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A study on the influence of some biogenic effectors on bread staling. Sensory evaluation

D. Diaconescu*, M., Zdremtan, M., Mester, L., Halmagean, M., Balint

"Aurel Vlaicu" University of Arad, 310130, Revolutiei Bd, no. 77, Romania

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Abstract

This work aims at studying the influence of the addition of some biogenic effectors on the bread staling rate. So, we use enzymes, natural substances, which can substitute chemical additives in order to improve the bread quality. Due to the fact that, added to starch retrogradation, the gluten also plays an important role in bread staling, we use starch- and non-starch degrading enzymes, such as: α-amylase, photolytic enzymes, xylanase, cellulase and combinations of them. We combine these enzymes to detect the potential synergistic effects which can provide better results as compared to their sole use. To study the influence of these effectors on bread staling we choose a sensory evaluation method. The obtained results show that all the effectors are more or less efficient in decreasing the rate of staling, but, the most efficient are the preparations that comprise combinations of enzymes.

Keywords: bread, staling, enzymes, sensory evaluation

1. Introduction

This paper is part of a larger study on the effects of the biogenous substances on the biotechnological process of breadmaking and especially on the quality of finite products. The enzymes are part of this category and they are beeing used as improvers in breadmaking from the first part of the last century. They are not "E's", they are natural improvers, without toxic potential; anyway, generally they aren't found in the finite product anymore, as they are inactivated during baking. The enzyme preparations used in our country correspond to the specifications recommended by *FAO/OMS* and *Food Chemical Codex* regarding the enzyme preparations of alimentary quality and they are produced by *GRAS (Generally Recognized As Safe)*. These are important issues in the current context of the demands of the consumers of "clean" products from the point of view of adage of chemical additives.

Aside from aspect and flavour, the freshness and the length of maintaining it there are decisive factors in the acquisition of a bakery product.

Unfortunately, because of the staling process, the length of the shelf-life of the bread is relatively short. In 1953, Bechtel et al. [2] define staling as the sum of qualitative alterations of bakery products, others than those produced by the spoilage microorganisms, which reduce the degree of accepting those products by the consumer. Mouneim et al. (2012) [19] state that the most important alterations for the consumer are flavour loss and the increase of firmness. Moreover, as stated by Goesaert et al. (2009) [11], the crumb loses its resilience and the crust its crispiness.

Taking into consideration these aspects connected to the common consumer, we have chosen to study the staling rate of white bread with different additives of enzymes through a simple method of organo-leptical analysis, which permits the sensorial perception of the physical and chemical alterations typical to the staling process.

From the physical-chemical and technological point of view, the staling process is based on the retrogradation of starch, a generally accepted fact [11, 18], especially in the retrogradation of the short

Corresponding author: *e-mail:* danieladiac@yahoo.com

amylopectin side chains, immediately after the bread gets cold [13,24]. However, an important role in staling is also held by the gluten and lipids [3,22], as well as the migration of the water between the biopolymers [11, 13]. Other authors found correlations between fermenting and staling. Thus, Gomez et. Al. (2008) [12] state that a longer period of fermenting determines a slower rate of staling. Moreover, the correlations have been made between the baking temperature and the retrogradation of starch. Giovanelli, G. et al. (1997) [9] stated that the higher the baking temperature is, the more intense the retrogradation will be.

There are numerous methods of delaying the stalling of the bread, such as the adage of anti-staling enzymes. Mouneim H. et al. (2012) [19] state that, in order to be efficient, an enzyme must be denaturized before baking; otherwise the bread will suffer unwanted sensorial alterations. For this study we have chosen to use the following enzymes: α-amylase, proteolityc enzymes, xylanase, cellulase and combinations of them. Because the gluten also plays an important role in bread staling, we use starch and non-starch degrading enzymes. We combine these enzymes to detect the potentials synergistic effects which can provide better results as compared to their sole use.

α-Amylase acts as an anti-aging effector by hydrolyzing amylpectin in oligosaccharides and α-dextrin of low weight, thus affecting starch retrogradation [10] state that specific amylase, namely a *Bacillus stearothermophilus* maltogenic amylase, is an efficient anti-aging agent. Similar results have also been obtained by other authors as well [13,16].

More authors, such as Gambaro, A. et al. (2002) [7] reported that the adage of α-amylase together with xylanase has a better anti-aging effect than that of α-amylase used alone. Gil, M.J. et al. (1999) [8] have used purified endo 1,4-beta-xylanase (hemicellulase) and have not noticed clear effects on the staling of the bread, but, by adding α-amylase together with xylanase, the crumb has maintained its elasticity, the firming rate was lower and the shelf-life increased by two days. Similar results have been obtained by other authors [1,15].

