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Abstract

The Romanian flours obtained by annual wheat crop are, in general, flours with low proteolitic activity, with low gluten deformation values, respectively. It means that these flours require an improvement of this activity because of bread quality (structure, volume, crust, etc.).

The study evaluates the possibilities of improving the flours with low proteolitic activity.

This improvement can be made by proteolitic enzymes or other reducer agents. In our study we tested bacterial proteases by different methods: baking tests and rheological tests, respectively.

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Keywords: improvement, gluten deformation, proteolitic activity, wheat flours, bacterial proteases, etc.

Introduction

The improvement of Romanian flours gluten deformation is a necessity taking into account that more than half of our wheat crop has a low proteolitic activity.

Therefore, the objectives of this research were to determine: 1) baking performances of bacterial proteases (ALPHAMALT LQ 4020, ALPHAMALT BK 5020, bacterial protease) 2) its effect on the rheological characteristics and 3) improving quality bread.

This study takes into account the influence of bacterial proteases on bread quality.

Materials and Methods

Commercial flours were obtained from Baneasa S.A. (F1 and F4 flours) and Plevnei S.A. (F2 flour). The physic-chemicals and rheologicals indicators are given in the (**Table 1** and **Table 2**).

Indicators	UM	Values				
		F1	F2	F4		
Moisture	%	15.7	13.9	12.4		
Ash	% s.u.	0.44	0.58	0.36		
Acidity	degrees	2.0	2.4	2.3		
Wet gluten	%	22.39	23.36	26.0		
Gluten Index		98	98	-		
Gluten deformation	mm	4.0	6.0	4.0		
Falling Number	sec.	398	363	345		

Table 1. Physic-chemicals indicators of flours.

Indicators	UM		Values			
		F1	F2	F4		
Hydration capacity	%	52.6	59.0	59.5		
Development	min.	2.0	1.9	1.9		
Stability	min.	5	4	3.5		
Elasticity	uB	140	120	120		
Softening	uB	80	80	70		
Power	-	44	44	48		

Table 2. Rheological indicators of flours.

The following bacterial proteases were used: ALPHAMALT LQ 4020 and ALPHAMALT BK 5020 supplied from Muhlenchemie, Germany and bacterial protease supplied from S.C. Elton Corporation S.A., Romania.

Pakmaya yeast was used from Rompak Ltd., Romania.

Baking

The bacterial proteases were added to the baking formula during the mixing stage. Breads were made with commercial bread flour 480 and 650 type, respectively. By the pup loaf formula uses a 90-min. fermentation, straight-dough process (Romanian Standard STAS Baking Test). We also used the indirect method, with 2 stages, sponge and dough. The proteases were added in dough stage.

The dough were proofed and then baked. The loaf volume of bread was measured using rapeseed displacement.

Physic-chemical indicators of all the breads were determined and the internal and external characteristics evaluated sensorial properties.

Rheological test

The straight-dough formula with 300 g of commercial flour and water were used for the rheological test.

Bacterial proteases (Alphamalt LQ 4020, Alphamalt BK 5020, bacterial protease) were added to some dough in different quantities to notice the optimal doses which can be used for a better quality of bread. The control dough had no additives. Farinograph Brabender was used for rheological test.

Results and Discussion

To compare the performance of bacterial proteases on bread quality in baking systems technological parameters and doses of additives have to be optimized.

1. ALPHAMALT LQ 4020

1.1. INFLUENCE OF ALPHAMALT LQ 4020 ON BREAD QUALITY

There were used the wheat flours presented in the (Table 3 and Table 4).

Flour F1

Raw materials and		Di	rect method -	F1	
technological	M1	P1	P2	P3	P4
parameters					
Flour, kg	1	1	1	1	1
Water, l	0.530	0.530	0.530	0.530	0.530
Yeast, g	30	30	30	30	30
Salt, g	15	15	15	15	15
Alphamalt LQ 4020, g	-	0.2	0.3	0.4	0.5
Mixing time, min.	2	2	2	2	2
Fermentation time,	90	90	90	90	90
min.					
Proofing time, min.	50	50	50	50	50
Baking time, min.	40	40	40	40	40
Baking temperature, °C			225°C		

Table 3. Recipes and technological parameters in baking test.

