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Synergistic Effect of Bacterial and Fungal Amylases on Breadmaking

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Abstract

For decades enzymes have been used for bread making. Due to the changes in the baking industry and the demand for more varied and natural products, enzymes have gained more and more importance in bread formulations.

The study evaluates the synergistic effects of these enzymes when these enzymes are combined with each other.

Bread staling is responsible for significant financial losses. A maltogenic α-amylase is a true anti-staling enzyme that affects neither volume or crumb structure. Therefore, it is more feasible to use this enzyme in combination with enzymes such as fungal α-amylase to ensure the other bread quality parameters such as volume, dough stability, and crumb structure.

Concerning the interaction of enzymes substrates in dough and bread is rather complex, using of enzyme combinations should have synergistic effects that are not observed if only one enzyme is used – even at high dosages.

The added enzymes (Novamyl with Fungamyl 2500 BG, Novamyl with fungal α-amylase, respectively) influence have been established by baking tests.

The maltogenic α-amylase has the synergistic effect together with two fungal α-amylases: 45 ppm of maltogenic together with other enzymes gave a softer crumb during storage than using 75, 100 or 200 ppm of the maltogenic α-amylase alone.

Key words: bacterial α-amylase, fungal α-amylase, synergistic effect, breadmaking, etc.

Introduction

Bread is the most common traditional food in the world, with low cost. Today, bread is closely related to biotechnology, the term that relates to these two subjects is enzyme.

Using enzyme combinations for breadmaking is not new. Enzymes are used for dough conditioning, for extending shelf life and improving crumb softness and elasticity, and for dough strengthening.

The maltogenic α-amylase has a unique anti-staling effect.

This article reviews the synergistic effects of maltogenic α-amylase with traditional enzymes such as fungal α-amylase.

Therefore, the objectives of this research were to determine: 1) the synergistic effects of Novamyl and Fungamyl 2500 BG on bread quality parameters; 2) the synergistic effects of Novamyl and fungal α-amylase from S.C.Enzymes & Derivates Romania S.A. on bread

quality parameters; 3) the effects of enzyme combinations on the gases power and retention in dough; 4) the results repeatability.

Materials and Methods

Commercial flours were obtained from S.C. PLEVNEI S.A. and S.C. BANEASA S.A. The physico-chemical and rheological indicators are given in the **(Table 1** and **Table 2)**.

Table 1. Physico-chemical indicators of flours.

Qualitative Indicators	**UM**	**Values**	
		F1	**F2**
Moisture	%	14.81	15.31
Ash	% d.m.	0.69	0.44
Acidity	degrees	2.4	2.4
Wet gluten	%	24.3	22.4
Gluten Index		95	99
Gluten deformation	mm	3.5	3.0
Falling Number	sec.	383	347

Table 2. Rheological indicators of flours.

Rheological Indicators	**UM**	**Values**	
		F1	**F2**
Hydration capacity	%	55.9	52.8
Development	min.	2.0	1.8
Stability	min.	4	3.5
Elasticity	uB	130	130
Softening	uB	110	90
Power	-	40	42

The amilolitics enzymes used were the following:
Maltogenic α-amylase: Novamyl, Novo Nordisk, Denmark.
Fungal α-amylases: Fungamyl 2500 BG, Novo Nordisk, Denmark; fungal α-amylase, S.C. Enzymes & Derivates Romania S.A.
Pakmaya yeast was used from Rompak Ltd., Romania.

Baking

The enzymes were added to the baking formula during the mixing stage. Breads were made with commercial bread flour 480 and 650 type. Employing the pup loaf formula with a 90-min. fermentation, straight-dough process (Romanian Standard STAS 90-88 Baking Test).

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

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

Freshness determination

The Freshness analysis was performed with an L MIM Penetrometer. The method consists in measurement of the resistance opposed, to an applied force, for 10 seconds, on

bread crumb. The measurements of freshness are made at 3, 24, 48, 72 and 96 hours, respectively, the results being compared with the control sample at the same time periods.

