

## COMPARISON OF THE EFFECTS OF MICROBIAL $\alpha$ -AMYLASES AND SCALDED FLOUR ON BREAD QUALITY

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**Abstract.** Effect of  $\alpha$ -amylase from fungi and bacteria on extending bread freshness was compared to this of scalded flour. The dough prepared by using Bulk Fermentation Process was supplemented with bacterial and fungal amylases or scalded flour. After baking and cooling loaf volume was measured and bread mechanical properties were estimated using a texture analyser. Texture studies were then repeated after 1, 3, 4 and 7 days. The obtained results show the relation between dosage of enzyme or scalded flour and bread volume as well as the rate of bread staling. Source of enzyme was found important, because bacterial  $\alpha$ -amylase lowered bread quality, while fungal  $\alpha$ -amylase extended shelflife not showing detrimental effects. Replacement of 2-6% of flour with scalded flour improved bread volume and retarded crumb firming. The influence on bread was similar to fungal  $\alpha$ -amylase. The results prove that substitution of fungal  $\alpha$ -amylase by scalded flour is possible. There is a need for such an exchange because in the last years many cases of asthma, induced by occupational exposure to  $\alpha$ -amylase derived from *Aspergillus oryzae*, were reported.

**Key words:** scalded flour,  $\alpha$ -amylase, baker's asthma, staling

### INTRODUCTION

$\alpha$ -amylase is an extracellular hydrolytic enzyme present in plants and produced by several bacteria, yeasts and fungi [Pandey et al. 2000]. It is routinely added to bread dough in order to supply it with dextrans with degree of polymerisation 3-9 which are intermediate products in the conversion of starch to maltose. These low molecular weight dextrans are effective in decreasing bread hardness [Bowles 1996, Defloor and Delcour 1999, Kulp and Ponte 1981, Leon et al. 1981, Martin and Hosney 1991, Min et al. 1998, Ziobro et al. 1998].

The hydrolysis of starch in dough is carried out by the combined action of both  $\alpha$ - and  $\beta$ -amylases.  $\alpha$ -amylases act by an endosplitting mechanism, producing dextrans,

oligosaccharides and maltose.  $\beta$ -amylase is an exospliting enzyme and produces maltose and  $\beta$ -limit dextrins [Robyt 1984]. The profiles of remaining oligosaccharidies depend on the source of the amylases which may be functionally important [Gerrad et al. 1996].

Enzymes that are used in bakeries have different characteristics – mainly thermal range of activity. Bacterial  $\alpha$ -amylases are usually much more heat stable than those found in other microbes and plants, can function after both fungal and cereal  $\alpha$ -amylase are inactivated by heat [Stear 1990, Kulp 1993, Pandey et al. 2000].

Powdered fungal  $\alpha$ -amylase from *Aspergillus oryzae* is added routinely to baking flour or bread improvers to compensate for the low natural contents of amylase and carbohydrates fermentable by yeast. Its supplement stimulates the growth of yeast, improving dough rising and bread quality [Kamel and Stauffer 1993].

On the other hand among bakers, millers and grain workers exposed to dust from powdered fungal  $\alpha$ -amylase the frequency of asthma is elevated and varies from 2 to 40% [Flindt 1979, Baur et al. 1986, 1988]. There are also some reports bakers asthma induced by  $\alpha$ -amylase can be accompanied with problems upon bread ingestion [Kanny and Moneret-Vautrin 1995] although it seems, that allergenic activity of amylase is mostly destroyed under baking conditions so the obtained bread should not be harmful [Carmona 2000, Sander et al. 2000]. Nevertheless it seems rational to avoid excessive employment of enzymatic preparations and replace them with safer additives, such as processed cereals.

Scalded flour – prepared by treating flour or meal with hot water and cooling the mixture – may also be used to improve bread quality [Jankowski 1969]. Formerly, scalding was often used in Sweden to enable bread to be made from sprouted rye [Ask et al. 1990]. The residual amylase activity depends mainly on the mixing temperature. The optimum temperatures required for  $\alpha$ - and  $\beta$ -amylase activity in rye are 54-63°C and 48-51°C respectively [Robyt 1984].

Through the proper selection of parameters (time and temperature) of flour scalding or addition of sufficient quantity of  $\alpha$ -amylase to flour, it is possible to obtain dough that contains large amount of low molecular weight dextrins which are able to retard bread firming [Ambroziak 1992].