The following quantities of ALPHAMALT LQ 4020 were used: 0.2; 0.3; 0.4 and 0.5 g/kg flour, respectively.

Table 4. Quality indicators of bread after 3 hours from the baking.

Sample/Indicators	M1	P1	P2	P3	P4
Weight, g	530	526	529	522	543
Volume, cmc/100 g	259	363	350	356	338
Height, (H), cm	9.1	11.6	11.5	11.1	11.3
Diameter (D), cm	15.7	15.7	15.9	15.8	15.85
H/D	0.58	0.74	0.72	0.70	0.71
Porosity (crumb	77	85	85	84	86
structure), %					
Elasticity, %	97	97	98	98	98
Moisture, %	41.81	42.51	40.70	41.91	41.5
Acidity, degrees	1.0	0.8	0.8	0.8	0.8
Bread note	81	96	94	92	93



Figure 1. The Alphamalt LQ 4020 influence on physic-chemical parameters of bread (flour F1).

The sample with 0.2 g/kg flour Alphamalt LQ 4020 added (P1) the best results presented:

- 40 % volume increasing;
- 10 % porosity improving;
- 28 % H/D;
- 19 % note improving.

Also by increasing enzyme dose the indicators values decreased, but all of these had been higher than control.

Flour F2

Table 5. Recipes and technological parameters in baking test.											
Raw materials and		Direct method - F2									
technological parameters	M2	P1'	P2'	P3'	P4'						
Flour, kg	1	1	1	1	1						
Water, 1	0.590	0.590	0.590	0.590	0.590						
Yeast, g	30	30	30	30	30						
Salt, g	15	15	15	15	15						
Alphamalt LQ 4020, g	-	0.2	0.3	0.4	0.5						
Mixing time, min.	2	2	2	2	2						
Fermentation time, min.	90	90	90	90	90						
Proofing time, min.	50	50	50	50	50						
Baking time, min.	40	40	40	40	40						
Baking temperature, °C			225°C								

Table 5. Recipes and technological parameters in baking test

Sample/Indicators	M2	P1'	P2'	P3'	P4'
Weight, g	531	536	523	526	528
Volume, cmc/100 g	273	316	323	306	309
Height, (H), cm	8.7	10.0	9.8	9.2	9.1
Diameter (D), cm	15.9	15.7	15.65	15.65	15.70
H/D	0.55	0.64	0.63	0.59	0.58
Porosity (crumb	76	83	82	83	82
structure), %					
Elasticity, %	97	98	98	95	98
Moisture, %	43.41	43.4	42.9	42.99	43.09
Acidity, degrees	1.0	1.2	1.0	1.0	1.0
Bread note	82	91	90	84	89

Table 6. Quality indicators of bread after 3 hours from the baking.



Figure 2. The Alphamalt LQ 4020 influence on physic-chemical parameters of bread (flour F2).

In the case of flour F2, the optimal dose was again 0.2 g/kg flour (for almost all indicators) and 0.3 g/kg flour, respectively (for the volume). It was a volume increasing with 18 % than control.

Table 7. Recipes and technological parameters in baking test.

Raw		Indirect method – F4													
materials	Total				Sponge				Dough						
and	Μ	P1	P2	P3	P4	Μ	P1	P2	P3	P4	Μ	P1	P2	P3	P4
technological															
parameters															
Flour, kg	1	1	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Water, 1	0.595	0.595	0.595	0595	0.595	0.30	0.30	0.300	0.300	0.300	0.295	0.295	0.295	0.295	0.295
Yeast, g	25	25	25	25	25	25	25	25	25	25	-	-	-	-	-
Salt, g	15	15	15	15	15	-	I	-	-		15	15	15	15	15
Alphamalt LQ	-	0.2	0.3	0.4	0.5	-	-	-	-	-	-	0.2	0.3	0.4	0.5
4020, g															
TFr, min.	-	-	-	-		2	2	2	2	2	-	-	-	-	
TFe, min.	-	-	-	-		120	120	120	120	120	-	-	-	-	
TFr, min.	-	-	-	-		-	-	-	-		4	4	4	4	4
Td, min.	-	-	-	-		-	I	-	-		45	45	45	45	45
Tc, min.	-	-	-	-		-	-	-	_		30	30	30	30	30
Tbaking, °C							2	202	230°C						

TFr = mixing time sponge, dough ; TFe = fermentation time sponge ; Td = proofing time dough; Tc = baking time dough; Tbaking = baking temperature dough.

