrate to the weight of flour and 1% dry lamium leaves to the weight of flour, which was added to recipe of wheat bread. Sunflower lecithin is used as emulsifier and source of phospholipids. Rice protein concentrate with 74.2% of protein was extracted from rice bran by enzymatic method (Shevchenko et al., 2023). Bread without sunflower lecithin, rice flour, rice protein concentrate and dry lamium leaves was the control. Bread was obtained using preparing dispersed phase and dough on its base.

Methods

Brittleness of bread

Two pieces of 5 g of bread crumb were put into conical flask and mixed for 5 minutes. Then the mass of the whit formed by rubbing two pieces of bread was weighed. Brittleness was calculated as relation of weight of the whit to the initial weight of the bread pieces. Analyzis was done after 4, 24 and 48 hours after baking.

Amount of water absorbed by the crumb of bread

Crumb, 3 g, was crushed and weighed. The crumb was put onto a sieve and 17 ml of distilled water was slowly added to them from a pipette over 5 minutes. The soaked crumb was collected and weighed.

Deformation of the bread crumb

The total deformation of the bread crumb was studied after 4, 24 and 48 hours after baking using automatic penetrometer AP-4/2 (Germany). For measurement, a piece of bread crumb was put on the plate and a body with a certain mass for a certain time immerse into the crumb. The data was in expressed in penetrometric units (Zlateva and Chochkov, 2019).

Water activity of bread

Water activity of bread crumb was determined on a water analyzer at a temperature of 20 °C in the measurement range of 0 – 1 a_w (0 – 100%). A piece of bread crumb was placed in a container and a measuring chamber. A water activity probe was located on its top. The

measurement cycle lasts 3–5 minutes, after which the water activity and temperature values for each probe were shown on the display (Kochubei-Lytvynenko et al., 2018).

Bread firmness

Bread firmness was determined using TA.XT Plus Texture Analyser (Stable Micro Systems, Surrey, UK). The loaves of bread were cut into equal slices of 20 mm thick. For slices of this thickness, one slice was used for each test sample, and the end crust slices of the loaf were discarded. The measurement was performed in 3 replicates for each sample. Firmness is defined as the force (in grams) required to compress the product by a pre-set distance (i.e. force taken at 25% compression of 25 mm). A 25% compression of a 25 mm thick sample = 6.25 mm compression distance at which point the compression force value (CFV) is taken (Wongsagonsup et al., 2015).

Moisture bond forms in the crumb of bread

Moisture bond forms in the crumb of bread during its storage was determined by the thermogravimetric method on a Q-1500 derivatograph. The sample and the standard are loaded into the working volume and heated at a constant rate. The temperature, the difference between the temperatures of the sample and the standard reference temperature, sample mass change, the difference in masses of the reference and working samples were measured. 1 g of the standard sample and a test sample was loaded in two crucibles. They are heated in the temperature range of 20–250 °C and at a rate of 1.25 °C/min. The recording device captures the graphs (Pivovarov et al., 2018).

Total polyphenol content in bread

Total phenolic content was determined spectrophotometrically using Folin-Ciocalteu procedure in a microplate method according to (Xiong et al., 2020) with slight modifications. In a 96 well-plate, 50 µL of distilled water were added, followed by 25 µL of the sample or standard (in triplicate), and 25 µL of Folin-Ciocalteu reagent. After 6 min, 100 µL of 7.5% sodium carbonate solution were added to all wells. The plate was then left in the dark for 90 min at room temperature and then read using a UV–Vis plate reader (Glomax Multi+, Promega Corp., Madison, USA) at 765 nm. The UV–vis measurements were calibrated against gallic acid curve (0 – 500 mg/L; $R^2=0.9989$), and the results were presented as gallic acid equivalent (GAE) in mg per g of dry weight.