Power Gas

The straight-dough formula with 250 g of commercial flour, 5.0 g of salt, 5.0 g of yeast and water was used for the power gas test (a strengthened dough is obtained).

Bacterial and fungal alpha-amylases were added to some dough were in different quantities to notice the optimal doses which can be used for a better quality of bread. The control dough had no additives. Zymotachigraph Chopin was used for power and retention gas.

Results and Discussions

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

The Influence of Novamyl and Fungamyl 2500 BG on bread quality

Flour F1

Table 3. Recipes and technological parameters in baking test.

Raw materials	**Direct method**				
	F1				
	Control	**P14**	**P15**	**P16**	**P17**
Flour, kg	2	2	2	2	2
Water, l	1.12	1.12	1.12	1.12	1.12
Yeast, g	60	60	60	60	60
Salt, g	30	30	30	30	30
Novamyl, g +	-	0.09	0.09	0.09	0.09
Fungamyl 2500 BG, g	-	0.01	0.02	0.04	0.1
Mixing time, min.	3	3	3	3	3
Fermentation time, min.	90	90	90	90	90
Proofing time, min.	65	65	65	65	65
Baking time, min.	30	30	30	30	30
Baking temperature, °C	230				

The following quantities of Novamyl were used: 0.045 g/kg flour, which correspond with 450 units MANU/kg flour and Fungamyl 2500 BG: 0.005; 0.01; 0.02 and 0.05 g/kg flour, respectively, which correspond to 12.5; 25; 50 and 125 units FAU/kg flour, respectively.

The bread was packed 3 hours from the baking.

Table 4. Quality indicators of bread after 24, 48, 72 and 96 hours, respectively from the baking.

Sample/Parameters	**Control**	**P14**	**P15**	**P16**	**P17**
Weight, g	520 529 537 532	527 524 526 521	534 526 529 516	537 525 527 528	532 530 536 533
Volume, cmc/100 g	313 294 302 312	359 344 349 365	343 342 340 365	340 347 338 345	334 340 327 336
Height, cm	9.4 9.8 10.0 9.9	11.1 11.2 10.7 10.9	11.1 10.7 11.0 10.7	10.7 10.3 11.3 11.0	10.7 10.3 10.8 11.2
Diameter, cm	15.45 15.55 15.55 15.4	15.45 15.05 15.35 15.2	15.65 14.9 15.25 15.45	15.95 15.3 15.2 15.45	15.35 15.35 15.05 14.9
H/D	0.61 0.63 0.64 0.64	0.72 0.74 0.70 0.72	0.71 0.72 0.72 0.69	0.67 0.67 0.74 0.71	0.70 0.67 0.72 0.74
Porosity (Crumb structure), %	82 82 82 81	86 86 85 85	85 85 84 82	84 82 84 83	83 86 85 85
Elasticity, %	98 97 95 95	98 98 98 97	98 100 97 98	97 97 98 98	98 98 98 98
Moisture, %	44.19 43.31 43.89 43.90	44.0 44.4 42.89 43.91	44.50 43.91 44.1 42.80	44.21 44.20 44.4 44.01	44.40 43.80 44.40 44.31
Acidity, degrees	1.4 1.2 1.2 1.2	1.2 1.4 1.2 1.6	1.6 1.4 1.4 1.6	1.2 1.0 1.2 1.6	1.0 1.2 1.2 1.4
Bread score	90 87 84 85	97 96 95 96	96 96 92 93	95 92 91 93	90 94 94 94
Freshness	518 481 536 485	596 592 527 513	521 574 516 508	554 525 474 513	634 602 545 557

