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Study of Pectin Substances from Apple and Grape Husks and Assessment of their Effect on the Activity of Microbiological Processes in Wheat Dough

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ABSTRACT: At present, in the technology of creating functional food products, there is a stable tendency to use secondary raw materials from local plant materials and their processed products. In the article, the results of the experimental studies on the quality of the secondary raw materials obtained at the processing enterprises of the Krasnodar region when processing apples and grapes have been provided. The following objects were used as the objects of the study: the dry and frozen husks from apples and grapes, pectin extracts based on them and yeast dough samples. Generally accepted standard research methods were applied in the study. The quality of dry and frozen apple and grape husks and pectin extracts (PEs) based on them has been studied. The dependence of quantitative and qualitative characteristics of PEs (solids content, solids concentration, pectin purity) on the type, variety, and method of preserving husks (drying, freezing) has been illustrated. It has been found that the method of the husks preservation does not have a significant effect on the content of pectin substances. The analysis of these characteristics allowed to find rational parameters for the extraction of pectin substances. The expediency of using PEs in baking technology and their influence on the rate of reproduction of yeast cells during the dough fermentation have been established. The efficiency of using PEs from apple and grape husks at a dosage of 10% by flour weight to prevent microbiological damage of bread from wheat flour has been illustrated. The obtained results suggest that the use of PEs in the baking industry is promising.

Keywords: Apple Husks, Dough, Extraction, Fermentation, Grape Husks, Pectin Containing Raw Materials, Pectin Extracts, Yeast Cells.

Abbreviations: PE, pectin extract.

I. INTRODUCTION

According to the modern requirements of the nutrition science, the food industry has to provide the population with various foods in sufficient quantities and range to form a proper and balanced diet [1, 2]. The introduction of the modern food processing technology into production and the output of refined long-term storage products led to a decrease in the consumption of useful active ingredients that were derived directly from food. As such, one of the main tasks of food manufacturers is to enrich products with biologically active components [3, 4]. Accordingly, the directions such as the search for new physiologically active ingredients that have a beneficial effect on human health, the development of products for dietary and preventive nutrition, and the creation of innovative technology to produce food products with desired properties are now relevant for manufacturers [5].

There is a global trend to increase the number of consumers who prefer food products enriched with natural herbal supplements today [6-8].

Modern consumers show an increasing interest in food products based on natural raw materials, which include micronutrients capable of restoring cells for their normal functioning [2, 9, 10].

Due to the problem of environmental pollution, much attention is paid to the creation of low-waste and non-waste technology in the food and processing industries at this stage of the food industry development [11-14].

The introduction of waste-free technology at enterprises will allow to obtain the maximum amount of useful components from raw materials and convert them into consumer value.

The integrated use of raw materials is one of the priority areas in the development and implementation of low-waste technology [15].

The secondary raw materials got while processing fruits and berries – apple and grape husks, as well as the PEs obtained from them are of practical interest in solving the above problems [16, 17].

It is known that pectin substances are carbohydrates of the highest order of plant origin. They are part of the group of nutrients that have a therapeutic and preventive effect, since they have a bactericidal effect on pathogenic bacteria. Once in the intestine, they make the pH more acidic, bind and remove heavy metals from the body, and contribute to the elimination of radionuclides [18-20].

The demand for pectin has been increasing in recent years due to the production of new progressive food products focused on a healthy lifestyle.

Therefore, the task of the food industry in Russia is to introduce such new technologies that will allow to abandon the import of pectin and reduce the cost of its acquisition.

To date, a large number of studies have been carried out to develop the technology of dry pectin and to study its effect on the quality of bread. The work on establishing the feasibility of using PEs in the technology of baking and their influence on the activity of microbiological processes in wheat dough is not enough.

The goal of the article is to study PEs from apple and grape husks and assess their influence on the activity of microbiological processes in wheat dough.