Total phenolic acid content in bread

Total phenolic acid content was determined using method of Jain et al. (2017). A 0.5 ml of sample extract was mixed with 0.5 ml of 0.5 M hydrochloric acid, 0.5 ml Arnova reagent (10% NaNO_2 + 10% Na_2MoO_4), 0.5 ml of 1 M sodium hydroxide (w/v) and 0.5 ml of water. Absorbance at 490 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid (1 – 200 mg/L, $R^2 = 0.999$) was used as a standard and the results were expressed in mg/g caffeic acid equivalents.

Total flavonoid content in bread

Total flavonoid content was determined using the modified method of Willett, (2002).

The sample (0.5 ml) was mixed with 0.1 ml of 10% (w/v) ethanolic solution of aluminium chloride, 0.1 ml of 1 M potassium acetate and 4.3 ml of distilled water. After 30 min in darkness the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Quercetin (0.5-20 mg/L; $R^2=0.989$) was used as the standard and the results were expressed in mg/g quercetin equivalents.

Antioxidant activity of bread

Antioxidant activity was determined spectrophotometrically using DPPH assay described by Brand-Williams et al., (1995) adapted to microplate protocol. Briefly, to prepare the 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent, 25 mg was dissolved in 100 mL of 96% ethanol and adjusted to an absorbance of 0.7. Into a 96 well-plate, 25 μ L of the sample, blank (DPPH) or standard (in triplicate) were added, followed by 180 μ L of DPPH solution. The plate was then left in the dark for 10 min at RT, while simultaneously shaking at 500 rpm, and read using a UV-Vis plate reader (Glomax Multi+, Promega Corp., Madison, USA) at 515 nm. Determination of AA was calculated as a percentage of DPPH inhibition according to the formula (1):

$$\text{DPPH inhibition (\%)} = [(\text{AbsBlank} - \text{AbsSample}) / \text{AbsBlank}] \times 100 \quad (1)$$

Subsequently, the ability to scavenge DPPH radical was expressed as Trolox equivalent antioxidant capacity (TEAC) in mg per g of dry weight (DW), calculated using a Trolox standard curve (0 – 100 mg/L; $R^2=0.9982$).

Oxidative stability of bread

The oxidative stability was determined in 892 Rancimat apparatus from Metrohm (Switzerland) according to ISO 6886:1997 utilizing a sample of 0.5 ± 0.01 g. All samples were studied in temperature 120 °C, under a constant airflow (20 L/h). The induction times were printed automatically by apparatus software with the accuracy of 0.005.

Statistical analysis

The data represents the mean of a minimum three replicates $\pm$ standard deviation (S.D.). Graphical presentation of experimental data was performed using program Microsoft Excel 2010.

Results and discussions

The influence of recipe components (mixture of 3% sunflower lecithin by weight of flour, 5% rice flour instead of wheat flour, 4% rice protein concentrate to the weight of flour and 1% of dry lamium leaves to the weight of flour) on the shelf life of bread was determined by indicators of crumb deformation, crumbliness, water absorption, water activity and redistribution of moisture in the crumb.

Studies of crumb deformation using a penetrometer during 48 h of storage showed (Table 1) that the total deformation of the samples with additives was lower than in the control.

Table 1

Indicators of deformation of the crumb of the products according to the penetrometer

Indicator	Control bread	Experimental bread
General deformation of the pulp, units of device		
after 4 hours	47±1	58±2
after 24 hours	32±1	48±1
after 48 hours	24±2	34±1
Degree of preservation of freshness relative to initial freshness, %		
after 24 hours	68±1	83±3
after 48 hours	51±2	58±1

Results are given as M ± SD (mean ± standard deviation) for triplicate trials.

It was proved that the crumb of the developed bread was less susceptible to deformation by 23.4% after 4 hours of storage, by 50.0% and 41.6% after 24 and 48 hours, respectively, and faster regained form. This is explained by the higher water absorbing and moisture-retaining capacity of the rice flour (Shevchenko and Litvynchuk, 2022a), rice protein concentrate and dry lamium leaves.

