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THE INFLUENCE OF USING DIFFERENT PROTEOLYTIC ENZYMATIC PRODUCES OVER THE CHARACTERISTICS OF FLOUR BREADING PRODUCTS

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Abstract

The utilization of enzymatic produces is a way of improving the process of breading products, moreover of the properties of the cereal products. Normal flour has a reduced proteolytic activity, while the flour made of sprouted wheat or attacked by the wheat bugs which has a special proteolytic activity, such that the resulted produces from the processing of such flour do not have the required quality. To improve this flour a supply of proteolytic enzymes will be used which gives the possibility of adjusting rheological indicators of the dough according to the technical requirements. After the analyzes being done, it has been noticed that for equal proteolytic activities, the enzymatic produces of various origins have a different effect on bread volume and porosity. The best results are obtained from the addition of fungal protease (Aspergillus oryzae, Aspergillus niger), bacterial protease (Bacillus subtilis) reducing the volume, even at minimal doses.

Key words**:** *bread, flour, protease, rheological indicators*

INTRODUCTION

During the last decades, due to the development of scientific research in bakery and cereal products appeared a diversification and modernization of processing technologies, a broadening of the product range, an increased use of additives for different purposes. In parallel with these, the risk of contamination with harmful substances of different foods increased, including bread, and the requirement of consumers to quality and food safety. All these factors have imposed a requirement to increase the control of products, in terms of their composition and safety [2].
The control strategies aim to know and use the most appropriate and accurate methods and analysis techniques, allowing the evidence of product quality indices. The aimed researches had as a highlight the influence of two proteolytic preparations from two different sources, namely a fungal and a bacterial enzyme preparation, over the quality of bread made from flour of type 680.

MATERIAL AND METHOD

In experiments, a wheat flour of type 680 has been used, for which the physic-chemical and rheological indicators have been determined. To achieve the technological tests compressed yeast from bakery has been used, according to the formulation.
For laboratory experiments the following proteolytic enzyme preparations have been used:
1. The Fungal Protease from the Mirpain company, Turkey, is an enzyme preparation containing peptihydrolase obtained by controlled fermentation with Aspergillus oryzae. The preparation is a mixture of acid proteases, neutral and alkaline, with both

exopeptidasic activity and endopeptidasic activity over the protein molecules.

2. The panazyme, from the Zytex company, India, is a bacterial enzyme preparation containing protease obtained by controlled fermentation of Bacillus subtilis.

During the research have been analyzed 7 recipes for the preparation of bread, respectively a control and 6 experimental samples, which were introduced in different amounts of protein and Panazyme Fungal enzyme preparations (Table 1).

In Table 2 are presented the recipes and technological parameters that used during the experimental period

Table 1. The experimental scheme

No. Sample	The used amount of enzyme preparation	The followed aims
Control sample	Without enzyme supplement	- indicators of quality of bread (nominal mass, volume, height, diameter, porosity, acidity, humidity) - physical indicators of bread (softening, elasticity)
Exper. sample 1	With addition of 3.0 g protein Fungal / 10 kg flour	
Exper. sample 2	With addition of 3.5 g protein Fungal / 10 kg flour	
Exper. sample 3	With addition of 4.0 g protein Fungal / 10 kg flour	
Exper. sample 4	With addition of 3.5 g Panazyme / 10 kg Veron GX meal preparation	
Exper. sample 5	With addition of 4.0 g Panazyme / 10 kg Veron GX meal preparation	
Exper. sample 6	With addition of 4.5 g Panazyme / 10 kg Veron GX meal preparation	

Table 2. The recipes and technological parameters used during the experimental period