The following tasks must be solved to achieve this goal:

– to study the qualitative characteristics of the dry and frozen apple and grape husks obtained from the processing of fruits and berries recognized in the Krasnodar region;

– to determine the dependence of the qualitative characteristics of PEs on the type, variety, and method of the husks preservation; and

– to establish the expediency of using PEs in baking technology and assess their influence on the activity of microbiological processes in wheat dough.

The joint research on the secondary raw materials got while processing apples and grapes and the possibility of their use in the production of PEs for functional nutrition was carried out in the research laboratories of the Kuban State Agrarian University named after I.T. Trubilin and the Krasnodar Research Institute for Storage and Processing of Agricultural Products.

II. METHODS

Due to the fact that apple and grape husks are a promising and underused pectin-containing secondary raw material, the following objects were used as the objects of the study:

– the dry and frozen husks from apples of the following varieties: Florina, Freedom, Liberty, Sochi-3, Renet Simirenko, and Golden Delicious;

– the dry and frozen husks of white grapes: Bianca, Viorica, Pinot Blanc, Chardonnay, Pervenets Magaracha, and Citron Magaracha;

– PEs; and

– yeast dough samples.

The PEs were obtained by extraction, citric acid was used as a hydrolyzing agent for the apple husks, and autohydrolysis was used for the grape husks [21, 22].

The conditions for the hydrolysis of PEs are presented in Table 1.

Table 1: Parameters of obtaining PEs.

Parameter	Husks	
	apple	grape
hydrolyzing agent	citric acid	water
hydrolysis time, hours	3	3
temperature,ºC	90	90
hydraulic module	1 : 6	1 : 6
Active pH of the extract	3.2	2.5 – 3.5

Hydrolysis was carried out in a thermal bath equipped with a glass thermometer, with regular stirring.

The research was conducted using standard analysis methods. The humidity of the husks was determined by the gravimetric method. The concentration of soluble solids in the PEs was determined by the refractometric method [23, 24], the acidity was determined by the titrometric method, and pectin substances were determined by the titration-based method based on titration of previously isolated and pectin substances prepared with alkali before and after hydrolysis.

Microbiological studies were performed using an Axiolmeger microscope at 400x magnification. A Goryaev counting chamber was used to count the number of yeast cells [25].

III. RESULTS AND DISCUSSION

According to the plan of the experiment, the initial studies were aimed at studying the qualitative characteristics of the dry and frozen apple husks obtained from processing the fruits of various varieties of apple trees recognized in the Krasnodar region, Tables 2 and 3.

Table 2: Quality indicators of the dry apple husks.

Parameter	Variety					
	Florina	Freedom	Liberty	Sochi-3	Renet Simirenko	Golden Delicious
Titratable acidity, degrees	2.08	2.20	2.81	1.54	2.02	1.34
Humidity, %	7.90	7.70	7.50	7.50	7.60	7.50
Soluble pectin, % d.m.	1.14	2.58	2.23	1.68	1.99	1.28
Protopectin, % d.m.	4.87	6.41	5.29	4.47	4.72	5.10
Total pectin, % d.m.	6.01	8.99	7.52	6.15	6.71	7.26
Total pectin, % a.d.m.	6.53	9.74	8.13	6.61	7.26	6.90

Table 3: Quality indicators of the frozen apple husks.

Parameter	Variety					
	Florina	Freedom	Liberty	Sochi-3	Renet Simirenko	Golden Delicious
Titratable acidity, degrees	0.60	0.64	0.80	0.60	0.90	0.34
Humidity, %	54.30	56.60	52.30	53.00	51.20	52.00
Soluble pectin, % d.m.	0.97	1.18	1.24	0.96	0.81	0.86
Protopectin, % d.m.	2.10	2.96	2.62	2.27	2.76	2.29
Total pectin, % d.m.	3.07	4.14	3.86	3.23	3.57	3.15
Total pectin, % a.d.m.	6.71	9.53	8.09	6.87	7.32	6.56

The results of the studies illustrate that in all options of the experiment, both with the dry and frozen apple husks, the content of the protopectin fraction prevailed over the soluble fraction.