Proteolitical enzymes are added in the dough to achieve a partial gluten hydrolysis for improving machinability, to reduce ropiness and increase shelf-life. Caballero, P. A., et al. (2009) [5] have noticed that the adage of protease determines a decrease of the tenacity and an insignificant increase in the extensibility of the dough. Similar results have been obtained by Wikström, K. şi Eliasson, A. C. (1998) [23]: the protease increases the relaxation speed of the dough and it decreases its resistance to extension. For clearer effects upon the quality of the bread, the protease can be added together with the α-amylase, the volume, elasticity and texture of the crumb being improved. The dextrin resulted after the action of the α-amylase can intervene in the interaction between the granules puffed by the starch and the gluten network, thus altering the tolerance of the dough to machinability [6].

Apart from the positive effects of xylanase upon the volume and porosity, this also determines a delay in the aging of the bread by reducing the hardening of the core [4,17,21]. The xylanase is an enzyme which degrades the cellular walls and as they, together with the hemicelluloses, contain cellulose, it should be added together with the cellulase. The cellulase, as well as the xylanase, frees mono- and oligosaccharides which can intervene in the interaction of starch and the proteins during the depositing of the bread as well as specific dextrin which intervene in the retrogradation of amylopectin [20]. Haros M. and his collaborators (2002) [14] state the way in which the xylanase and the cellulase delay aging, namely by reducing the speed of the hardening process of the crumb during depositing.

2. Materials and Methods

Materials: We used a regular commercial white flour with the following properties: 13.20% water content, 61.40% hydration percentage, mineral substances 0.65%, 13.18% proteins, wet gluten content 28%, gluten index 39, extensibility index under 30cm, deformation index 9mm. We also used Pakmaya yeast and regular kitchen salt with iodine.

The following enzyme combinations were used:

Grindamyl A 1000 – a fungal α-amylase produced by fermentation of selected strains of *Aspergillus oryzae*, standardized to 1,000FAU enzymatic activity/g (10,000 SKB) (Danisco Ingredients – OG&G International SRL Sibiu);

Alphamalt A 6003 – an enzyme preparation which comprises of fungal α-amylase produced by fermentation of selected strains of *Aspergillus*

oryzae, with hemicellulases activity, standardized to 2500 SKB enzymatic activity/g (Mühlenchemie);

Bel`Ase R – a bacterial photolytic enzyme preparation (Beldem Food Ingredients Romania);

Grindamyl PR 59 – a photolytic enzyme complex which also comprise amylase activity, produced by fermentation of selected strains of *Aspergillus oryzae*, (Danisco Ingredients – OG&G International SRL Sibiu);

Bel`Ase C – a pure concentrate of bacterial xylanase (Beldem Food Ingredients Romania);

Multifect CSG – an enzyme preparation extracted from Trichoderma reesei, which comprise cellulase with 8000 IU enzymatic activity/g (EDR Food Ingredients – OG&G International SRL Sibiu);

Fermizyme HE 400 – a standard mixture of xylanase and cellulase with 4000 LYX/g, 400 XVU/g enzymatic activity (Overseas Bakery & Ingredients Romania SRL Bucureşti).

Baking Test: The recipe used for making bread consisted of: 800g of flour, 13g of yeast, 13g of salt, 491g of water according to the water absorption determined by farinograph, and the established level of enzyme preparation. The dough was kneaded with the aid of a laboratory mixer for 3 minutes. After 60 minutes of fermentation at 28-30^{0}C, 1000g dough pieces were hand shaped into elongated forms and were left in the leavening chamber for 60 minutes at 28-30^{0}C. The final product was baked for 35 min. at 250^{0}C.

Thus, we have baked eight breads, out of which one is a control, without adage of enzymes and the other seven with the elements mentioned above. The usage limits for the enzyme preparations were determined through a trial-error process and only intervals in which the enzymes registered effects were chosen. Addition levels for each enzyme preparations represent arithmetical averages of the double trials results. Only levels with maximum impact on sample quality were chosen. They are, respectively: 4g *Grindamyl A 1000*/100kg flour, 11g *Alphamalt A 6003*/100kg flour, 50g *Bel`Ase R*/100kg flour, 10g *Grindamyl PR 59*/100kg flour, 13g *Bel`Ase C*/100kg flour, 7g *Multifactor CSG*/100kg flour, 25g *Fermizyme HE 400*/100kg flour.