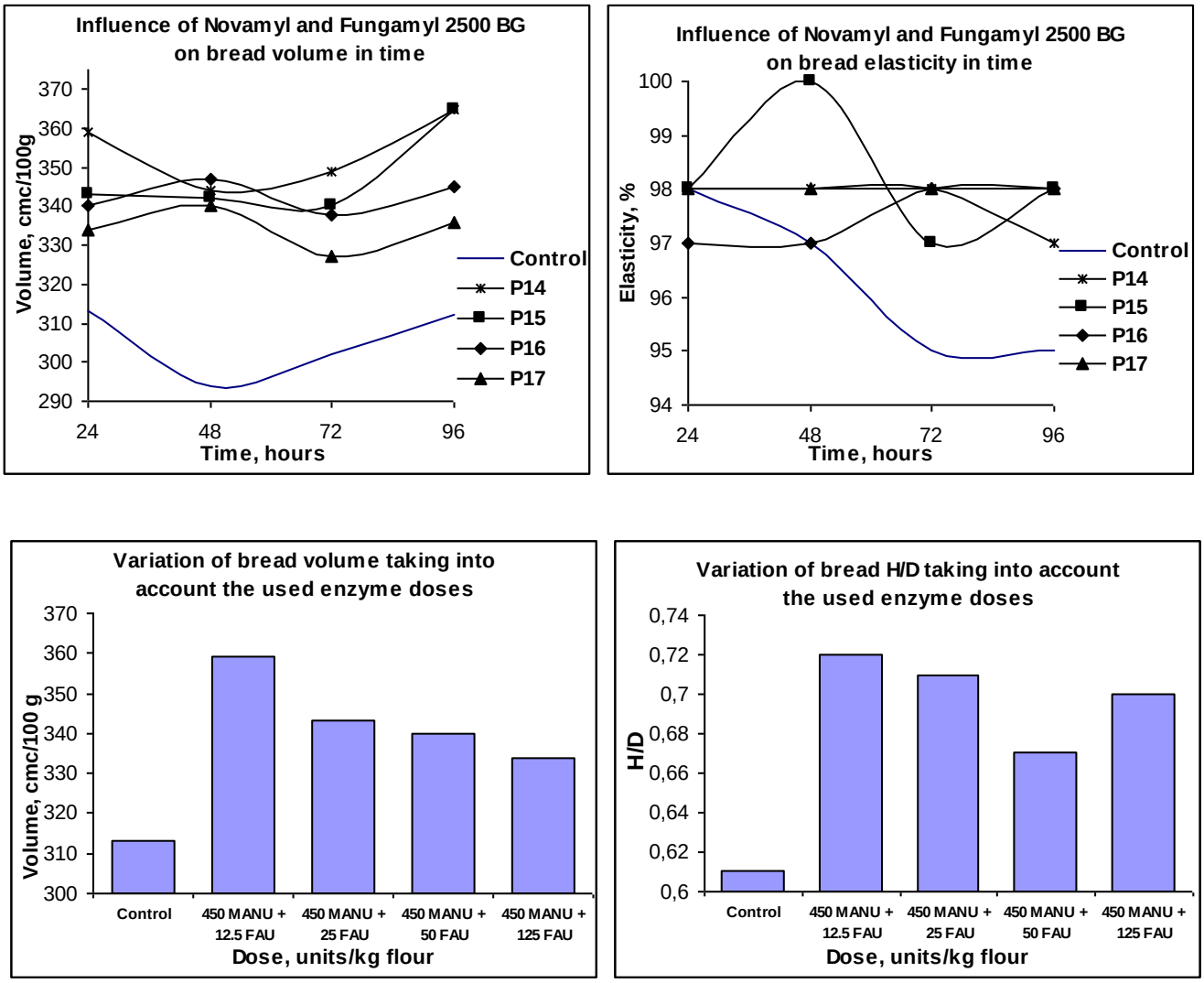

Figure 1. Variation of physico-chemical parameters of bread obtained from baking tests with Novamyl and Fungamyl 2500 BG.