Pectin content by a.d.m. (absolute dry mass) in the dry husks varied depending on the variety and ranged from 6.53 % to 9.74 %. This indicator was significantly higher for Freedom and Liberty varieties compared to other varieties and amounted to 9.74 % and 8.13 %, respectively. The Freedom variety also stood out among other varieties in the content of soluble pectin (2.58 %) and protopectin (6.41 %). The Liberty variety ranked second by these parameters with the content of soluble pectin of 2.23 % and protopectin of 6.25 %. According to a set of quality indicators, these varieties are optimal for producing PEs; this is confirmed by the high content of the soluble fraction of pectin [26].

The studies of the frozen husks illustrated that the content of pectin substances in them remained almost the same as in the dry husks. The husks preservation method did not have a significant effect on the content of pectin substances. The best results were observed in a Freedom variety with pectin substances content by a.d.m. of 9.53 % and Liberty variety with pectin substances content by a.d.m. of 8.09 %.

Based on the data after the study of the qualitative characteristics of the husks preserved by drying and freezing, it can be concluded that the content of pectin substances is more influenced by the varietal specifics of apples, rather than by the husks preservation method [27, 28].

The husks obtained from the processing of grapes are of no less interest. The Krasnodar region has a developed branch of winegrowing, and up to 20 % of the wastes are generated as a result of grapes processing. The husks of the grape varieties, preserved by drying and freezing methods, were studied, and their quality indicators were evaluated in Tables 4 and 5.

The results of the analysis illustrated that the content of the insoluble fraction of pectin prevailed over the soluble fraction in all options of the experiment. The content of pectin substances by a.d.m. ranged from 4.40 % to 6.97 % in the dry husks, and from 4.49 % to 7.03 % in the frozen husks. At the same time, the largest amount of pectin substances was found in the grape husks of Bianca and Citron Magaracha varieties.

The next stage of the study was to obtain PEs from all types of the husks preserved by drying and freezing and to analyze their quality.

The PEs were obtained by hydrolysis from the husks of recognized and prospective apple varieties in the Krasnodar region, and their yield [29] and qualitative characteristics were determined in Table 6.

Table 4: Quality indicators of the dry grape husks.

Parameter	Variety					
	Bianca	Viorica	Pinot Blanc	Chardonnay	Pervenets Magaracha	Citron Magaracha
Titratable acidity, degrees	4.16	3.15	5.48	3.23	5.25	3.56
Humidity, %	11.50	11.50	11.30	11.40	11.40	11.50
Soluble pectin, % d.m.	2.88	2.03	1.53	1.49	1.84	2.67
Protopectin, % d.m.	3.29	2.91	2.37	2.75	2.60	3.13
Total pectin, % d.m.	6.17	4.94	3.90	4.24	4.44	5.80
Total pectin, % a.d.m.	6.97	5.58	4.40	4.79	5.01	6.55

Table 5: Quality indicators of the frozen grape husks.

Parameter	Variety					
	Bianca	Viorica	Pinot Blanc	Chardonnay	Pervenets Magaracha	Citron Magaracha
Titratable acidity, degrees	1.35	1.80	1.43	1.28	1.88	1.73
Humidity, %	64.10	63.50	61.30	62.70	60.40	63.50
Soluble pectin, % d.m.	1.02	0.76	0.69	0.65	0.75	1.15
Protopectin, % d.m.	1.50	1.36	1.05	1.14	1.22	1.25
Total pectin, % d.m.	2.52	2.12	1.74	1.79	1.97	2.40
Total pectin, % a.d.m.	7.03	5.80	4.49	4.80	4.97	6.57

Table 6: Quality indicators of the PEs from the apple husks.