*Methods:*The flour quality analysis was made according to STAS 90-77 and STAS 6283/1-83 [25]. The bread quality analysis was made according to STAS 91-83 [25]. The bread staling was analyzed by sensory evaluation and expressed with the following scale, reported on control, at 24, 48 and 72 hours after baking:

0 – altered parameter
1 – highly modified parameter
2 – moderately modified parameter
3 – slightly modified parameter
4 – unmodified parameter.

The analyzed organoleptic parameters were smell, taste, aroma, crust crispness, crumb reliance and crumb firmness. The bread samples were stored at room temperature.

3.Results and Discussion

The alterations of the bread, both sensory and physic-chemical, appear immediately after baking and they are considered positive up until 4 hours. They take place during the whole depositing period. We have done the first sensory analysis 24 hours after taking it out of the oven and then every 24 hours for three days. The results obtained are presented in table no. 1 as arithmetical means of the points given for each parameter separately.

When we added α-amylase (*A1000*) we noticed, a slight slowing of the alteration of smell, taste and aroma of the bread, after 24 hours of depositing due to the acceleration of fermentation. The crispness of the crust is lost as it is in the case of the control bread. However, the elasticity and firmness of the crumb are much less affected: after 72 hours the elasticity is at the level of the control bread after 24 hours, and the elasticity is even weaker.

By adding α-amylase together with xylanase (*A6003*) we haven't noticed differences from the adage of α-amylase alone in terms of smell, taste and flavour of the bread. The maintenance of the crispness is slightly prolonged. There is a slight improvement of the elasticity of the crumb and a weaker hardening after 24 hours, as compared to the addition of the α-amylase alone.

Table 1. Points given after the sensorial evaluation

Parameter		*Control*	Enzyme preparation						
			A1000	*A6003*	*R*	*PR 59*	*C*	*CSG*	*HE400*
Smell	24h	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
	48h	2.6	2.8	2.8	2.6	2.8	2.6	2.8	2.8
	72h	2.4	2.6	2.6	2.4	2.4	2.6	2.4	2.6
Taste	24h	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
	48h	3.0	3.2	3.2	3.0	3.0	3.0	3.0	3.0
	72h	2.6	2.8	2.8	2.6	2.8	2.6	2.6	2.8
Aroma	24h	3.4	3.6	3.6	3.4	3.4	3.4	3.4	3.6
	48h	3.0	3.2	3.2	3.0	3.0	3.2	3.0	3.2
	72h	2.6	2.8	2.8	2.6	2.8	2.6	2.6	2.8
Crust crispiness	24h	2.6	2.6	2.8	2.6	2.6	2.8	2.6	2.8
	48h	2.2	2.2	2.4	2.2	2.2	2.4	2.2	2.4
	72h	1.6	1.6	1.8	1.6	1.6	1.8	1.6	1.8
Crumb resilience	24h	2.8	3.4	3.6	3.2	3.6	3.4	3.4	3.8
	48h	2.0	3.2	3.4	2.8	3.0	3.2	3.0	3.6
	72h	1.2	2.8	3.2	2.2	2.8	3.0	2.8	3.4
Crumb firmness	24h	3.0	3.8	3.8	3.2	3.6	3.6	3.6	3.8
	48h	2.2	3.6	3.8	3.0	3.4	3.4	3.6	3.8
	72h	1.2	3.2	3.4	2.6	3.2	3.2	3.2	3.6

The addition of protease (R) did not modify the evolution of the smell, taste and flavour of the bread nor the crispness of the crust. The elasticity of the crumb is better after 3 days of depositing than that of the witness after 2 days, but not as weak as the sample with α-amylase. The firmness of the crumb is weaker after 3 days of depositing than that of the witness after 2 days, but not as weak as the sample with α-amylase.

If we add protease together with α-amylase (PR 59), the scores given to smell, taste, aroma and crispness are insignificantly modified than those given at the adage of protease alone. As for the elasticity and hardening of the crumb, the scores are practically maintained at the level of those given to the addition of α-amylase alone, but are substantially better than those given to the addition of protease alone.

Xylanase (C) does not seem to modify the evolution of smell, taste and flavour of the bread either. However, the crispness of the crust is slightly prolonged. The elasticity of the crumb is substantially improved, with a slightly higher score than that in the case of adding α-amylase alone and slightly smaller than that in the case of adding α-amylase and xylanase.

The hardening of the crumb is highly prolonged, at the end of the 3 depositing days having a higher score than after 24 hours in the case of the control bread.

