

## **Development of gluten-free bread with unconventional raw ingredients of high nutritional value and antimicrobial activity**

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**Abstract.** Two types of rowan powder (botanical species *Sorbus aucuparia*) as unconventional raw ingredients of high nutritional and biological value as well as three types of dietary fiber and pectin were used in development of gluten-free bread. These raw ingredients have high water-holding capacity and a rich biochemical composition that makes it possible to use them not only as thickeners and structure forming agents, but also as enriching additives. It was experimentally found that the citrus fiber and pectin improved the bread specific volume and the crumb compressibility if compared to the control sample. The content of vitamins A, E, PP, C, B complex, minerals - iron, magnesium, calcium, potassium, selenium, organic acids, including preservatives rendering action (citric, lactic, sorbic, benzoic) were found in rowan powder. It was found that rowan powder and citrus fiber had a significant effect on the increase in the content of dietary fiber in 2.5–5.4 fold and iron in 2–3.5 fold. The content of dietary fiber in bread with 4% of rowan fruit powder is 3.6 g 100g<sup>-1</sup> while in bread with 8% rowan pomace it is 4.3 g 100g<sup>-1</sup>, which corresponds to the dietary fiber daily needs satisfaction respectively by 10.0% and 21.5%. It was found that bread with rowanberry powder had 66.7% more water-soluble antioxidants. The contamination of the main gluten-free raw material (soy protein, rice flour and corn starch extrusion and corn, rowan powder) and its influence on ropy disease of gluten free bread were established. Four spore forming bacteria strains were isolated from gluten-free raw materials and its ability to cause ropy disease of gluten free bread was proved. It was also found that using of rowan powder slow down ropy disease and mold spoilage due to the organic acids in its composition and the bread acidity increase.

**Key words:** Gluten-free bread, rowan powder, quality, nutritional value, antioxidant activity, microflora, microbial resistance

### **INTRODUCTION**

Due to environment adverse effects, genetic and allergic diseases growth, the dietary and functional products development is one of main food industry objectives. Today bakery products traditionally form the basis of the population diet both in Russia and Europe being the most consumed grain foods. Therefore, in their production special attention is paid to the issues of quality, safety and assortment of gluten-free bread due

to the wide variety of food ingredients that provide the human body with the necessary physiologically active substances. The improved diagnostic methods have led to the identification of one of the 21st century genetic diseases – celiac enteropathy (coeliac disease). It is a serious genetic autoimmune disorder where gluten ingestion leads to chronic inflammation of the mucous membrane with complete atrophy of the intestinal villi. Coeliac disease is caused by a reaction to gluten that is a protein found in wheat and other grains such as barley, rye, triticale and oats in the early stages (Troncone & Jabri, 2011). At present there is no cure and the only effective treatment for this disease is a lifelong gluten-free diet. It leads to the intestinal mucosa recovery, improves symptoms, and reduces risk of developing complications in most people (Rashtak & Murray, 2012). Bread is included into the daily diet of all population segments of Russia. But a large group of the population of Russia suffering from a celiac disease is forced to abandon the traditional bread consumption and to start consuming gluten-free products (Vokhmyanin, 2009). Therefore, the demand for gluten-free products, produced from natural gluten-free raw materials (rice, corn, buckwheat flour, corn starch and other gluten-free cereal, soy protein, lupine, amaranth, legumes, etc.) is constantly growing. However, existing gluten-free products have a number of disadvantages, namely: low nutritional value, mild flavor and odor, susceptibility to microbiological spoilage (Thompson, 2000; Hager et al., 2012; Javaria et al., 2016). This is due to the fact that for the production of gluten-free products, including bread, raw materials with low nutritional value are mainly used (do Nascimento, 2013; Pellegrini & Agostoni, 2015). Gluten-free bread does not have traditional odor of freshly baked bread and does not satisfy consumers' flavor demands (Pszczola, 2012). In addition, gluten-free bread stales quickly because of the high content of starch in the recipe. The Russian gluten-free bread rapidly undergoes microbial spoilage because it has very low acidity (pH above 6). Today a lot of scientists and food industry specialists are developing ways of improving gluten-free bread quality (Krasilnikov et al., 2013; Nachay, 2013; Kuznetsova & Dubrovskaya, 2014; Matos & Rosell, 2014).