Parameter	Apple variety					
	Florina	Freedom	Liberty	Sochi-3	Renet Simirenko	Golden Delicious
Output of PEs from 100 g of raw materials, ml: from dry husks from frozen husks	310.00 545.80	470.00 555.6	516.00 570.60	440.00 495.50	400.00 540.40	440.00 510.50
Mass fraction of solids, %: from dry husks from frozen husks	3.00 2.80	3.20 2.90	3.20 2.60	3.60 2.80	3.40 2.70	3.20 2.60
Titratable acidity, degrees: from dry husks from frozen husks	2.85 3.12	2.78 3.99	2.11 3.72	2.71 3.18	2.78 3.09	2.71 3.68
Mass fraction of pectin substances, %: from dry husks from frozen husks	0.67 0.74	0.83 0.89	0.84 0.92	0.81 0.84	0.74 0.79	0.75 0.79
PEs purity A_p: from dry husks from frozen husks	0.21 0.26	0.26 0.31	0.26 0.35	0.23 0.30	0.22 0.29	0.23 0.30

The yield of PEs varied depending on the variety. The Liberty and Freedom varieties stood out, as their extracts from the dry husks had yields of 516 ml and 470 ml. The PEs from the frozen husks of these varieties also had large yields: 570.6 ml for the Liberty variety and 555.6 ml for the Freedom variety.

The taste varied greatly from sour (Florins, Freedom, and Sochi-3) to slightly acidic (Golden Delicious) in the extracts from the dry husks, depending on the variety. The Liberty extract had a medium acid taste. A distinct flavor of dried apples was observed in the Sochi-3 variety.

The smell of the PEs did not vary significantly.

A wide variety of shades must be noted when evaluating the color of extracts. The extract color varied from brown in Florina to light brown in Liberty and light yellow in Golden Delicious. Extracts from the husks of Sochi-3 and Freedom varieties had an amber color.

Extracts from the frozen apple husks varied by organoleptic characteristics.

Extracts of Sochi-3, Golden Delicious, and Renet Simirenko varieties had acidic taste. A medium acid taste was noted in Liberty and Freedom varieties. Florina had a slightly acidic taste.

The extracts did not vary significantly by smell. In this case, the color did not differ much depending on the variety of apples and varied from pale yellow to light brown.

Extracts from the husks of the Liberty and Freedom varieties were the best in terms of a set of indicators of organoleptic evaluation.

The titratable acidity in extracts from the frozen apple husks was higher compared to extracts from the dry husks – depending on the variety, it ranged from 3.09 to 3.68 degrees, while in extracts from the dry husks it ranged from 2.11 to 2.85 degrees. The use of freezing significantly increases the degree of the PEs purity, because pectin substances do not degrade when frozen.

The PEs from Liberty and Freedom varieties are recommended for use in functional food technology, taking into account the results of complex studies and the obtained data on the content of pectin substances, the fraction of soluble fraction in their total content, the yield of pectin substances by ethanolic precipitation, and the purity index of pectin (Ap).

The grape PEs were obtained from the husks (frozen and dry) of recognized white grape varieties, which varied in the quality and yield of the finished product (Table 7).

Table 7: Quality indicators of the PEs from the grape husks.

Parameter	Variety					
	Bianca	**Viorica**	**Pinot Blanc**	**Chardonnay**	**Pervenets Magaracha**	**Citron Magaracha**
Output of PEs from 100 g of raw materials, ml: from dry husks from frozen husks	540.00 592.50	480.00 570.00	486.70 565.00	477.30 574.00	500.00 567.50	512.00 575.00
Mass fraction of solids, %: from dry husks from frozen husks	5.10 4.00	5.40 4.30	6.0 4.50	5.3 4.40	5.4 4.20	5.0 3.90
Titratable acidity, degrees: from dry husks from frozen husks	2.85 3.80	2.70 3.30	2.93 3.25	2.80 3.80	2.40 3.95	2.74 3.60
Mass fraction of pectin substances, %: from dry husks from frozen husks	0.75 0.79	0.64 0.69	0.68 0.70	0.67 0.74	0.65 0.71	0.74 0.77
PEs purity A_p: from dry husks from frozen husks	0.15 0.20	0.12 0.16	0.11 0.15	0.13 0.17	0.12 0.17	0.15 0.20

The largest yield of the PEs from the dried husks was noted in Bianca (540 ml) and Citron Magaracha (512 ml from 100 g of raw material).