Raw materials and technological regime	Indirect method																			
	Total							Yeast lees							Dough						
	M	P1	P2	P3	P4	P5	P6	M	P1	P2	P3	P4	P5	P6	M	P1	P2	P3	P4	P5	P6
Flour, kg	8	8	8	8	8	8	8	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Water, l	4	4	4	4	4	4	4	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Yeast, kg	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	-	-	-	-	-	-	-
Salt, kg	0.12	0.12	0.12	0.12	0.12	0.12	0.12	-	-	-	-	-	-	-	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Fungal Protease, g	-	3	3.5	4	-	-	-	-	3	3.5	4	-	-	-	-	-	-	-	-	-	-
Panazyme, g	-	-	-	-	3.5	4	4.5	-	-	-	-	3.5	4	4.5	-	-	-	-	-	-	-
TFr, min.				-	-	-	-	2	2	2	2	2	2	2	-	-	-	-	-	-	-
TFe, min.				-	-	-	-	120	120	120	120	120	120	120	-	-	-	-	-	-	-
TFr, min.				-	-	-	-	-	-	-	-	-	-	-	4	4	4	4	4	4	4
Td, min.				-	-	-	-	-	-	-	-	-	-	-	40	40	40	40	40	40	40
Tb, min.				-	-	-	-	-	-	-	-	-	-	-	30	30	30	30	30	30	30
Toven, °C				-	-	-	-	-	-	-	-	-	-	-	-	220…230					

TFR = time kneading yeast, dough, yeast fermentation; TFE = time; Td = time leavening dough baking; Tb = time baking; Toven = temperature oven.

The used fungal protein and Panazyme doses had, in average, the following enzymatic activity: 484; 569; 644 SKB units / kg flour.

In the performed experimental tests have been used:

- 4 kg of flour for yeast and 4 kg of flour for the dough, therefore a total of 8 kg for each of the five samples and control sample;
- water at a rate of 2 liters and 2 liters of yeast dough, so 4 liters in total for each sample;
- 200 grams of yeast in the yeast lees for each sample;
- salt, 120 grams in the dough;
- no enzyme preparations have been used for the control sample; at the first test in the dough have been used 3.0 g of Fungal protease, for sample 2, 3.5 g of protease Fungal protease preparation, for sample 3, 4.0 g of protease Fungal protease preparation, for sample 4, 3.5 g of Panazyme protease preparation, for sample 5, 4.0 g of Panazyme protease preparation and for sample 6 were introduced 4.5 g of Panazyme protease preparation.

The time of leaven dough was two minutes. The leaven fermentation time was 120 minutes. The kneading of the dough was 4 minutes. Dough leavening took 40 minutes for the control sample and for the other samples, 30 minutes. Baking time was for each of samples, including the control one, 30 minutes. The temperature at which cooking was done for all the samples was $220\text{-}230^0$.

On each experimental group have been analyzed 16 loaves, which were obtained by the indirect procedure. The followed objectives were the determination of the physic-chemical indicators of quality, respectively nominal weight, volume, height, diameter, porosity, acidity, humidity, softening, elasticity.

RESULTS AND DISCUSSIONS

Quality indicators of the baking bread samples obtained after 3, 24, 48 and, respectively 72 hours out of the oven, are presented in Tables 3 and 4.

Table 3. Quality indicators of bread on their size

Quality indicator	*Time after baking (hours)*	*M*	*P1*	*P2*	*P3*	*P4*	*P5*	*P6*
Nominal weight. g	3	526.5	531.5	539.5	511.2	527.2	525.1	524.1
	24	523.1	528.4	536.3	508.4	525.1	522.3	520.6
	48	519.3	524.1	532.7	505.9	521.2	518.6	517.2
	72	511.7	518.2	525.4	500.1	518.5	512.0	509.7
Volume. cmc/100 g product	3	336	337	340	327	337	333	329
	24	328	334	337	323	330	325	325
	48	325	328	334	322	328	322	321
	72	323	328	332	316	326	320	318
Height (H). cm	3	9.9	10.3	10.8	10.1	10.4	9.9	9.7
	24	9.7	10.1	10.7	9.9	10.2	9.6	9.4
	48	9.5	10.1	10.5	9.8	10.0	9.5	9.2
	72	9.1	10.0	10.3	9.5	9.9	9.0	8.9
Diameter (D). cm	3	15.40	15.45	15.50	15.35	15.48	15.39	15.35
	24	15.21	15.32	15.35	15.24	15.25	15.21	15.20
	48	15.12	15.20	15.25	15.15	15.23	15.13	15.10
	72	14.92	15.15	15.17	15.05	15.16	15.04	15.00
H/D	3	0.64	0.67	0.70	0.73	0.67	0.64	0.63
	24	0.64	0.66	0.70	0.70	0.67	0.63	0.62
	48	0.63	0.66	0.69	0.70	0.66	0.63	0.61
	72	0.61	0.66	0.68	0.68	0.65	0.60	0.59