Table 8. Quality indicators of bread after 3 hours from the baking.

Sample/Indicators	Μ	P1	P2	P3	P4
Weight, g	456	465	465	469	471
Volume, cmc/100 g	340	382	353	349	348
Height (H), cm	10.35	11.5	10.4	10.5	10.6
Diameter (D), cm	16	15.55	15.6	15.5	15.48
H/D	0.65	0.74	0.67	0.68	0.68
Porosity, %	81	85	83	83	84
Elasticity, %	96	98	98	98	97
Bread note	90	97	93	92	93



Figure 3. The Alphamalt LQ 4020 influence on physic-chemical parameters of bread (flour F4).

In the case of flour F4, the optimal dose was again 0.2 g/kg flour (volume, H, D, H/D, P, and E). By increasing enzyme dose the indicators values decreased, but all of these had been higher than control.

The sample with 0.2 g/kg flour Alphamalt LQ 4020 added the best results presented:

- 12 % volume increasing;

- 5 % porosity improving;

- 8 %, note improving.

1.2. INFLUENCE OF ALPHAMALT LQ 4020 ON RHEOLOGICAL INDICATORS

It was tested the influence of Alphamalt LQ 4020 on rheological indicator's F1 and F2 flours. The following quantities of Alphamalt LQ 4020 were used: 0.2; 0.3; 0.4 and 0.5 g/kg

flour, respectively. At the moment of these tests, we made again the flours'farinograms, after over ten days from the last one. In this period the maturation of wheat flours had taken place, the

days from the last one. In this period the maturation of wheat flours had taken place, the internal balance equilibration of flour, as we can see from the rheological indicators.

Table 9. Rheological indicators of F1 and F2 flours before and after maturation.

Flour/Indicator	Flou	r F1	Flour	F2
	Before	After	Before	After
Hydration capacity, %	52.6	57.7	59.0	61.8
Development, min.	2.0	1.5	1.9	2.4
Stability, min.	5.0	8.2	4.0	5.1
Elasticity, uB	140	140	120	140
Softening, uB	80	50	80	100
Power	44	58	44	44

Table 10. The influence of Alphamalt LQ 4020 added on rheological indicators values of tested flours.

Sample/Indicator	F1				F2					
	M1	P1	P2	P3	P4	M2	P1'	P2'	P3'	P4'
Hydration capacity, %	57.7	58.1	58.3	58.5	58.5	61.8	61.9	62.5	62.6	63.0
Development, min.	1.5	1.5	2.0	1.8	1.7	2.4	2	2	2	2
Stability, min.	8.2	3.2	2.4	2.1	1.8	5.1	4	2.5	3	2.4
Elasticity, uB	140	140	140	140	140	140	140	120	110	140
Softening, uB	50	90	140	160	210	100	130	130	170	160
Power	58	38	33	31	26	44	38	36	32	32







By increasing enzyme dose, rheological indicators values had been worse. Therefore, the stability and power decreased and softening increased, as we can see in the figure 4.



Figure 5. Flour's F1 and F2 farinograms, with Alphamalt LQ 4020 adding.



Figure 6. Tests with Alphamalt LQ 4020 (flour F1).



Figure 7. Tests with Alphamalt LQ 4020 (flour F2).

Rheological indicators values confirmed the results obtained on technological tests. Therefore, increasing enzyme dose gled to worse bread qualitative indicators, as a result of breaking peptide links from gluten network. The sample P1 had, as on the technological test, the best results.

2. ALPHAMALT BK 5020

2.1. INFLUENCE OF ALPHAMALT BK 5020 ON BREAD QUALITY

There were used the wheat flours presented in the Table 1 and Table 2 (flour F1 and F2). The following quantities of ALPHAMALT LQ 4020 were used: 0.5; 0.6 and 0.7 g/kg flour, respectively.

Flour F1

 Table 11. Recipes and technological parameters.