The aim of research is to develop gluten-free bread using unconventional raw ingredients of high nutritional and biological value – dietary fiber and pectin, as well as the rowan powder (botanical species *Sorbus aucuparia*). These ingredients have high water-holding capacity and a rich biochemical composition that makes it possible to use them not only as thickeners and structure forming agents, but also as enriching additives. The effect of non-traditional raw ingredients on biotechnological processes in the dough and on the quality, freshness, structural and mechanical properties of the crumb, nutritional value, antioxidant activity and resistance to mold growth and the ropy disease of gluten-free bread with fiber and pectin and rowan powder were studied.

## MATERIALS AND METHODS

Two types of rowan powder (botanical species - *Sórbus aucupária*) – the ordinary rowan fruit powder and the powder from the pomace of the fruits of rowan varietal phoenicea – were used in this research. The ordinary rowan is characterized by bitterness and astringency of the fruit. The rowan varietal phoenicea, a new variety grown in the Michurin All-Russian Research Institute of genetics and breeding of fruit plants, has, as

its distinctive feature, the lack of bitterness and astringency and high content of biologically active substances, especially vitamins, micro- and macroelements.

Acidity, content of volatile acids and organic acids (malic, succinic, sorbic etc., including fatty and phenolic acids), vitamins, and minerals were determined in the rowan powder. Gluten-free bread was evaluated by organoleptic and physico-chemical parameters such as moisture, acidity, porosity and specific volume.

Acidity was determined by titration, using 0.1 n. solution of NaOH. Content of volatile acids was determined by neutralization of evaporated volatile acid using 0.1 n. solution of NaOH.

Analysis of biochemical composition of the rowan powder was carried out using gas-liquid chromatography with mass spectrometry (GC-MS) on a chromatograph 'Agilent 6850' (USA). Antioxidant activity of rowanberry powder and gluten free bread was determined using amperometric method.

As thickeners and the texture forming substances the citrus fiber Citri-Fi 100, Citri-Fi 200, Herbacel AQ Plus and citrus pectin (a Genu pectin type BIG) were studied. Corn extrusion starch was used as control.

Water holding capacity of the thickener is studied in the following way. 5 g of the thickener were mixed with 30 ml of water (or more), then it was mixed for 1 min at 1000 rpm, left at rest for 30 min, centrifuged for 25 min with the imminent 3200 rpm, feeding the separated water. The increased water-holding capacity was calculated according to the ratio of the mass of released water to the initial weight and multiplied by 100%

Optimization of doses of rowan powder in the recipe was carried out by a laboratory test baking. The formulation of control bakery products was: bakery mix total 100% (including corn starch – 57.8%, extrusion corn starch – 10.0%, rice flour – 19.7%, soy protein – 9.7%, salt – 0.8%, sugar – 2.0%) and yeast – 2.5%, vegetable oil – 4.0%.

The enrichment of bakery products is achieved by adding rowan powder instead of corn starch. The content of additive varied from 2 to 10% with the increment of 2 %. All test samples were prepared by using the straight dough method. Test bakery products weighing 250 g were baked in laboratory at the temperature of 210 °C during 18 min.

The assessment of bakery products quality was carried out by following properties: Organoleptic – appearance (shape, surface, crumb color), condition of crumb (porosity and texture), flavor and odor; physico-chemical and physical - mass proportion of moisture was determined by drying at a temperature of 130 °C during 45 minutes, acidity was determined by titration, using 0.1 n. solution of NaOH, porosity was determined as the ratio of pore volume to the total volume of products, pore volume – as the difference between the volume of product and the volume of non-porous mass, specific volume – as the ratio of product volume to 100 g of bread. The contents of the spore-forming bacteria number was determined by plating the heated samples of meat-peptone agar. It was suspended in 100 cm<sup>3</sup> of sterile water and heated in a water bath for 10 minutes at a temperature of 90–94 °C in order to inactivate the vegetative cells of 10 g of the studied raw materials. The prepared dilutions 10<sup>-1</sup>–10<sup>-2</sup>. 1 ml of each dilution were added to Petri dish and flooded with 10 ml mycopathologia agar, and then for 2 days were cultured in a thermostat at 37 °C (Afanasjeva, 2003).