In the following study trial was made to substitute  $\alpha$ -amylase with scalded flour in order to minimise the risk of allergy. To this end the effects of bacterial and fungal  $\alpha$ -amylase and scalded flour on bread quality and shelflife were compared and the optimal levels of these components in order to achieve a maximum volume and bread freshness were established.

## MATERIAL AND METHODS

Bacterial  $\alpha$ -amylase A6814 (*Bacillus* species) and fungal  $\alpha$ -amylase A0273 (*Aspergillus oryzae*) were obtained from Sigma. Activity unit (U) was the enzyme dosis liberating the equivalent of 1 mg of maltose ( $2.9 \times 10^{-3}$  mmol) from starch in 3 min at pH 6.9 and 20°C. Yeast, salt and fat were obtained from local suppliers. Commercial wheat flour (type 2000 and wholemeal) was used.

Water absorption of the flour was determined by means of a Brabender farninograph (Brabender, Duisburg, Germany). Quantity of water used in breadmaking recipe was adjusted basing on dough performance in preliminary trials.

### Scalding procedure

Flour (or wholemeal) was scalded by mixing it with hot water (75°C or 100°C) in a stainless steel pot with a spoon, at a ratio 1:2 (w/w) (flour/water) to obtain homogeneous suspension. The temperature after mixing was about 60°C. When mixing process was completed, the covered pot with the mixture was left overnight (18 h) in the proving cabinet at 40°C.

### Breadmaking process

Control formulation contained 2.5 g of dry yeast, 2 g of salt, 58.5 g of water and 1.5 g of fat per 100 g of flour. The dough was prepared in spiral mixer. The dough temperature after mixing was 27°C, the mixing time was 9 min 40 s. During the bulk fermentation at room temperature, which lasted 1 hour, the dough was remixed over 1 min after 1 h. Dough after bulk fermentation was divided by hand to 250 g pieces which were moulded into a cylinder shape and put into grassed pan as single pieces. The dough pieces were proved at 40°C and 80% relative humidity in proving cabinet. Then bread was baked for 30 min without steam. The oven was set up at 225°C. The loaves were cooled for 30 min and bread volume was measured by rapeseed displacement. Each value is an average for 6 loaves.

Anti-staling agents were used in the following dosage:

- fungal  $\alpha$ -amylase 2, 4, 8 U per gram of flour,
- bacterial  $\alpha$ -amylase 0.25, 0.5, 1, 2, 4 U per gram of flour,
- scalded flour 0, 2, 4, 6, 8, 10 g of flour in the recipe was replaced with scalded flour (added as flour/water mixture).

For texture analysis TA-XT2 with XT R1 software package was used. The slice 25 mm thick was cut from the centre of the loaf. The slice was compressed to 15 mm at a plunger speed of 1 mm·s<sup>-1</sup>. Plunger diameter was 24.9 mm and height 30.55 mm. Each measurement was done in duplicate.

$\alpha$ -amylase activity in wheat flour (type 2000) was measured by the Neolting-Bernfeld method and expressed as the mg of anhydrous maltose produced by 1 g of flour per minute at 30°C.

Statistical treatments were performed using Gnumeric spreadsheet. Least significant difference was calculated at  $\alpha = 0.05$ .

## RESULTS

### Influence of the enzymes on dough and bread quality

It was noticeable that during the mixing process the dough became more adhesive to the mixer bowl or chamber when enzyme was added, especially if bacterial  $\alpha$ -amylase was used. Bacterial  $\alpha$ -amylase has considerable influence on the bread quality, because it produces a substantial quantity of relatively long dextrans (larger in size than those developed by fungal amylase) which are known to cause bread stickiness [Martinez-Amaya and Jimenez 1997]. Thick layer of the bread crumb stayed on the knife when bread containing bacterial  $\alpha$ -amylase was being cut. This indicates that starch had been

excessively hydrolysed to medium size dextrans that broke continuity of the starch matrix. Their presence caused deeper colour of crust. The rate of bread firming was lower when bacterial enzyme was used even at the lowest applied level (Fig. 1). Higher amounts of the enzyme resulted in excessive stickiness of the crumb, so texture measurements were unreliable. Initial hardness of bread with bacterial amylase was considerably lower than of control bread and after the first day it was almost constant.