Changes in the physico-mechanical properties of crumb, which affect aging, were determined by indicators of brittleness and water absorption capacity (Table 2).

Table 2

Brittleness and water absorption capacity of bread

Indicator	Control bread	Experimental bread
Brittleness of bread, % to mass of crumb		
after 4 hours	2.4±0.1	1.5±0.1
after 24 hours	9.2±0.2	7.1±0.3
after 48 hours	11.8±0.1	9.9±0.1
Water absorption capacity of the crumb of products, % dry matter		
after 4 hours	456±2	498±2
after 24 hours	416±1	439±2
after 48 hours	336±2	388±2

Results given as M ± SD (mean ± standard deviation) of triplicate trials.

It was found that the brittleness of the experimental bread decreased by 22.8% after 24 hours and by 16.1% after 48 hours, compared to the control. This is explained by a smaller loss of hydrophilic properties of bread crumb, which led to a delay in the aggregation of amylose and amylopectin of flour starch (Scott and Awika, 2023).

Determination of the hydrophilic properties of bread crumb showed that the water absorption capacity of experimental bread was higher after 24 hours by 5.5% and by 15.5% after 48 hours compared to the control sample. At the same time, the developed bread lost its hydrophilic properties more slowly. This is explained by the higher water absorption and water retention capacity of the added complex plant supplement, so bread had a higher moisture content, which helps to delay the staleness.

Staleness significantly determined by the state of moisture in bread, which is affected by the activity of water (De Luca et al., 2021). It was established (Figure 1) that bread belong to products with intermediate water activity because the value of the indicator lies within the

limits 0,9 – 0,6, the water activity index of the developed product was lower compared to the control by 2.0%. This may be due to a lower indicator of water activity of raw materials included in the recipe. This is positive considering the fact that products with high values of this indicator undergo microbiological spoilage more intensively (Tapia et al., 2008). From the point of view of preservation of freshness, it is important to analyze the change in water activity values in bread during storage. After 48 hours, the value of water activity for the control did not change, but for the developed product it increased slightly by 0.6%. However, the value was lower by 1.4% than for the control bread after 48 h, which is a positive factor considering the lower microbiological activity in this product.

The freshness of bread was also determined by changes in its firmness after 48 hours of storage. The firmness of bread after baking and after 48 h of storage was found (Figure 2).

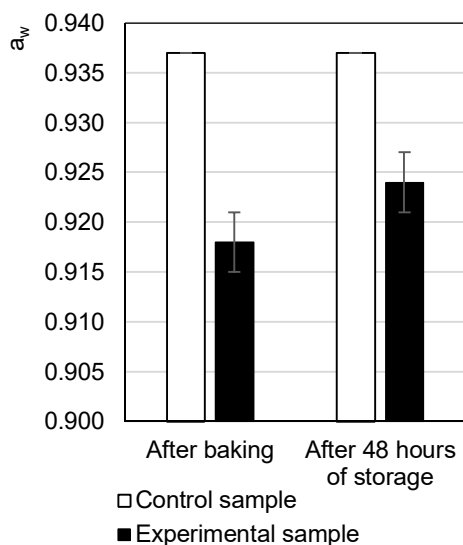


Figure 1.
Activity of water of bakery products during storage



Figure 2.
Firmness of bakery products

It was established that complex plant supplement increased the firmness of bread after baking, which is associated with the difference in the fractional composition and properties of proteins and starch of rice and wheat flour (Shevchenko and Litvynchuk, 2022b). However, after 48 h, the increase of the firmness of bread with complex plant additive was lower compared to the control, which demonstrates the ability of this additive to contribute to the preservation of the textural properties of bread during storage.

The effect of addition of complex plant supplement on the duration of freshness of products can be estimated by the change in the amount of free and bound moisture during storage and the energy consumption of the dehydration process using the thermogravimetric method (Matejschuk, 2016). The research was carried out on a derivatograph (Figure 3, 4).