	I										
Raw materials and		Direct method - F1									
technological parameters	M1	P1	P2	P3							
Flour, kg	1	1	1	1							
Water, 1	0.530	0.530	0.530	0.530							
Yeast, g	30	30	30	30							
Salt, g	15	15	15	15							
Alphamalt BK 5020, g	-	0.5	0.6	0.7							
Mixing time, min.	2	2	2	2							
Fermentation time, min.	90	90	90	90							
Proofing time, min.	50	50	50	50							
Baking time, min.	40	40	40	40							
Baking temperature, °C		225	J°C								

Sample/Indicators	M1	P1	P2	P3
Weight, g	530	546	535	530
Volume, cmc/100 g	259	247	283	278
Height, (H), cm	9.1	7.2	7.7	7.8
Diameter (D), cm	15.7	15.6	16.25	15.75
H/D	0.58	0.46	0.47	0.49
Porosity (crumb structure),	77	74	76	80
%				
Elasticity, %	97	93	95	93
Moisture, %	41.81	41.01	41.40	41.6
Acidity, degrees	1.0	0.8	0.8	1.0
Bread note	81	72	76	78





Figure 8. The Alphamalt BK 5020 adding influence on physic-chemical parameters of bread (flour F1).

In this case it was not a benefic influence of enzymatic preparation used. Therefore, the volume values had been higher than control with 0.6 and 0.7 g/kg flour, respectively. All other indicators, less porosity at 0.7 g/kg flour, had been, in generally, under those of control.

Flour F2

Table 13. Recipes and technological parameters in baking test.

	1		U				
Raw materials and	Direct method - F2						
technological parameters	M2	P1'	P2'	P3'			
Flour, kg	1	1	1	1			
Water, 1	0.590	0.590	0.590	0.590			
Yeast, g	30	30	30	30			
Salt, g	15	15	15	15			
Alphamalt BK 5020, g	-	0.5	0.6	0.7			
Mixing time, min.	2	2	2	2			
Fermentation time, min.	90	90	90	90			
Proofing time, min.	50	50	50	50			
Baking time, min.	40	40	40	40			
Baking temperature, °C	225°C						

Table 14. Quality indicators of bread after 3 hours from the baking.

Sample/Indicators	M2	P1'	P2'	P3'
Weight, g	531	545	558	545
Volume, cmc/100 g	273	244	247	244
Height, (H), cm	8.7	7.5	7.3	7.2
Diameter (D), cm	15.9	15.75	15.75	15.75
H/D	0.55	0.47	0,46	0.46
Porosity (crumb structure), %	76	73	75	75
Elasticity, %	97	93	90	93
Moisture, %	43.41	42.49	42.49	42.41
Acidity, degrees	1.0	1.0	1.0	0.8
Bread note	82	73	74	73



Figure 9. The Alphamalt BK 5020 adding influence on physic-chemical bread indicators (flour F2).

The influence of Alphamalt BK 5020 was not good, like in the case of flour F1. Therefore, all quality indicators values of samples with enzymes had been under the control sample, with tendency to decrease as the enzyme dose increase.

2.2. INFLUENCE OF ALPHAMALT BK 5020 ON RHEOLOGICAL INDICATORS

It was tested the influence of Alphamalt BK 5020 on rheological indicator's F1 and F2 flours. The following quantities of Alphamalt BK 5020 were used: 0.5; 0.6 and 0.7 g/kg flour, respectively.

Sample/Indicator	F1			F2				
	M1	P1	P2	P3	M2	P1'	P2'	P3'
Hydration capacity, %	57.7	58.3	58.5	58.7	61.8	63.2	63.7	64.2
Development, min.	1.5	2.0	2.0	2.1	2.4	2	2	1.8
Stability, min.	8.2	3.0	2.2	2.1	5.1	3.1	2.8	2.6
Elasticity, uB	140	140	120	120	140	140	120	140
Softening, uB	50	250	270	280	100	270	290	290
Power	58	25	23	22	44	22	21	20

Table 15. The influence of Alphamalt BK 5020 added on rheological indicators of tested flours.

By increasing enzyme dose, rheological indicators values had been worse. Therefore, stability and power decreased and softening increased, as in the figure 10.





Figure 10. The influence of Alphamalt BK 5020 added on rheological indicators values of tested flours.



Figure 11. Tests with Alphamalt BK 5020 (flour F1).



Figure 12. Tests with Alphamalt BK 5020 (flour F2).



Figure 13. Flour's F1 and F2 farinograms, with Alphamalt BK 5020 adding.