The extracts from the frozen husks also had greater yield in the Bianca and Citron Magaracha varieties: 592.5 ml and 575 ml, respectively.

The grape PEs were evaluated by organoleptic and physicochemical parameters. There was a great difference in taste depending on the variety. It varied from sweet (the Bianca variety) to strongly acidic (the Chardonnay variety). The extracts from the remaining grape varieties had a sour taste.

The smell of the extracts also varied significantly, depending on the variety. The extract from the Bianca variety had the aroma of lemon and honey, the Chardonnay variety had the lemon flavor, the Viorika variety had the aroma of herbs, and the remaining varieties had a characteristic aroma of the dry grape husks.

The grape PEs varied by color, which directly depended on the variety. Their color ranged from light yellow in the Chardonnay variety to dark brown in the Citron Magaracha variety. The most harmonious organoleptic characteristics were noted in the PEs from the dry grape husks from the Bianca and Citron Magaracha varieties.

The extracts obtained from the frozen grape husks were also subjected to organoleptic evaluation.

The taste of this group of extracts varied from slightly acidic to acidic. The smell of the extracts from the frozen grape husks varied greatly. The color of the extracts ranged from light yellow to dark brown with a gray tint, depending on the grape variety.

An organoleptic evaluation indicated that the extract from the frozen husks of the Citron Magaracha variety was the best in this group of extracts, while the extract from the husks of Bianca was somewhat inferior to it.

Physicochemical parameters describing the quality were also determined in the extracts from the frozen husks. The extracts obtained from frozen raw materials had larger mass of pectin substances and higher degree of the extract purity than PEs from dry raw materials.

The PEs from the apple and grape husks that had the best qualitative characteristics were used as biologically active additives in the wheat flour doughing in order to assess their effect on microbiological processes.
The ethanolic fermentation caused by the baker's yeast Saccharomyces cerevisiae is known as the fundamental process in the bread technology. Therefore, the processes of reproduction of microorganisms and the accumulation of metabolites are of great importance in doughing (Fig. 1). The straight dough method was used in the experiment.
In the test dough samples, 10 % of the PEs from the frozen apple and grape husks were added to the flour weight. The option of making dough without additives served as the reference.
The number of yeast cells in the test samples was estimated using the generally accepted method in microbiology in the Goryaev chamber [20]. Samples were taken 1.5 and 3 hours after the start of fermentation; the data from the PEs from the apple husks are presented in Fig. 2.
The introduction of PEs in dough contributes to the intensification of microbiological processes, which is explained by an increase in sugars, vitamins, minerals and other components that serve as additional sources of energy necessary for the active life of the yeast. The most intensive microbiological processes took place in the test sample with the introduction of the PEs from the frozen apple husks of the Liberty variety.
The dynamics of the change in the activity of microbiological processes in the test with the PEs from the grape husks are illustrated in Fig. 3.

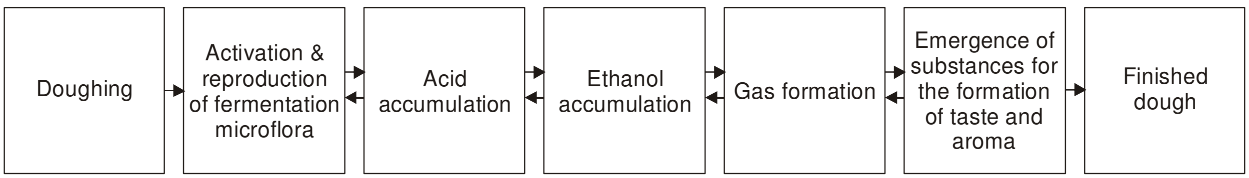


Fig. 1. Operator model of reproduction of microorganisms.