To determine the ability of the isolated spore-forming bacteria that cause ropy disease a bread crumb with the spores was prepared. Thus, the spore-forming bacteria on meat-peptone medium was added to the surface of the sliced gluten free bread and cultured at a temperature of 37 °C for 96 hours or until signs of disease. The diseased

bread was dried in an oven at a temperature of  $50 \pm 2$  °C and milled to obtain crumbs. 1% of infected crumbs were added while kneading the dough for gluten-free bread. The ready bread was stored at 37 °C until the appearance of ropy disease symptom.

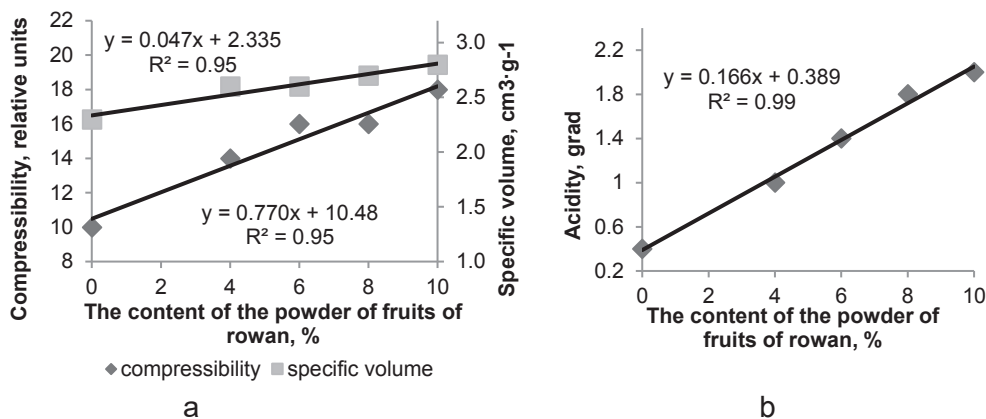
## RESULTS AND DISCUSSION

Gluten-free bread is characterized by a low content of vitamins, minerals and fiber, bland, and empty flavor, odor is not enough pronounced (Thompson, 2000; Hager et al., 2012). In addition, it quickly molds or becomes ropy (Gutierrez et al., 2011). In order to expand the range of gluten-free bread with the improved consumer properties and high nutritional value, the enriching supplements from non-traditional vegetable raw materials, which contain significant quantities of nutritionally valuable substances – vitamins, fiber, protein, macro- and microelements – as well as preservative and antimicrobial compounds were used.

The possibility of using the rowan powder of the Russian origin as an enriching and preservative additive in the formulation of gluten free bread was studied.

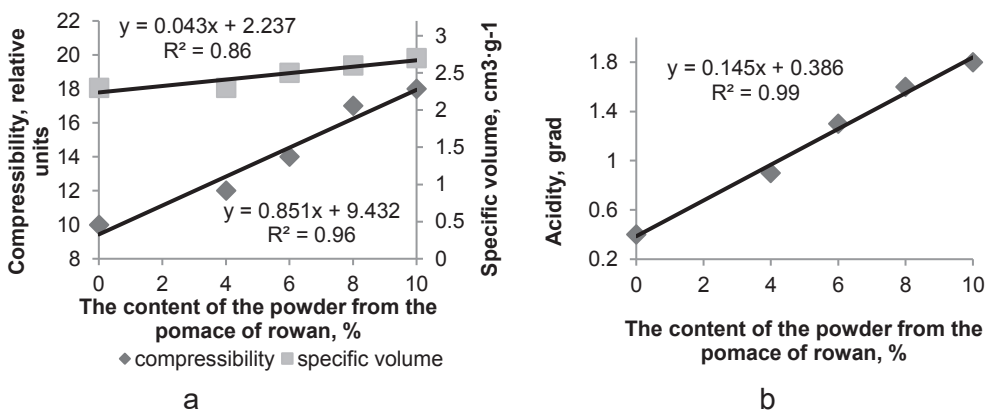
The use of rowan powder in the gluten-free mix is proved by its rich chemical composition, high acidity (50–55 deg) and a high content of volatile acids (of 2.18 and 2.75%), which significantly affects the increase in the nutritional value and the improvement of consumer qualities, the formation of a more pronounced gluten-free bread odor and flavor. The presence of sorbic, benzoic and acetic acids possessing preservative properties will help prolong the shelf life and inhibit the development of microbial spoilage. Furthermore, the use of rowan powder will result in improved processing characteristics due to the high water absorption ability (205%) and high degree of esterification (55–78%) (Dubrovskaya et al., 2013).