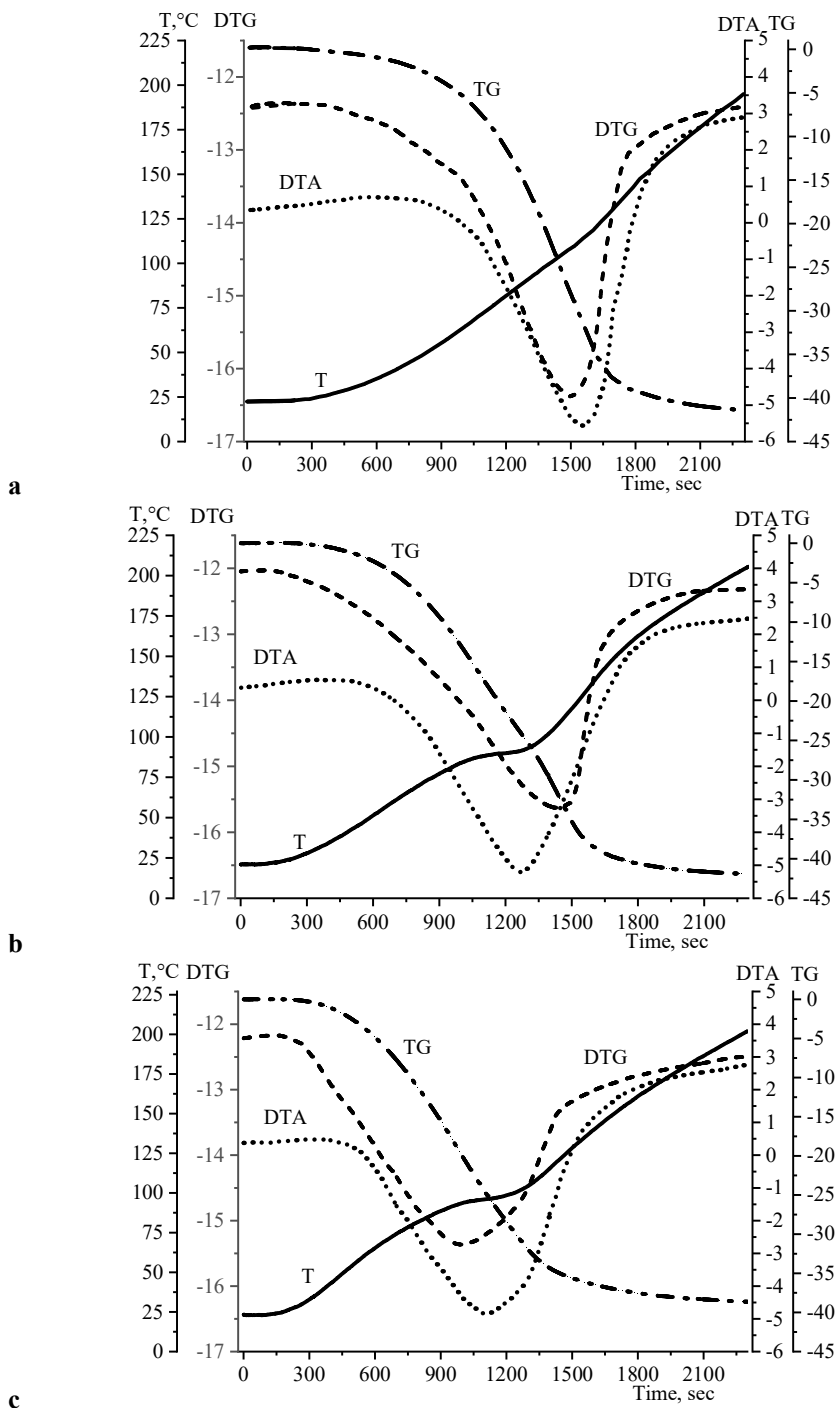


Figure 3. Derivatograms of control bread crumb thermolysis:
a, b, c – control after 4, 24, 48 hours of storage