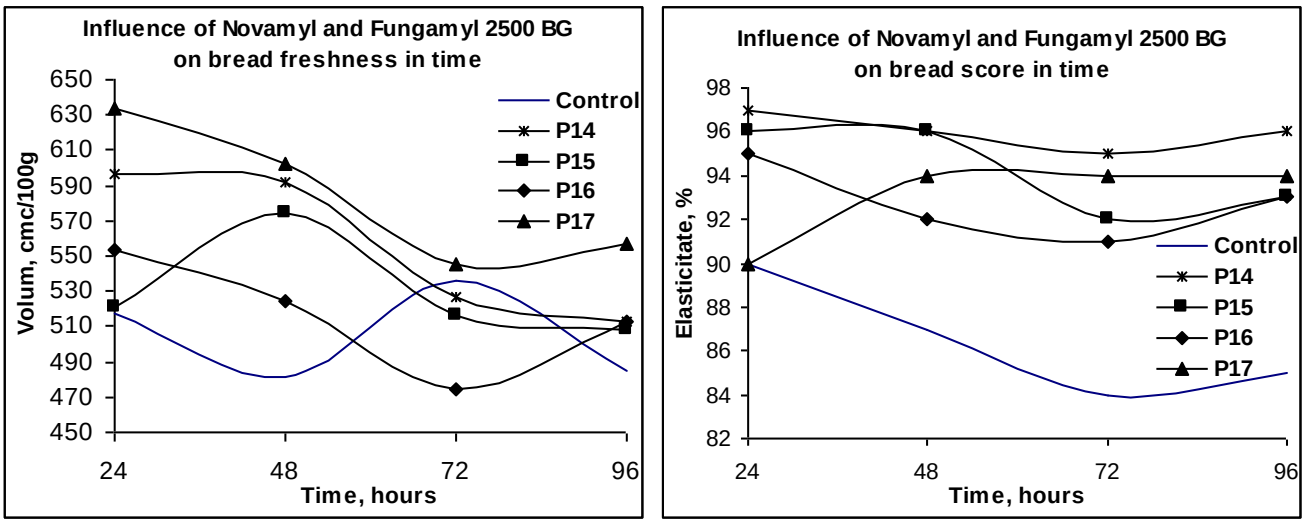

Figure 2. Variation of freshness and bread score, in time, of bread obtained from baking tests with Novamyl and Fungamyl 2500 BG.

By the analysis of the table information and the graphic representations it results that the highest values of the bread quality indices were obtained at P14 sample, with Novamyl addition (constant dose) and Fungamyl 2500 BG (the lowest dose). We should remark that the values obtained for the other samples are also higher than the values of the control sample.

By the analysis of the results we can come to the following conclusions: the values of the bread quality indices obtained by the simultaneous action of the two enzymes are higher than those resulted by the individual action of each enzyme preparation; on the other hand, the optimal utilization dose for each enzyme preparation, in case of simultaneous action, is much lower than in the case of separate utilization.

The Influence of Novamyl and Fungamyl 2500 BG on gas power

Experiments for gas power determinations were made.

Table 5. CO_2 volumes and retention surface (Flour F1).

Parameters	**Control**	**P14**	**P15**	**P16**	**P17**
Total CO_2 volume, cm^3	2700	2430	2490	2400	2535
Total surface, cm^2	180	162	166	160	169
Retention surface, cm^2	36	16	16	15	16
Time, hours	4	4	4	4	4
Temperature, °C	30	28	28	28	29

From the table above one can noticed that total volume of CO_2 is not higher than the control sample. Maltogenic α-amylase does not affect the dough rheological property because of its low activity at a temperature less than 35°C. The values obtained did not correlate with technological tests, where the best results are for sample P14.

The Influence of Novamyl and Fungamyl 2500 BG, Novamyl and fungal α-amylase, respectively, on bread quality

Flour F2

Table 6. Recipes and technological parameters in baking test.

Raw materials and technological regime	**Direct method**				
	F2				
	Control	**P12**	**P13**	**P14**	**P15**
Flour, kg	1.5	1.5	1.5	1.5	1.5
Water, l	0.8	0.8	0.8	0.8	0.8
Yeast, g	45	45	45	45	45
Salt, g	22.5	22.5	22.5	22.5	22.5
Novamyl + Fungamyl 2500 BG, g	-	0.0675 0.0075	-	-	-
Novamyl, g + Fungal α-amylase, g	-	-	0.0675 0.0015	0.0675 0.0034	0.0675 0.0068
Mixing time, min.	3	3	3	3	3
Fermentation time, min.	90	90	90	90	90
Proofing time, min.	65	65	65	65	65
Baking time, min.	30	30	30	30	30
Baking temperature, °C	230				