Rheological indicators values confirmed the results obtained on technological tests. Therefore, by increasing enzyme dose the bread qualitative indicators became worse, as a result of breaking peptide links from gluten network.

3. BACTERIAL PROTEASE

3.1. INFLUENCE OF BACTERIAL PROTEASE ON BREAD QUALITY

Commercial flours were obtained from Baneasa S.A. (flour F1) and Plevnei S.A. (flour F2 and F3). The physic-chemical and rheological indicators are given in the Table 14 and Table 15.

Indicators	U.M.	Values				
		F1	F2	F3		
Moisture	%	11.30	13.80	15.4		
Ash	% s.u.	0.47	0.46	0.59		
Acidity	degrees	2.0	2.2	2.2		
Wet gluten	%	24.7	24.0	19.90		
Gluten Index	-	97	97	99		
Gluten deformation	mm	2.5	6.5	8.0		
Falling Number	sec.	429	375	405		

Table 16. Physic-chemicals indicators of flours.

 Table 17. Rheological indicators of flours.

Indicators	U.M.	Values			
		F1	F2	F3	
Hydration capacity	%	59.7	61.5	61.5	
Development	min.	1.7	2	2.5	
Stability	min.	12	6.5	10.4	
Elasticity	uB	130	140	150	
Softening	uB	40	90	30	
Power	-	52	42	52	

Flour F1

Table 18. Recipes and technological parameters in baking test.

Raw materials and	Direct method				
technological parameters			F1		
	$\mathbf{M_1}$	P ₁	P ₂	P 3	P 4
Flour, kg	1	1	1	1	1
Water, 1	0.6	0.6	0.6	0.6	0.6
Yeast, g	30	30	30	30	30
Salt, g	15	15	15	15	15
Bacterial protease, g	-	0.2	0.3	0.4	0.5
Mixing time, min.	3	3	3	3	3
Fermentation time, min.	90	90	90	90	90
Proofing time, min.	35	35	35	35	35
Baking time, min.	40	40	40	40	40
Baking temperature, °C			230°C		

The following quantities of bacterial protease were used: 0.2; 0.3; 0.4 and 0.5 g/kg flour, respectively.

Sample/Indicators	F1				
-	M 1	P 1	P 2	P 3	P 4
Weight, g	528	546	528	530	543
Volume, cmc/100 g	301	341	367	346	342
Height (H), cm	9.4	10.5	11	11	10.9
Diameter (D), cm	15.5	15.55	15.9	15.5	15.75
H/D	0.61	0.68	0.69	0.71	0.69
Porosity, %	81	81	81	82	81
Elasticity, %	97	97	97	97	98
Moisture, %	43.51	44.09	44.2	44.11	43.20
Acidity, degrees	0.8	0.8	1.0	0.8	0.8
Bread note	87	92	94	93	93

Table 19. Quality indicators of bread after 3 hours from the baking.



Figure 14. The bacterial protease influence on physic-chemical indicators of bread (flour F1).

The sample with 0.3 g bacterial protease/kg flour (P2) added the best results presented:

- 22 % volume increasing;
- 8 % note improving;

Table 20.	Table 20. Recipes and technological parameters in baking test.							
Raw materials and		Direct method						
technological			F2					
parameters	M ₂	P 1'	P 2'	P3'	P 4'			
Flour, kg	1	1	1	1	1			
Water, l	0.615	0.615	0.615	0.615	0.615			
Yeast, g	30	30	30	30	30			
Salt, g	15	15	15	15	15			
Bacterial protease, g	-	0.2	0.3	0.4	0.5			
Mixing time, min.	3	3	3	3	3			
Fermentation time, min.	90	90	90	90	90			
Proofing time, min.	35	35	35	35	35			
Baking time, min.	40	40	40	40	40			
Baking temperature °C			230°C					

Flour F2

1 1.

The following quantities of bacterial protease were used: 0.2; 0.3; 0.4 and 0.5 g/kg flour, respectively.

Table 21. Quality indicators of bread after 3 hours from the baking.