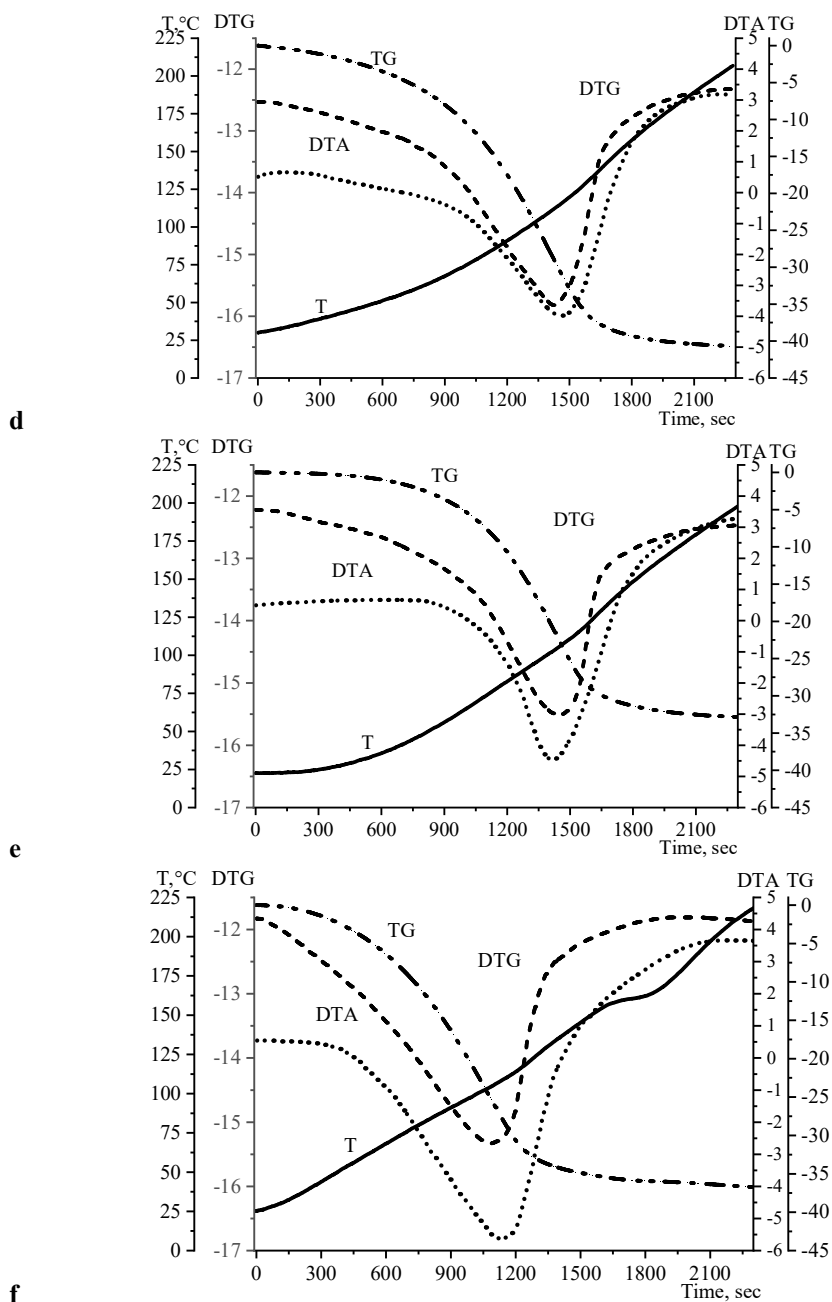


Figure 4. Derivatograms of experimental bread crumb thermolysis:
d, e, f – experimental sample after 4, 24, 48 hours of storage

Curves were built: DTG curve, which indicates the processes of decreasing the weight of the samples, DTA curve is a curve of temperature difference between the sample material and the reference material versus temperature and time, TG curve shows the change in sample weight as a function of temperature or time under controlled gas atmosphere, T curve shows temperature change.