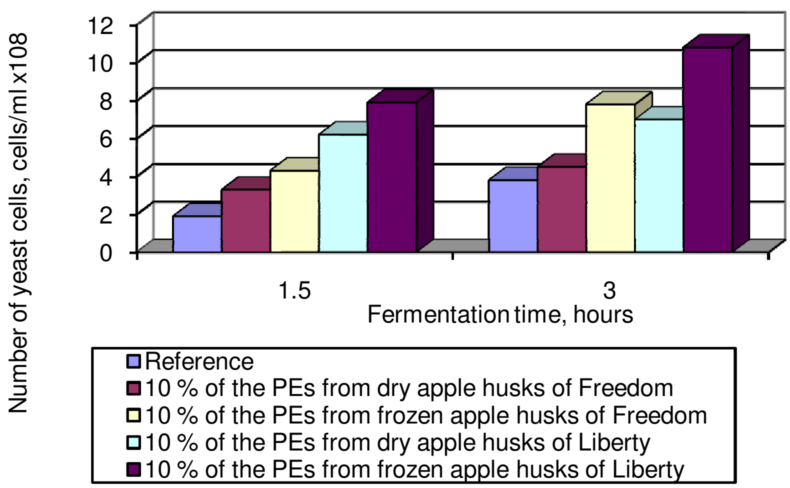


Fig. 2. Change in the activity of microbiological processes in the test with the PEs from the apple husks.

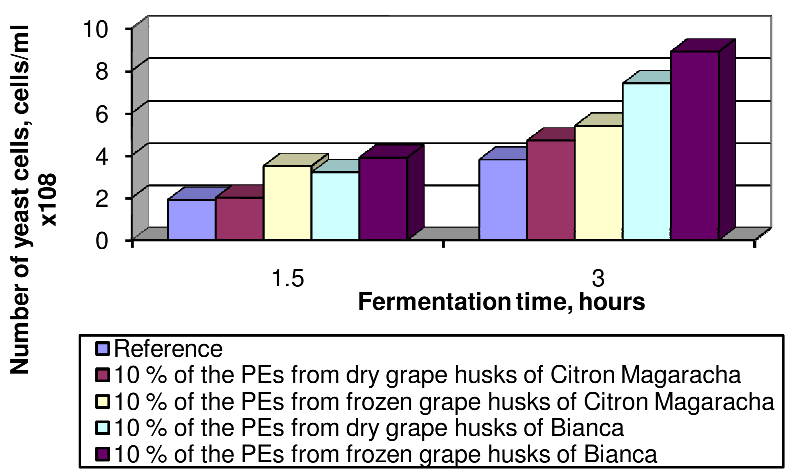
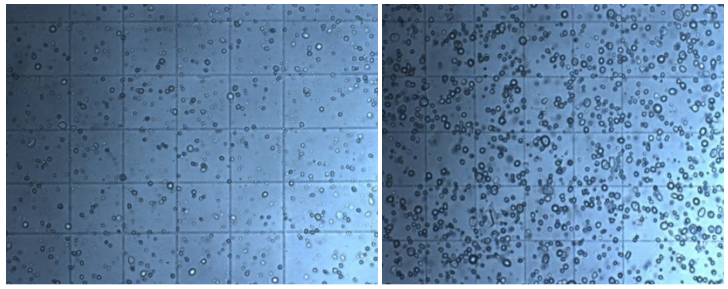


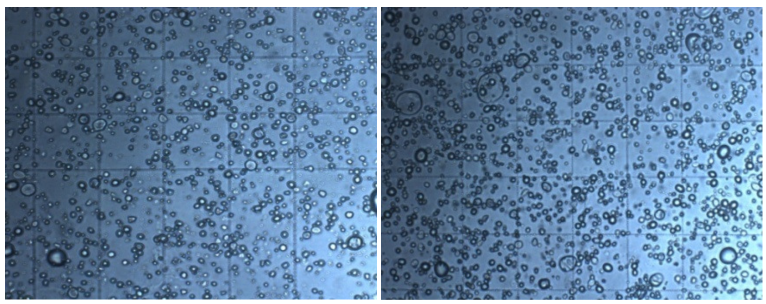
Fig. 3. Change in the activity of microbiological processes in the test with the PEs from the grape husks.