The following quantities of Novamyl were used: 0.045 g/kg flour, which correspond to 450 units MANU/kg flour and Fungamyl 2500 BG: 0.005 g/kg flour, respectively, which correspond to 12.5 units FAU/kg flour, respectively (sample P1); Novamyl 0.045 g/kg flour, which correspond to 450 units MANU/kg flour and fungal α-amylase from S.C. Enzymes & Derivates Romania S.A.: 0.001; 0.0023 and 0.0045 g/kg flour, respectively, which correspond to 40; 90 and 180 units SKB/kg flour, respectively.

The bread was packed after 3 hours from the baking.

Table 7. Quality indicators of bread after 3, 24, 48 and 72 hours, respectively from the baking.

Sample/Parameters	**Control**	**P12**	**P13**	**P14**	**P15**
Weight, g	518	490	521	515	525
	495	517	524	517	530
	517	513	529	486	489
	511	519	489	510	519
Volume, cmc/100 g	326	362	345	353	353
	328	350	312	329	328
	311	351	327	342	352
	333	345	340	353	334
Height, cm	10.2	9.8	10.7	11.0	11.0
	10.5	11.2	10.4	10.9	10.0
	10.5	11.5	11.1	10.5	10.2
	10.6	11.5	10.7	11.0	10.2
Diameter, cm	16	16.1	16.1	15.8	16.1
	15.3	15.05	15.0	15.05	15.45
	15.45	15.25	15.1	15.5	15.3
	15.3	15.55	15.75	15.4	15.6
H/D	0.64	0.61	0.66	0.70	0.68
	0.69	0.74	0.69	0.72	0.65
	0.68	0.75	0.74	0.68	0.67
	0.69	0.74	0.68	0.71	0.65
Porosity (Crumb structure), %	83.5	86.2	85.68	82.56	85.68
	83.4	84.8	81.4	84.82	85.3
	82.94	87.6	84.45	84.92	85.11
	83.79	85.4	83.97	84.73	84.73
Elasticity, %	95	95	98	98	97
	98	98	97	98	98
	98	100	98	98	97
	98	97	98	98	97
Moisture, %	43.8	43.90	43.01	44.19	44.10
	43.71	43.90	43.39	44.21	43.99
	43.11	43.50	44.70	42.71	43.5
	43.00	43.11	42.71	43.09	43.10
Acidity, degrees	1.0	0.8	0.8	0.8	1.0
	1.2	1.0	1.0	1.0	1.0
	1.0	1.0	0.8	1.2	0.8
	1.0	0.8	1.0	1.2	1.0
Bread score	88	93	93	93	95
	87	92	86	92	92
	85	92	91	93	93
	89	92	90	93	91
Freshness, mm	630	640	600	605	615
	522	635	563	612	573
	513	605	589	592	516
	554	603	532	555	562