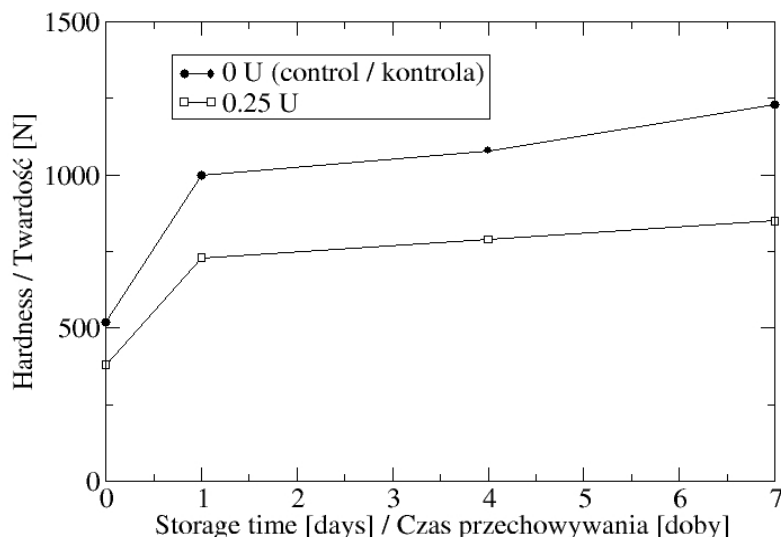


Fig. 1. Influence of bacterial  $\alpha$ -amylase on bread hardness  
Rys. 1. Wpływ  $\alpha$ -amylazy bakteryjnej na twardość chleba

The temperature range of activity of bacterial  $\alpha$ -amylase is 65-92°C and it can hydrolyse starch even after the endogenous amylase is inactivated [Achremowicz and Wójcik 2000]. Some bacterial  $\alpha$ -amylases can survive baking process and hydrolyse starch during bread storage [Hebeda et al. 1990], though in the present study no evidence of their activity was found after baking.

Although bacterial  $\alpha$ -amylase had beneficial effects on bread staling, organoleptic assessment revealed that bread baked from doughs with this level of enzymes is unacceptable as a commercial product. All quality parameters of bread were lowered due to the excessive hydrolysis of starch. When 0.25 unit dosage was used staling rate was higher than at previously discussed dosage, but bread was still too sticky and open crumb structure was created.

Springiness of bread containing only 0.5U bacterial  $\alpha$ -amylase was 69% while for control bread it was 94%. The adhesiveness after baking for 0.5U was 20 N×s whereas for control no adhesiveness was found. These two values suggest that an excessive quantity of dextrans produced by bacterial amylase caused stickiness of the bread crumb.

Influence of fungal  $\alpha$ -amylase on dough and bread properties differed from that of bacterial  $\alpha$ -amylase. During mixing process the dough adhered to the surface of mixer bowl at lower rate than the dough supplemented in bacterial  $\alpha$ -amylase. It was the most

noticeable when 4 and 8U of enzyme was used. The influence on bread crumb was also different than in case of bacterial enzyme. Crumb structure was only slightly more open than in the control bread.

As it is shown on Figure 2, fungal  $\alpha$ -amylase had a positive impact on bread volume. ANOVA proved that the differences in this parameter between loaves with various levels of the enzyme are statistically important at the significance level 0.1. The increase in volume depended on the dosage of enzyme. The change in volume after rising of the enzyme supplement from 2 to 4U was more pronounced than between 4 and 8U.