In this case, the introduction of the PEs from the grape husks to dough also leads to an acceleration of the reproduction of yeast cells and, as a result, an increase in their quantity.
The best result was noted in the option with the addition of the grape PEs from the frozen grape husks of the Bianca variety.
The reference and experimental samples from the frozen apple and grape husks released as a result of the experiment were additionally examined by microscopy using an Axio Imeger microscope (Fig. 4).

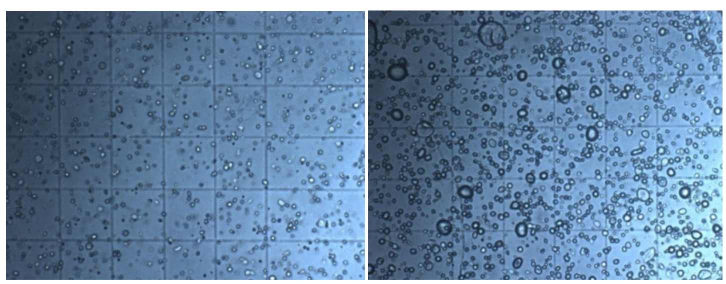
The carried out microscopic examination of wheat dough samples confirmed the dependence of the activity of yeast cells reproduction on the introduced PEs.
It is currently known that pectin substances have an antiseptic effect. As such, a need arose to study the effect of the PEs from the apple and grape husks on the microbiological contamination of wheat dough and bread (Table 8).



(a) (b)
Reference sample (no PEs added).



(a) (b)
Sample with the PEs from the apple husks.



(a) (b)
Sample with the PEs from the grape husks.

Fig. 4. Microphotos of dough samples (a) fermentation of 1.5 hours (b) fermentation of 3 hours.

Table 8: Microbiological contamination of wheat dough and bread.

Sample	**Quantity of microorganisms**					
	in dough					**in bread**
	Yeast and mold CFU/g	**Bacteria**			**Total quantity of microorganisms**	**Bacteria of the Escherichia coli group (coliforms), g**
		Bacillus subtilis, g	**Proteus, g**	**Total, g**		
Reference	$50 \cdot 10^5$	$30 \cdot 10^1$	Not detected	$45 \cdot 10^5$	$95 \cdot 10^5$	Not detected
10 % of the PEs from apple husks	$40 \cdot 10^5$	$8 \cdot 10^1$	Not detected	$22 \cdot 10^5$	$62 \cdot 10^5$	Not detected
10 % of the PEs from grape husks	$36 \cdot 10^5$	$8 \cdot 10^1$	Not detected	$24 \cdot 10^5$	$60 \cdot 10^5$	Not detected

The obtained experimental data have indicated the efficiency of using the PEs from the apple and grape husks at a dosage of 10 % to the flour weight to prevent microbiological damage of bread from wheat flour, because adding PEs partially inhibits the development of molds and bacteria.

IV. CONCLUSION

The comprehensive study of the quality of the secondary raw materials obtained at the food industry in processing apples and grapes has been conducted.

The results have been obtained which allow to determine the optimal method of preserving husks and rational parameters of the pectin substances extraction.

It has been proven that PEs from apple and grape husks can be used in baking as a biostimulant of baker's yeast due to their stimulating effect on the reproduction and growth of *Saccharomyces cerevisiae* yeast cells.

The results of this study can become the basis for the development of technological solutions aimed at the integrated processing of secondary raw materials.

The conclusions obtained in this work can be interesting and useful for food producers with functional orientation.

Conflict of Interest. There are no conflicts of interest to declare.

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