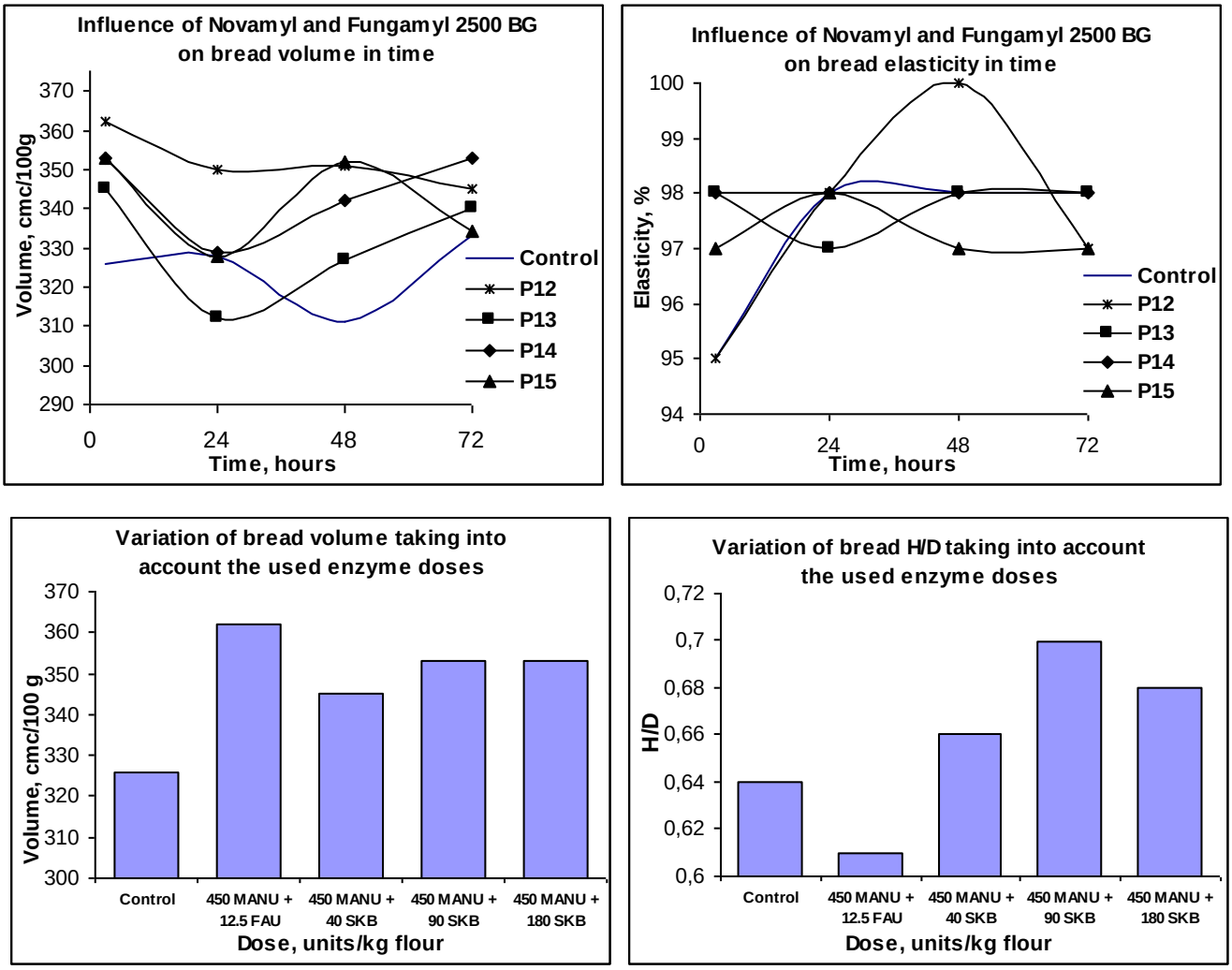

Figure 3. Influence of Novamyl and Fungamyl 2500 BG, Novamyl and fungal α-amylase from S.C. Enzymes & Derivates Romania S.A., respectively, on bread physico-chemical indicators.