Moreover, neither cellulases (CSG) nor the mixture of xylanase with cellulases (HE400) do not influence the smell, taste and flavour of the bread during the 3 days of depositing. The cellulases do not act upon the crispness of the crust, but the mixture of xylanase with cellulases brings it to the level of that obtained by adding xylanase alone. The elasticity of the crumb is weaker after 3 days than that of the crumb with xylanase, and the scores obtained for the hardening of the crumb are practically similar to those in the case of xylanase. The elasticity of the crumb with a mixture of xylanase and cellulases is much improved, so that after 72 hours it is at the level of that with α-amylase after 24 hours, and the hardness of the crumb is weaker after 72 hours, as opposed to that noticed in the case of any other enzyme combination, in the same interval of time.

In order to draw conclusions, we have processed the table above under the form of the following graphs, which present the evolution of the parameters which modify substantially, namely the crispness of the crust, and the firmness and elasticity of the crumb after 24, 48 and 72 hours

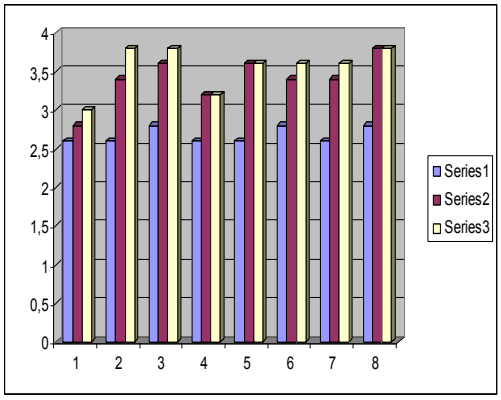

Figura 1. The evolution of parameters after 24 hours

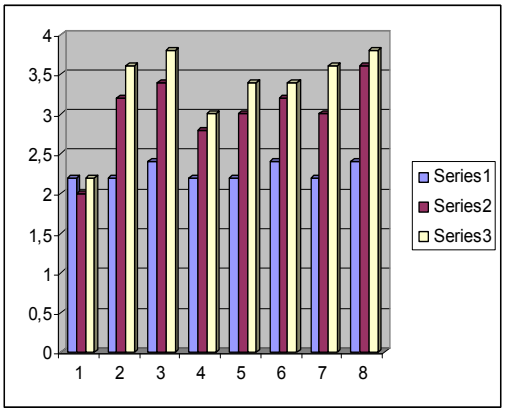

Figura 2. The evolution of parameters after 48 hours

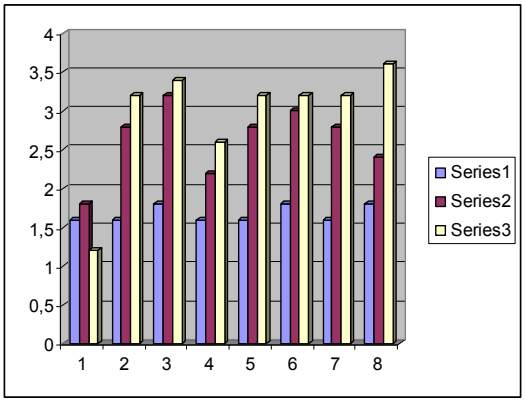

Figura 3. The evolution of parameters after 72 hours

Legend
Series 1 – Crust crispiness
Series 2 – Crumb resilience
Series 3 – Crumb firmness
1 – *Control*, 2 – *A1000,* 3 – *A6003,* 4 – *R,* 5 – *PR 59,* 6 – *C,* 7 – *CSG,* 8 – *HE400.*

4. Conclusion

During the aging process, the bread progressively loses its aroma. The simple or combined enzyme preparations do not cause alterations of the smell, taste and flavour and of their evolution during depositing. These qualitative parameters can be improved by other additions like fats, dairy products, wheat embryos etc. or by applying longer fermentative processes.

Looking at table no. 1 it is clear that, regardless of the addition used, all the qualitative parameters lose scores during depositing. Thus, the crispness of the crust disappears progressively, the elasticity of the crumb deteriorates and the firmness if the crumb increases. On the other hand, all enzyme mixtures used improve the qualitative parameters of the bread as compared to the control.

Looking at the figures 1-3, one can notice that the best effecters are α-amylase, the mixture of α-amylase and xylanase and the mixture of xylanase and cellulases. The effects of xylanase are relatively weak, but, added together with α-amylase or cellulases they improve significantly. The best results appear in the case of adding xylanase and cellulases together.

The protease used alone triggers weak effects, but added together with α-amylase the effects improve almost to the level of those caused by xylonite and celluloses, but do not reach the effects of the α-amylase.

Taking these observations into account, the choice of the proper enzyme mixture will be done firstly according to the type and quality of the flour used: for white flour we will use mixtures with α-amylase or enzyme mixtures with α-amylase; for white flour with strong gluten we will prefer the mixture of protease with α-amylase; for flours with a high content of fibre we will prefer the mixture of xylanase and cellulases.

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Compliance with Ethics Requirements

Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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