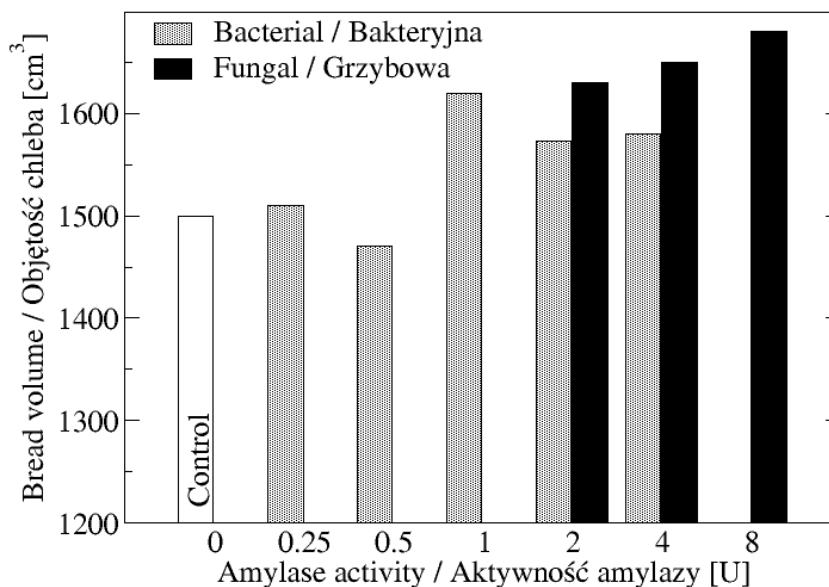


Fig. 2. Effect of bacterial and fungal  $\alpha$ -amylase on bread volume ( $LSD_{0.05} = 28.7$ )  
Rys. 2. Wpływ  $\alpha$ -amylazy bakteryjnej i grzybowej na objętość chleba ( $NIR_{0.05} = 28,7$ )

Addition of fungal  $\alpha$ -amylase preparation significantly affected bread staling. Hardness of loaves supplemented with 2U and 4U after 4 days was the same as of control bread after only 24 h (Fig. 3). The similar tendency was also found for other texture parameters (data not shown).

#### Influence of scalded flour on bread

There is a wide variety of flours that could be used in scalding procedures. They could be made from various wheat cultivars and contain different  $\alpha$ -amylase isoenzyme which have different properties like range of their activity and isoelectric point [Kruger et al. 1995]. Their balance in the particular flour could vary and products of their enzymatic activity could also differ.

In the present study scalded wholemeal was used, because it exhibited higher amylolytic activity (low falling number, Table 1) than white flours, where the outer parts of the grain, which are rich in enzymes, are removed during milling process.

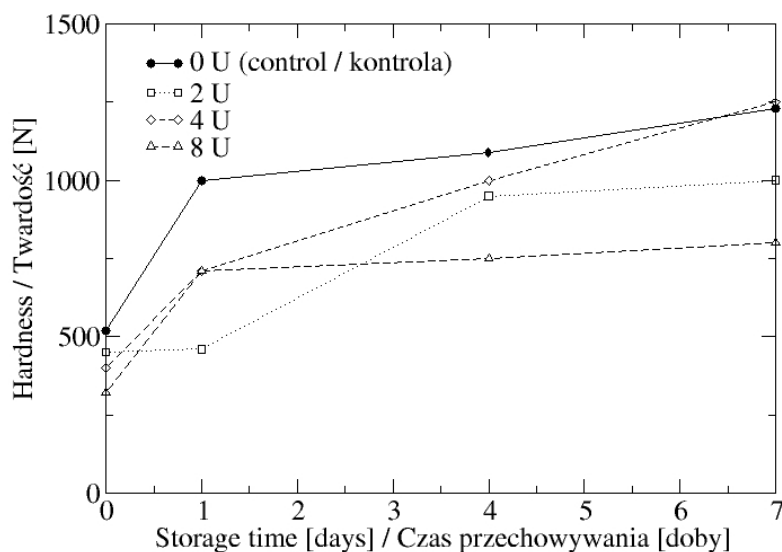


Fig. 3. Influence of fungal  $\alpha$ -amylase on bread hardness  
Rys. 3. Wpływ  $\alpha$ -amylazy grzybowej na twardość chleba

Table 1. Characteristics of flour used for scalding  
Tabela 1. Charakterystyka mąk zaparzanych

Flour type Typ mąki	Protein, % Białko, %	Moisture, % Wilgotność, %	Falling number, s Liczba opadania, s	Water absorption, % Wodochłonność, %
2000	11.8	12.6	346	60.8
Wholemeal	13.4	12.5	200	59.2

It was found that scalded flour generally improved bread quality. Small addition (2%) of scalded wholemeal significantly influenced bread staling. The hardness after 3 days was similar to that of the control bread after 24 h (Fig. 4). On the other hand the excess of scalded flour (10%) lead to the increase of staling rate, which is probably due to reduction in the amount of gluten (denaturation upon scaling).