The analysis of vitamin composition of the rowan powder showed high contents of ascorbic acid ( $40\text{--}70$  g  $100\text{g}^{-1}$ , which technology is used as bread improver that provides improved structural-mechanical properties and improves ability of gas retention of the dough, which will lead to the increase in the specific bread volume and structure of porosity. In rowan powder vitamins – A ( $9\text{--}14$  mg  $100\text{g}^{-1}$ ), B1 ( $28\text{--}29$  g  $100\text{g}^{-1}$ ), B2 ( $374\text{--}431$  g  $100\text{g}^{-1}$ ), E ( $9\text{--}9.2$  g  $100\text{g}^{-1}$ ) and minerals - potassium ( $780\text{--}980$  g  $100\text{g}^{-1}$ ), calcium ( $260\text{--}300$  g  $100\text{g}^{-1}$ ), magnesium ( $78\text{--}100$  g  $100\text{g}^{-1}$ ), iron ( $8\text{--}2.4$  g  $100\text{g}^{-1}$ ) etc. were discovered. Their presence will provide the opportunity to increase the content of macro- and micronutrients that are available in gluten-free bread. So rowan powder is a valuable biological raw material that can be used to improve processing characteristics and gluten free bread nutritional value. To identify the optimal doses of rowan powder in the mixture, the gluten-free bread was baked. The results indicate that with the increasing dosages of powder from both the fruits of ordinary rowan and pomace of varietal rowan compressibility indices, specific volume and acidity of bread were improved (Figs 1 and 2). A significant influence of rowan powder in almost all sensory characteristics, particularly flavor, odor and color of the crumb was also observed.



**Figure 1.** Influence of the rowan fruits powder on the compressibility, specific volume (a) and acidity (b) of gluten-free bread.

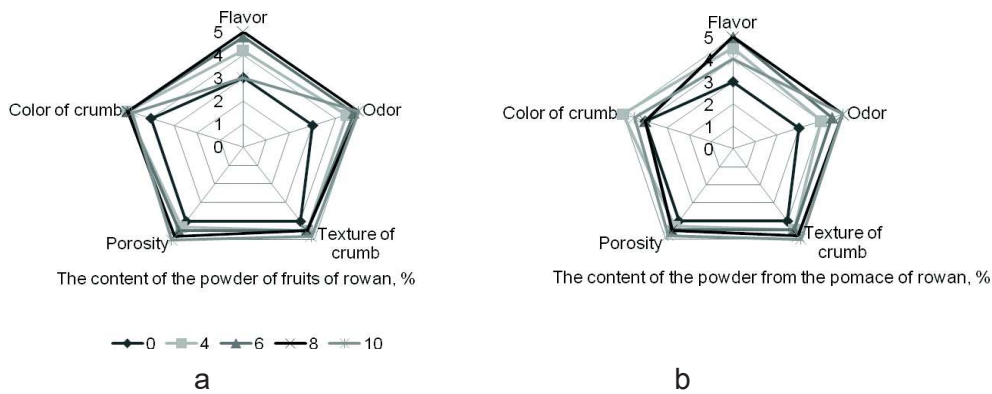
The crumb of the bread containing 4% rowan powder had a fine texture, porosity, and the odor and flavor was more pronounced and had fruit notes. It was found that the flavor depended on the type of rowan powder and its dosage. The powder of fruits of rowan, in contrast to the powder from the pomace of the rowan, had a bitterness, which manifests itself in the bread, and its intensity depends on the amount of rowan powder contained in the recipe gluten free mix. At the same time bread with powder from the pomace of rowan has gained a more pronounced fruity flavor and a harmonious acidity with the powder dosage increase (Fig. 3).



**Figure 2.** Influence of rowan fruits powder on the compressibility, specific volume (a) and acidity (b) of gluten-free bread.