During the research 7 recipes of bread making have been analyzed, respectively a control and 6 experimental samples in which different amounts of Fungal Protease and Panazyme enzyme preparations have been introduced.

After the results being obtained for the three samples in which the Fungal Protease proteolytic enzyme preparation has been introduced, it can be observed that the nominal weight increased in the case of the sample for which 3.5 g Fungal Protease / 10kg flour were introduced, respectively the sample 2 to the control sample for which no enzyme preparation was used.

In case of using the Panazyme product, it can be observed that the nominal weight increased for sample 4 for which Panazyme 3.5 g / 10 kg flour was introduced to the nominal weight of the control sample for which no proteolytic preparation was used.

At 3 hours after baking, the lowest value of the bread's volume is that of the same sample 3 with 327 cmc/100g product. The sample 2 has the highest value of product, 340 cmc/100g, followed by sample 1 with 337 cmc/100g of product and the control sample with 336 cmc/100g of product.

At 24 hours the sample 3 has the lowest volume 323 cmc/100g of product and sample 2 has the highest volume of 337 cmc/100g. The control sample has a quite low value. At 48 hours the lowest volume is that of the sample 6, 321 cmc/100 g, followed by the value belonging to sample 5 and 3 with 322 cmc/100g of product.

At 72 hours after baking the sample 4 presents further the highest volume of 326 cmc/100g of product, remarking the fact that the volume of the bread has decreased as the amount of preparation of bacterial proteolytic introduced in bread increased.

After the analyzes being performed, it can be observed that still the amount of 3.5 g Fungal Protease/10kg flour introduced in the recipe determines the volume increase compared with the control sample.

Regarding the height on analyzed bread, it can be noticed that the better performance is still present in the Fungal Protease preparation which carries higher values of the height at the samples where 3.5 g were introduced, determining increases in height as opposed to

the control sample for which no preparation supply was used.

The loaves diameter recorded the highest values when using Fungal Protease preparation, introduced at a dose of 3.5 g, values which were higher than those recorded when using the Panazyme product.

Table 4 presents the physic-chemical indicators of quality of the produced bread obtained during the experimental period.

Table 4. Physic-chemical indicators of quality of the produced bread obtained during the experimental period

Quality indicator	*Time after baking (hours)*	*M*	*P1*	*P2*	*P3*	*P4*	*P5*	*P6*
Porosity %	3	82.98	83.55	83.32	81.75	82.75	80.36	80.06
	24	81.06	82.52	82.12	80.36	81.26	79.65	78.85
	48	80.12	82.37	81.24	80.06	81.05	77.36	77.01
	72	79.07	81.81	80.19	80.01	80.97	77.14	76.14
Elasticity %	3	93	95	96	95	95	91	89
	24	91	92	93	92	90	88	85
	48	88	90	90	89	89	78	79
	72	79	87	86	77	81	76	69
Humidity %	3	43.69	42.79	42.95	42.30	43.38	42.33	41.89
	24	41.90	41.41	41.57	41.22	42.17	42.01	41.26
	48	40.41	40.24	40.36	41.01	41.13	41.69	40.25
	72	40.30	40.15	40.56	40.29	41.01	41.01	39.75
Acidity degrees	3	2.2	2.4	2.3	2.4	2.2	2.1	2.2
	24	2.1	2.4	2.2	2.3	2.1	2.1	2.0
	48	2.1	2.3	2.1	2.2	2.1	2.0	2.0
	72	2.0	2.1	2.0	2.2	2.0	1.9	1.8

After the results being obtained and the comparisons being made between the samples with supply of Fungal Protease and Panazyme and the control sample, it can be noticed that there are differences of porosity. Sample 1 with an addition of 3 g of Fungal Protease has the highest value in comparison with the one of the control sample.