The influence of scalded flour on bread volume was not so pronounced as in case of fungal  $\alpha$ -amylase but it was still statistically significant. Addition of 5% of scalded flour positively influenced bread volume, however 10% of scalded wholemeal caused slightly lower bread volume (Fig. 5). This fact can be attributed to the excess of minerals, pentosans and other constituents of the outer layers of the wheat kernel that impede development of gluten.

The same values of hardness were measured for loaves supplemented with scalded flour 24-72 h later than for control bread, which signifies that the content of low molecular dextrans was significantly increased. They were formed not only due to the higher susceptibility of partly gelatinised starch but also high amyolytic activity of scalded flour. Such activity has been shown to persist after thermal treatment even at higher temperatures (Table 2).

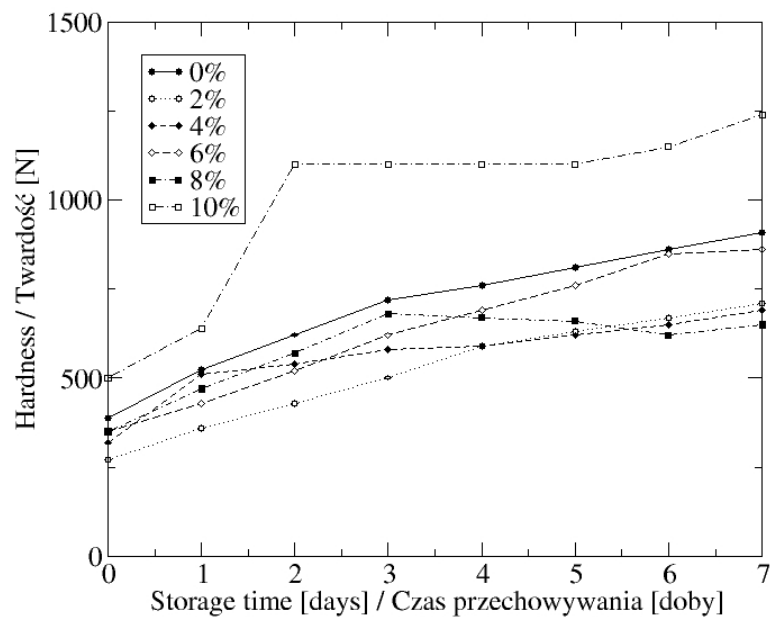


Fig. 4. Influence of scalded WM flour on bread hardness  
Rys. 4. Wpływ zaparzonej śruty na twardość chleba

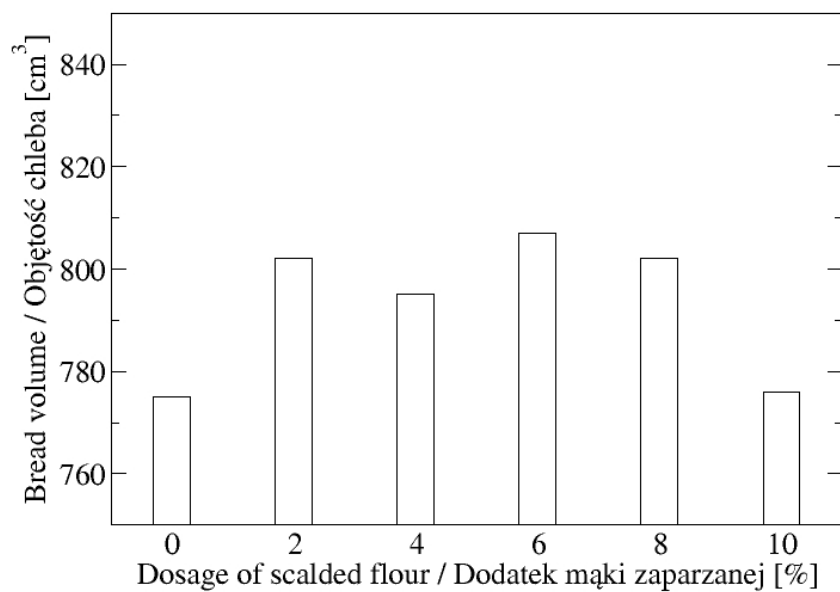


Fig. 5. Influence of scalded WM flour on bread volume (LSD<sub>0,05</sub> = 32.3)  
Rys. 5. Wpływ zaparzonej śruty na objętość chleba (NIR<sub>0,05</sub> = 32,2)