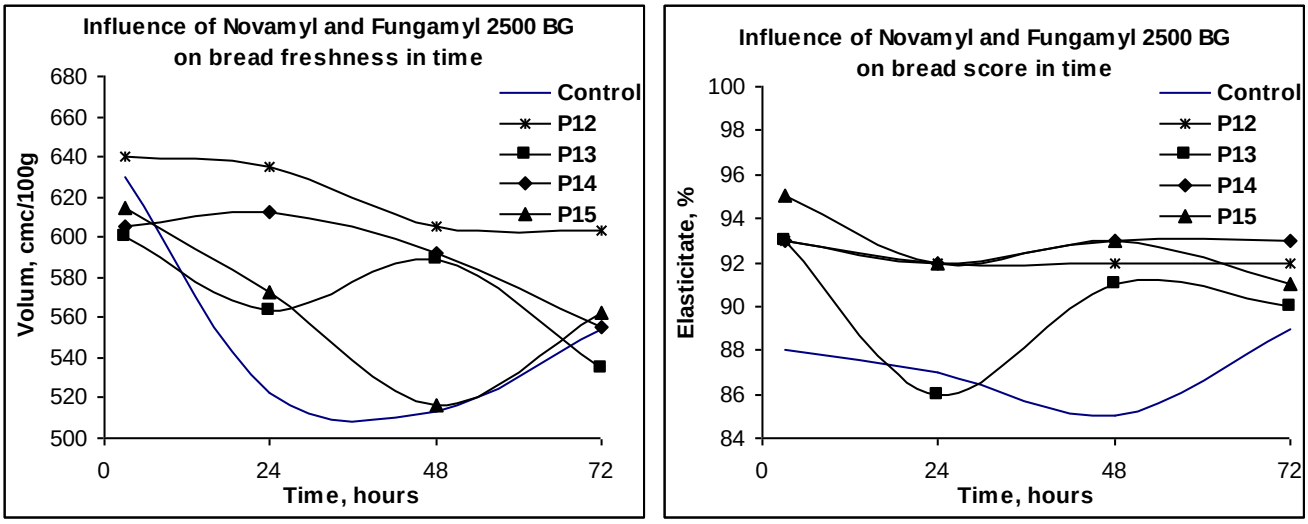

Figure 4. Influence of Novamyl and Fungamyl 2500 BG, Novamyl and fungal α-amylase from S.C.Enzymes & Derivates Romania S.A., respectively, on bread freshness and bread score in time.

In the case of flour F2 two possibilities were tested:

- the enzymes doses which gave the best results in the case of flour F1, for reproducibility;
- the same dose of Novamyl and different doses of fungal α-amylase.

The conclusions were:

- synergetic effect of the two enzymes is the same for the flour F1 and F2;
- for the flour F2, the best synergistic effect is for the sample with 450 units MANU/kg flour and 90 units SKB/kg flour.

Finally, the values of physico-chemical indicators for the samples with added enzymes are certainly higher than those of the control sample and have a good steadiness in time.