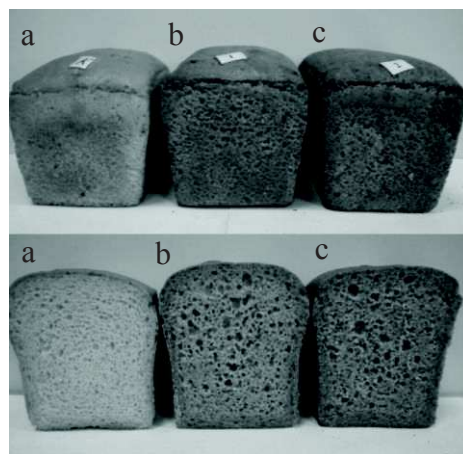
It was established that the optimum amount of rowan powder in the mixture was not more than 4% of rowan fruits powder and 8% of powder from the pomace of rowan. The samples of bread were distinguished by superior organoleptic characteristics: the color of the crusts had a bright coloring, and the flavor and odor was more pronounced.

The flavor was more intense, with acidity notes, resembling traditional bread. The odor was more intense, pleasant, with fruity notes. The increase in specific volume, acidity and compressibility were observed.



**Figure 3.** Influence of the rowan powder on the organoleptic profiles of gluten-free bread: a) rowan fruits powder; b) powder from the pomace of rowan.

To improve consumer properties of gluten free bread the research on the selection of new types of thickeners of vegetable origin of citrus fiber and pectin was conducted. Citrus fiber 'Citri-Fi 100', 'Citri-Fi 200', 'Herbaccel AQ Plus', obtained by drying the cell walls of citrus fruits, water extraction or mechanical treatment without the use of chemical reagents, and citrus pectin 'Genu pectin type BIG' – highly purified pectin from citrus pomace were investigated. High water-binding capacity (from 700 to 980%), emulsifying, stabilizing and structure-forming properties allow to apply them as a consistency stabilizer to bind water and give a certain structure to the product, prolong freshness, improve appearance, crumb structure and flavor and increase nutritional value. The quality parameters of used thickeners are presented in Table 1.



**Figure 4.** Images for gluten free bread: a) control without rowan; b) with 4% of rowan fruits powder; c) with 8% of powder from the pomace of rowan.

The parameter of water retention capacity of citrus fibres and pectin was in 2–2.6 times higher than this parameter for corn extrusion starch. Thus, it is possible to assume that the replacement of corn extrusion starch with citrus fiber and pectin will improve the structure of the dough, physico-chemical (specific volume, compressibility) and organoleptic indicators of gluten free bread quality. The use of these thickeners in the formulation of gluten free bread will increase dough water retention capacity, which will have a positive impact on the formation of crumb structure and on increasing of bread consumer advantages.

**Table 1.** Organoleptic and physico-chemical characteristics of thickeners (Mean  $\pm$  SD)

Quality parameters of used thickeners	Extrusion cornstarch	Citrus fibers		
		Citri-Fi 100	Citri-Fi 200	Herbacel AQ Plus
Mass proportion of moisture, %	9.8 $\pm$ 0.5	9.6 $\pm$ 0.5	9.2 $\pm$ 0.5	9.0 $\pm$ 0.5
Water-binding capacity, %	370 $\pm$ 2.0	705 $\pm$ 4.0	710 $\pm$ 4.0	978 $\pm$ 5.0
Content of dietary fiber, %	-	62 $\pm$ 0.5	75 $\pm$ 0.3	90 $\pm$ 0.3
Particle size, microns	98 $\pm$ 2.0	200 $\pm$ 4.0	200 $\pm$ 4.0	250 $\pm$ 4.5
Recommended dosage, % (by weight of the mixture)	9.5–10.0	0.1–3.0		0.3–1.5

On the basis of conducted experiments it was determined optimal dosage of thickeners in the formulation of gluten free bread, which was 1.5% – to 'Citri-Fi 100' and 'Citri-Fi 200'; 1.2% – 'Herbacel AQ Plus' and 1.3% for – 'Gene pectin type BIG' (Dubrovskaya, 2014).

In the second stage of research identified the degree of satisfaction of the average daily needs of an adult for nutrients when consuming 100 g of gluten free bread with rowan powder in accordance with the norms of physiological needs for energy and nutrients for different population groups of the Russian Federation (Tutelian, 2008).