Regarding the elasticity of bread belonging to the 7 lots, elasticity differences are found in case of addition of proteolytic preparation of fungal nature, respectively bacterial. The sample with the highest elasticity is found at sample 1, being of 89%, sample in which has been added the preparation of Fungal Protease of fungal nature and the highest value for the preparation of Panazyme of bacterial nature can be found at sample 4, being of 81% to the control sample which presents a low value in comparison with these, that of 72%. Elasticity increased in the upward direction of the dose of enzyme and it decreased over time. The smallest difference between the values at 3 hours and those at 72 hours was the P5 (sample with the highest dose of enzyme).

After the analyzes being performed to determine humidity, it has been shown that in comparison with the control sample, better results were obtained from samples in which the bacterial enzyme Panazyme preparation of 3.5 g and 4g was added.

The analyzed bread has recorded high values of acidity for the lots where an addition of 4 g of Fungal Protease preparation of fungal nature was used. In general, the bread has recorded close values at the experimental lots compared with the control sample without an addition of proteolytic enzymes, while there is a slight decrease in acidity.

Some authors think that for equal activities of proteases of different origins, the effect over the loaf volume is different. Better results are obtained by adding fungal proteases (Aspergillus oryzae, Aspergillus niger) [1, 4]. At low doses of 150-200 H/100 g flour units, the bread's volume increases, after which, increasing the dose to 500-600 H / g flour units, the volume does no longer modify and

at higher doses it decreases. The bacterial protease (B. Thermo-proteoliticus) visibly reduces the volume even for minimal doses. The addition of proteolytic enzymes also influences the bread porosity. The effect is also different for different proteases [3].
To adjust the dough plasticity, proteases are often used. The action of the proteases is more complex than the one of the amylases; the proteases causing the peptisation of the proteins from dough. The addition of proteolytic enzyme adjustes the rheological qualities of dough, according to technological needs. The proteolytic action starts during the kneading process and it continues throughout the fermentation until the enzymes are inactivated by heat [5].

CONCLUSIONS

After the research being done on the influence of a proteolytic enzyme preparation in order to improve the baking properties of flour. the following conclusions have been taken:
♦ the addition of proteolytic enzymes followed an increase in volume. The most visible increase was observed at the sample with a supply of 3.5 g of enzymatic Fungal Protease preparation/10 kg flour (sample 2) and at the one with an addition of 3.5 g of enzymatic Panazyme preparation /10 kg flour (sample 4).
♦ the bread height recorded a slight decrease in time of all samples. A higher value of bread height was observed for sample 2 (with an addition of 3.0 g enzymatic Fungal protease preparation/10 kg flour) and sample 4 (with an addition of 3.5 g enzymatic Panazyme preparation/ 10 kg flour).
♦ the diameter and the ratio H / D of the bread presented insignificant differences from the control sample during the baking period until 72 hours.
♦ the bread porosity decreased over time. The highest value recorded for the bread made from flour to which were added 3.0 g Fungal Protease/10 kg product (sample 1) and 3.5 g enzymatic Panazyme preparation/10 kg flour (sample 4).
♦ the elasticity of bread was not significantly influenced by the introduction of proteolytic preparations, the elasticity of the loaves obtained for the experimental samples being similar to that observed at the control sample without addition of proteolytic enzymes.
♦ the bread humidity showed a slight decrease tendency in humidity over time, the supply of enzymatic preparation not influencing this indicator of bread quality.
♦ The bread acidity registered close values at the experimental lots than the control sample without addition of proteolytic enzymes. In time there is a slight decrease in these values of the bread's acidity.
Summarizing the results of research on the influence of addition of proteolytic enzymes on flour quality, it is noticed that they can be used to improve the baking qualities of wheat flour of type 680, the recommended dose being of 3.5 g enzymatic Fungal Protease preparation/10 kg flour, which ensures obtaining high quality bread.

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