In both samples, one endoeffect was recorded on the DTG curves. In the bread samples after 4 and 24 hours of storage, the final stage of free moisture removal occurred at the same temperature - 115°C. After 48 h of storage, the trend changed and the free moisture of the control was removed at a lower temperature - 92°C, while for the developed sample at 105°C. This is explained by the need for more energy to remove free moisture from the developed bread, which will contribute to longer preservation of moisture inside the product and a lower degree of its evaporation. In the control sample a more rapid loss of moisture was observed, and in the developed product, the slowing down of moisture loss occurred due to its binding by proteins under the influence of temperature. This indicates that the energy supplied to the samples was spent largely on the course of destructive processes in the control than in the developed sample.

Added complex plant supplement, especially dry lamium leaves, have a high content of compounds with antioxidant properties. Therefore, it is advisable to investigate their content in the developed products.

Table 5

Antioxidant compounds in bakery products

Indicator	Control sample		Experimental sample	
	After baking	After 48 hours of storage	After baking	After 48 hours of storage
Total polyphenol content, mg GAE/g	2.15±0.02	1.69±0.02	3.09±0.03	2.49±0.03
Total phenolic acid content, mg CAE/g	0.19±0.01	0.04±0.01	1.62±0.04	1.34±0.04
Total flavonoid content, mg QE/g	2.15±0.03	0.27±0.03	3.06±0.03	2.01±0.03
Antioxidant activity, (mg TEAC /g)	0.32±0.01	0.07±0.01	0.55±0.03	0.13±0.03
Oxidative stability, induction time (hours)	35±0.25	32±0.25	7.8±0.28	6.5±0.28

Results given as M ± SD (mean ± standard deviation) of triplicate trials.

It was proved that the addition of rice flour, sunflower lecithin, rice protein concentrate and dry lamium leaves to the recipe showed a stable antioxidant effect in relation to the processes of free radical oxidation of lipids. An increase in the content of phenolic compounds, phenolic acids and flavonoids was found in bread samples after baking compared to the control bread because of their higher content in added complex plant supplement. At the same time, their decrease in the developed products during the storage process was more rapid, compared to the control. A decrease in the content of phenolic

compounds correlates with an increase in antioxidant activity in the developed bakery product.

Therefore, the added complex plant supplement due to their higher hydrophilic and antioxidant properties are able to extend the shelf life of bread and save its freshness longer. This is a promising way to obtain bread with good consumer properties.

Conclusions

1. The crumb of bread with added rice flour, sunflower lecithin, rice protein concentrate and dry lamium leaves was less susceptible to deformation by 23.4% after 4 hours of storage, by 50.0% and 41.6% after 24 and 48 hours, respectively, and faster regained form.
2. The brittleness of the developed bread with added rice flour, sunflower lecithin, rice protein concentrate and dry lamium leaves decreased by 22.8% after 24 hours and 16.1% after 48 hours, compared to the control bread because the loss of moisture of the crumb was less.
3. Bread with additional components had a higher water absorption capacity during storage by 5.5% after 24 hours and by 15.5% after 48 hours compared to the control and lost its hydrophilic properties more slowly.
4. Water activity index of the developed product was lower compared to the control by 2.0%.
5. Added components increased the firmness of bread after baking, but after 48 hours, the increase in the firmness was smaller compared to the control, which indicates the ability of this complex plant supplement to contribute to the preservation of the textural properties of bread.
6. Determination of moisture bond forms in the crumb of bread showed that after 48 hours of storage the free moisture in the control bread was removed at a lower temperature – 92°C, while for the developed sample – at 105°C. In the control, a more rapid loss of moisture was observed.
7. The addition of the proposed recipe components in the complex showed a stable antioxidant effect in relation to the processes of free radical oxidation of lipids.

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