It was found that rowan powder in combination with citrus fiber had a significant impact on the increase in the content of dietary fiber and iron (Table 2). It is important because of iron deficiency in the gluten-free diet (Thompson, 2000). The content of dietary fiber with 4% of rowan powder is 3.6 g 100 g<sup>-1</sup> and when using 8% pomace of rowan is 4.3 g 100 g<sup>-1</sup> which corresponds to the satisfaction of the daily needs for them respectively by 10.0% and 21.5%.

The iron content in the test samples compared with the control increased 2–3.5 fold, and the satisfaction of daily requirement reached 11%. Bread with 8% ash powder was characterized by a marked increase in the content of mineral elements – calcium, manganese, and potassium that corresponds, respectively, 2.4%, 14% and 4.6% of daily value.

The main sources of bio antioxidants for humans are known to be food products based on vegetable raw materials. The ash powder also contains antioxidant sources, such as vitamins C and E, selenium and carotenoids, etc. in its composition.

Consequently, the use of rowan powder in the recipe gluten free bread leads to the higher antioxidant activity, which positively influences the human health.

When determining the total content of water-soluble antioxidants on the device 'Color Jauza – 01 – AA', it was found that bread with rowanberry powder had 66.7% more water-soluble antioxidants.

**Table 2.** Satisfaction of daily requirement for nutrients

Substance	Consumption norm (Tutelian, 2008)	Value of the indicators of satisfaction of daily requirement for nutrients in the rice gluten-free bread, %		
		Control	With rowan powder,	
			From the fruits, 4.0 % in mixture	From the pomace, 8.0 % in mixture
Proteins, g day <sup>-1</sup>	58–117	5.6–11.4	5.7–11.5	5.8–11.7
Fats, g day <sup>-1</sup>	60–154	1.4–3.7	1.4–3.7	1.4–3.7
Digestible carbohydrates, g day <sup>-1</sup>	257–586	8.2–18.7	8.0–18.2	7.5–17.2
Dietary fiber, g day <sup>-1</sup>	20	4.0	10.0	21.5
<u>Vitamins:</u>				
C, mg day <sup>-1</sup>	90	-	0.7	0.8
A, mg day <sup>-1</sup>	900	-	0.2	0.4
E, mg/day	15	-	1.4	2.5
B <sub>1</sub> , mg day <sup>-1</sup>	1.5	0.9	0.8	0.8
B <sub>2</sub> , mg day <sup>-1</sup>	1.8	0.7	1.1	1.7
PP, mg day <sup>-1</sup>	20	1.6	1.6	1.6
<u>Minerals:</u>				
Sodium, mg day <sup>-1</sup>	1,300	15.2	15.3	15.5
Magnesium mg day <sup>-1</sup>	400	1.2	1.6	2.3
Potassium, mg day <sup>-1</sup>	2,500	3.2	4.2	4.6
Calcium, mg day <sup>-1</sup>	1,000	1.1	1.7	2.4
Phosphorus, mg day <sup>-1</sup>	800	3.5	4.6	5.7
Iron, mg day <sup>-1</sup>	10–18	1.7–3.0	6.1–11.0	3.3–6.0
Selenium, µg day <sup>-1</sup>	55–70	-	0.06–0.07	0.1–0.2
Manganese, mg day <sup>-1</sup>	2	-	3.3	14.0
Zinc, mg day <sup>-1</sup>	12	-	0.2	0.4

Due to the fact that rowan powder contains organic acids, which have preservative action, the effect of rowan powder on increasing of bread microbiological stability during storage was studied. The contamination of raw materials by spore-forming bacteria of the genus *Bacillus* is known to be the main cause of ropy disease.

The degree of bacteriological contamination of the main gluten-free raw material (soy protein, rice flour and corn starch extrusion and corn, Rowan powder) and the effect of the rowan powder on the quality of gluten free bread and its resistance to the ropy disease development were investigated.

The highest number of spore-forming bacteria was detected in the corn starch, rice flour and rowan powder (Table 3). Four strains of spore forming bacteria were isolated from gluten-free raw materials. It is known that a large number of bacteria can form spores, but not all of them are the causative agents of bread ropy disease. Regarding this, laboratory baking with bread crumbs, infected with spores of these bacteria was conducted to identify the ability of the isolated spore-forming bacteria that cause gluten-free bread ropy disease. A control bread sample was made from gluten free raw materials without spore infected crumbs.