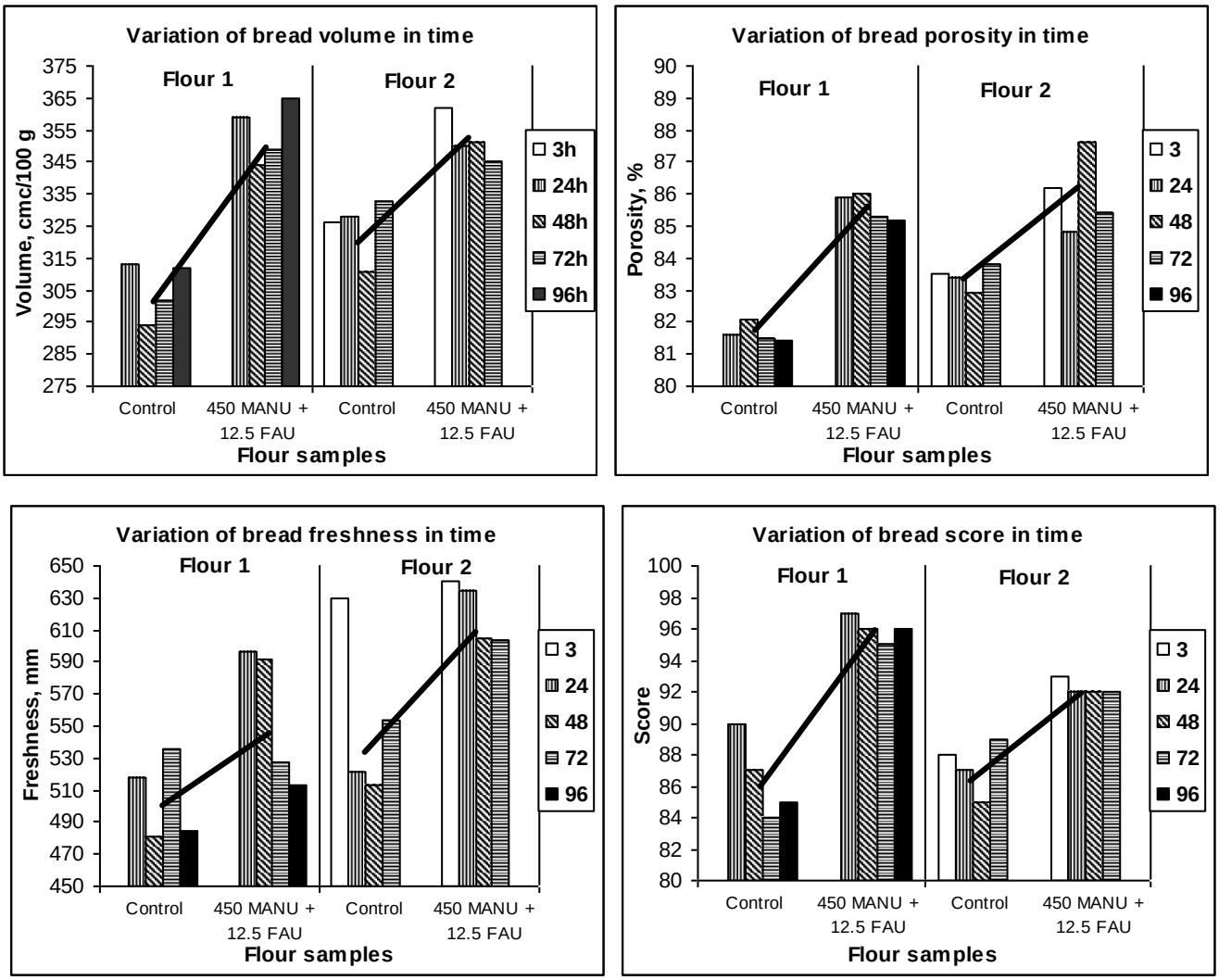

Figure 5. Synergistic effect of Novamyl and Fungamyl 2500 BG.

Figure 5 presents the variations of the quality indices for the samples obtained from the two flours with enzyme preparation supplements, in optimal doses experimentally established for the case of their simultaneous action.

From the statistical analysis of the data it results that the synergic effect of the utilization of the two types of enzyme preparations, having beneficial effects on the quality sensory indices, as well as on the bread freshness.

Conclusions

In the following table it is represented the synergistic effect of the enzymes used on the experiments.

Table 8. Synergistic effect of bacterial and fungal amylases on breadmaking

Name of commercial product	**Activity**	**Flour Falling Number, sec.**		**Optimal dose (to flour)**	
Novamyl + Fungamyl 2500 BG	10000 MANU/g 2500 FAU/g	383	347	450 MANU/kg flour 12,5 FAU/kg flour	450 MANU/kg flour 12,5 FAU/kg flour
Novamyl + Fungal alpha-amylase	10000 MANU/g 40000 SKB/g	347		450 MANU/kg flour 90 SKB/kg flour	

1. Optimal doses for the flours used in experiments were determined, as we can see in table 8.
2. The quality indices (volume, porosity, elasticity, bread mark, etc.) have improved. The values of bread quality indices resulted by the simultaneous action of the two enzyme preparations are higher than those resulted by the individual action of each of them separately.
3. The sensory characteristics generally improved, fact which reflects the variation of the bread mark.
4. The influence of the combination enzyme, Novamyl and Fungamyl 2500BG on the total CO2 volume produced within the dough was examined. The resulted values are not correlated to the results of the technological samples, when the best results were obtained at P14 sample. higher than those of the control sample and are constant in time.
5. In parallel, the influence of the enzyme combination on the bread freshness was examined. The obtained values are higher than those of the control sample and remain constant in time.
6. The synergic effect of the two enzymes, Novamyl and Fungamyl 2500BG, was ascertained on both flour number 1 and number 2. A good experimental reproduction of the enzyme doses was obtained, as well as of the results, which can constitute a data base, subsequently used at industrial level.
7. In the case of the second enzyme combination, Novamil and fungi α-amylase, at flour number 2, the synergic effect resulted for lower enzyme doses than those established in the case of their individual action.

Acknowledgements

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References

1. Romanian Standard 90-88. Baking Test
2. Zymotachigraphic method.