Table 2. Activity of  $\alpha$ -amylase in scalded flour  
Tabela 2. Aktywność  $\alpha$ -amylazy w mące zaparzonej

Flour sample Próbka mąki	Amylase activity (U) per gram of flour Aktywność amylazy na gram mąki
Untreated – Bez zaparzania	13.3
Scalded at 75°C – Zaparzana w 75°C	13.4
Scalded at 100°C – Zaparzana w 100°C	5.4

## DISCUSSION

As it was already shown by many authors the products of starch depolymerisation produced by  $\alpha$ -amylase have antifirming properties [Leon et al. 1981], caused by the interactions of low molecular weight dextrans with starch and gluten [Martin and Hoseney 1991]. These findings were confirmed in the present study. Staling of bread prepared from dough supplemented with  $\alpha$ -amylase occurred at a slower rate than without supplementation.

The improved bread volume after addition of enzymatic preparations was caused by the increased amount of fermentable sugars. The content of mono- and oligosaccharides in wheat flour is quite small, generally not exceeding 0.5%. This amount is not sufficient to sustain the fermentation requirements to give good loaf volume, consequently bread quality depends on the generation of maltose by the  $\alpha$ - and  $\beta$ -amylase of the flour. Usually wheat flour contains a sufficient amount of  $\beta$ -amylase [Jankowski 1969, Ambroziak 1992]. However, only  $\alpha$ -amylases are able to hydrolyse native undamaged starch although the degradation rate is low [Jankowski 1969]. During the dough making process, the damaged starch granules are quickly attacked by both  $\alpha$ - and  $\beta$ -amylase. The number of such granules depends on the milling intensity and increases rapidly with increasing roll pressure, typical flour contains 5-9% damaged starch.

The scalding procedure was carried out in a manner that provides a maximum activity of  $\alpha$ -amylase and minimum activity of  $\beta$ -amylase. The temperature of water used to scald flour was 70°C and the temperature of mixture was 60°C after mixing process was completed. This temperature range permits retention of activity of  $\alpha$ -amylase between 70-95% and 20-70% activity of  $\beta$ -amylase for 70°C and 60°C respectively [Robyt 1984]. The presented data show that such conditions are suitable for the preparation of natural anti-staling agent. Scalded flour seems to be a good source of low molecular dextrans (comparable to microbial  $\alpha$ -amylases) that can be helpful in retarding bread firming.

Scalding causes denaturation of gluten proteins, so when too much scalded flour is added to dough there occurs an apparent decrease in gluten quantity. In consequence, the dough has lower volume and can not retain a sufficient quantity of CO<sub>2</sub>. However, supplementation with 6% of scalded flour did not significantly diminish gluten quantity and provided a substantial amount of gelatinised starch, that was utilised to form fermentable saccharides. Bread volume was improved which signifies that the risen amount of CO<sub>2</sub> still could be held within matrix.



## CONCLUSIONS

Taking into account the similar effects of microbial amylases and scalded flour it could be concluded that the use of powdered enzymes can be to some extent replaced by utilisation of scalding procedure. As reported by several authors, inhalative uptake of fungal  $\alpha$ -amylase is associated with a risk of developing allergic respiratory diseases [Flindt 1979, Baur et al. 1986, Baur et al. 1988, Lazaro 2000]. Awareness of this risk is especially important for the baking industry, where extensive use of various powdered enzymes, predominantly from the genus *Aspergillus*, is common. The successive trial to substitute addition of  $\alpha$ -amylase by applying scalded flour should encourage bakers to employ it as a routine procedure.