Sample/Indicators	F2				
	M ₂	P 1'	P 2'	P 3'	P 4'
Weight, g	542	550	548	548	542
Volume, cmc/100 g	293	315	318	303	304
Height (H), cm	9.6	10	10.2	9.8	9.5
Diameter (D), cm	15.7	15.4	15.55	15.65	16
H/D	0.61	0.65	0.66	0.63	0.59
Porosity, %	79	80	81	78	78
Elasticity, %	97	97	95	95	93
Moisture, %	44.4	43.5	43.8	44.0	43.21
Acidity, degrees	1.0	1.0	1.0	1.0	0.8
Bread note	86	89	88	87	84





- The sample with 0.3 g bacterial protease/kg flour (P2') added the best results presented:
- 9 % volume increasing;
- 3 % porosity improving.



Figure 16. Tests with bacterial protease (flour F2).

Flour F3

Fable 22. Recip	bes and technol	ogical parameters	in baking test.
1			0

Raw materials and	1	Direct method					
technological	F3						
parameters	M ₃	P ₁ "	P2"	P3"	P4"		
Flour, kg	1	1	1	1	1		
Water, l	0.615	0.615	0.615	0.615	0.615		
Yeast, g	30	30	30	30	30		
Salt, g	15	15	15	15	15		
Bacterial protease, g	-	0.2	0.3	0.4	0.5		
Mixing time, min.	3	3	3	3	3		
Fermentation time, min.	90	90	90	90	90		
Proofing time, min.	35	35	35	35	35		
Baking time, min.	40	40	40	40	40		
Baking temperature, °C			230°C				

The following quantities of bacterial protease were used: 0.2; 0.3; 0.4 and 0.5 g/kg flour, respectively.

Table 23. Qualit	y indicators	of bread	after 3 hou	irs from the	baking test.
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Sample/Indicators	F3				
	M3	P 1"	P2"	P 3"	P4"
Weight, g	546	544	547	544	542
Volume, cmc/100 g	281	304	300	303	292
Height (H), cm	9.2	9.8	9.5	10	9.3
Diameter (D), cm	15.75	15.75	15.25	15.45	15.65
H/D	0.58	0.62	0.62	0.65	0,59
Porosity, %	78	79	79	5	76
Elasticity, %	95	97	95	95	95
Moisture, %	44.29	44.21	44.30	43.91	44.0
Acidity, degrees	1.0	1.2	1.0	1.0	1.2
Bread note	83	86	86	83	83



Figure 17. The bacterial protease influence on physic-chemical parameters of bread (flour F3).

The sample with 0.2 g bacterial protease/kg flour (P_1 ") added the best results presented:

- 8 % volume increasing;
- 2 % elasticity improving;
- 4 % note improving.



Figure 18. Tests with bacterial protease (flour F3).

Conclusions

1. A lot of bacterial proteases were identified and characterized.

Table 24. Commercial bacterial proteases used in experiments.						
Name of commercial product	Flour Gluten deformation, mm			Optimal dose (to flour) g/kg flour		
	F 1	F2	F4	F1	F2	F4
Alphamalt LQ 4020	4.0	9.0	4.0	0.2	0.2 - 0.3	0.2
Alphamalt BK 5020	4.0	9.0	4.0	0.6	-	-
				(volume)		

Table 24. Commercial bacterial proteases used in experiments.

Table 25. Commercial bacterial protease used in experiments.						
Name of commercial	Flour			Optimal dose		
product	Gluten deformation, mm			(to flour)		
				g/kg flour		
	F1	F2	F3	F1	F2	F3
Bacterial protease	2.5	6.5	8.0	0.3	0.3	0.2

Table 25. Commercial bacterial protease used in experiments.

- 2. Different enzymes doses were tested as a function of their activity. Therefore:
- For Alphamalt LQ 4020: 0.2; 0.3; 0.4 and 0.5 g/kg flour, respectively;
- For Alphamalt BK 5020: 0.5; 0.6 and 0.7 g/kg flour, respectively;
- For bacterial protease: 0.2; 0.3; 0.4 and 0.5 g/kg flour, respectively.

As a function of the quality indicators of used flours it was established the optimal doses, as we can see in the Table 24 and Table 25.

3. The quality indicators have improved (volume, porosity, elasticity, bread note, etc.).

4. The sensorial properties have improved, crumb and crust color, especially.

5. It was determined the adding bacterial proteases influence on dough rheological properties. The obtained values confirmed the baking tests results.

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7. Zymotachigraphic method	
8. STAS 985-79	Baking yeast
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