**Table 3.** Microflora of gluten-free raw materials (Mean ± SD)

Raw materials	Spore-forming bacteria x 10 <sup>-2</sup> , CFU g <sup>-1</sup>
Corn flour	8.0 ± 0.4
Corn starch	13.0 ± 0.6
Rice flour	12.0 ± 0.6
Soya protein	2.0 ± 0.1
Starch extrusion	2.0 ± 0.1
Rowan powder	10.0 ± 0.5

It was found that all four test samples prepared with the crumb containing strains of spore-forming bacteria isolated from raw material fell ill after 18–24 h, while in the control sample signs of the ropy disease appeared in 48 hours. It confirms the ability of the studied spore-forming bacteria to cause ropy disease of gluten free bread. Thus, the experiment has shown that gluten-free raw materials are the primary source of ropy disease.

In order to establish the effect of rowan powder on the ropy disease of bread the laboratory baking with rowan powder and breadcrumbs infected by spores were carried out. Bread was laid deposited in the precipitating conditions at 37 °C and humidity 70 ± 5%.

It was found (Table 4) that in the samples with the rowan powder in the amount of 4% signs of ropy disease appeared 12 hours later than in the controls. In the samples of gluten free bread with 8% rowan powder and infected crumbs signs of ropy disease were noted 6 hours later than in controls ones with crumbs, and in samples prepared without spore crumbs the signs of the disease were missing for 48 hours.

**Table 4.** Influence of rowan powder on the ropy disease of gluten free bread

Indicator	Storage periods (day) to microbial spoilage in bread when making rowan powder in the amount,%					
	0		4		8	
	Without spore crumbs	1% of spore crumbs	Without spore crumbs	1% of spore crumbs	Without spore crumbs	1% of spore crumbs
Acidity, degrees	0.4	-	1.2	-	1.4	-
Weak odor	24	18	36	24	nd	24
Strong odor	36	24	-	-	nd	-
The stickiness of crumb	36	24	48	36	nd	36

nd – not detected for 48 hours

It was also found that the use of rowan powder helped to slow down the development of colonies of mold on bread surfaces for 12 hours.

Safety of bread with rowan powder for nutrition of people suffering from coeliac was evaluated by the content of gluten in bread by enzyme-linked immunosorbent assay (Akobeng & Thomas, 2008). The content of immunoreactive gluten was less than 10 mg per 1 kg of bread that meets the requirements of diet therapy in coeliac disease (20 mg 1 kg<sup>-1</sup>).

Thus, it was proved that the use of rowan fruit powder and citrus dietary fiber would expand the assortment of gluten free bread, improve its physico-chemical and

organoleptic characteristics and increase the nutritional value and microbiological safety.

## CONCLUSIONS

The optimum amount of rowan powder in the gluten-free bread recipe, which is not more than 4% of rowan fruits powder and 8% of powder from the pomace of rowan, was established. It was proved that the use of rowan powder improved the organoleptic characteristics of gluten free bread and increases the acidity, specific volume and compressibility. The flavor and odor was more intense, pleasant, with acidity and fruity notes due to rowan powder in comparison to gluten free control bread.

It was experimentally established that citrus fiber and pectin improved the specific volume of the bread, the compressibility of the crumb compared to the control sample.

The content of vitamins A, E, PP, C, group b, mineral substances – iron, magnesium, calcium, potassium, selenium, organic acids, including providing the preservative effects (citric, lactic, sorbic, benzoic) was established in the rowan powder. It was established that rowan powder and citrus fiber had a significant impact on the increase in the content of dietary fiber and iron. The content of dietary fiber in bread with 4% of powder of fruits of rowan is 3.6 g 100 g<sup>-1</sup> and in bread with 8% pomace of rowan it is 4.3 g 100g<sup>-1</sup> which corresponds to the satisfaction of the daily needs for dietary fiber respectively by 10.0% and 21.5%.

It was found that bread with rowanberry powder had 66.7% more water-soluble antioxidants.

The contamination of the main gluten-free raw material (soy protein, rice flour and corn starch extrusion and corn, rowan powder) and its influence on ropy disease of gluten free bread were established. Four strains of spore forming bacteria were isolated from gluten-free raw materials and their ability to cause ropy disease of gluten-free bread was proved. It was found that the use of the powder of rowan slowed down ropy disease and molds due to organic acids in its composition and the increase in acidity of bread.

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