## REFERENCES

- Achremowicz B., Wójcik W., 2000. Enzymy amylolytyczne i inne hydrolazy O-glikozydowe. [Amylolytic enzymes and other O-glycosidic hydrolases]. Wyd. AR Lublin [in Polish].
- Ambroziak Z., 1992. Technologia piekarstwa [Breadmaking technology]. WSiP Warszawa [in Polish].
- Ask L., Nair B., Asp N.-G., 1990. Effect of scalding procedure on the retrogradation of starch in rye products. *Cereal Chem.* 13, 15-26.
- Baur X., Fruchmann G., Hang B., Rusche B., Reicher W., Weiss W., 1986. Role of aspergillus amylase in baker's asthma. *Lancet*, 42-43.
- Baur X., Sauer W., Weiss W., 1988. Baking additives as new allergens in baker's asthma. *Respiration* 54, 70-72.
- Bowles L., 1996. Amylolytic enzymes. baked good freshness: Technology, evaluation, inhibition of staling. Marcel Dekker New York, 105-129.
- Carmona J.B., 2000. Immunochemical characteristics of alpha-amylase sensitization. *Immunol. Clin.* 15, 42-45.
- Defloor I., Delcour J., 1999. Impact of maltodextrins and antistaling enzymes on the differential scanning calorimetry staling endotherm of baked bread doughs. *J. Agric. Food Chem.* 47, 731-741.
- Flindt M., 1979. Allergy to alpha-amylase and papain. *Lancet*, 1407-1408.
- Gerrad J., Every D., Sutton K., And M., 1996. The role of maltodextrins in the staling of bread. *J. Cereal Sci.* 26, 201-209.
- Hebeda R., Bowles L., Teague W., 1990. Developments in enzymes for retarding staling of baked good. *Cereal Foods World* 35, 453-457.
- Jankowski S., 1969. Zarys technologii zbóż i strączkowych jadalnych. Cz. III. Technologia piekarstwa, makaronu, preparatów zbożowych i pasz [Outline of the technology of cereals and edible legumes. Part III Technology of baking, pasta, cereal preparations and animal feed]. PWN Warszawa [in Polish].
- Kamel B., Stauffer C., 1993. Advances in Baking Technology. Blackie Academic and Professional Glasgow.
- Kanny G., Moneret-Vautrin D.-A., 1995. Alpha-amylase contained in bread can induce food allergy. *J. Allergy Clin. Immunol.* 95, 132-133.
- Kruger J., MacGregor A., Marchylo B., 1995. Endogenous Cereal Enzymes in Food Enzymology. Elsevier Appl. Sci. London.
- Kulp K., 1993. Enzyme as dough improvers. Blackie Academic and Professional Glasgow, 153-178.
- Kulp K., Ponte J., 1981. Staling of white pan bread: Fundamental causes. *Crit. Rev. Food Sci. Nutr.* 15, 1-48.

- Lazaro J.F., 2000. Occupational respiratory allergy to alpha-amylase in bakers. *Alerg. Immunol. Clin.* 15, 37-42.
- Leon A., Duran E., de Barber B., 1981. Firming of starch gels and amylopectin retrogradation as related to dextrin production by alpha-amylase. *Z. Lebensm. Unters. Forsch.* 205, 131-134.
- Martin M., Hosenev R., 1991. A mechanism of bread firming ii. role of starch hydrolysis enzyme. *Cereal Chem.* 68, 503-507.
- Martinez-Amaya M., Jimenez T., 1997. Functionality of enzymes that hydrolyse starch and non-starch polysaccharide in breadmaking. *Z. Lebensm. Unters. Forsch.* 205, 209-214.
- Min B., S.H., Y., Lee Y., Kim Y., Park K., 1998. Functionality of enzymes that hydrolyse starch and non-starch polysaccharide in breadmaking. *J. Agric. Food Chem.* 46, 779-782.
- Pandey A., Nigam P., Soccol C., Soccol V., Singh D., Mohan R., 2000. Advances in microbial amylases. *Biotechnol. Appl. Biochem.* 31, 135-152.
- Robytt J., 1984. Enzymes in hydrolysis and synthesis of starch. *starch chemistry and technology.* 135-152.
- Sander I., Raulf-Heimsoth M., Kampen V.V., Baur X., 2000. Is fungal alpha-amylase in bread and allergen? *Clin. Exp. Allergy* 30, 560-566.
- Stear C., 1990. Effect of dough additives. *Handbook of bread making technology.* Elsevier Sci. Publ. London.
- Ziobro R., Gambuś H., Nowotna A., Bala-Piasek A., Sabat R., 1998. Starch extrudates as a source of low molecular dextrans slowing down bread staling. *Żywn. Technol. Jakość* 17, 251-258.

## PORÓWNANIE WPLYWU $\alpha$ -AMYLAZ MIKROBIOLOGICZNYCH I MĄKI ZAPARZANEJ NA JAKOŚĆ CHLEBA

**Streszczenie.** W pracy porównano wpływ  $\alpha$ -amylazy bakteryjnej i grzybowej oraz dodatku mąki zaparzonej na przedłużenie świeżości pieczywa. Ciasto z udziałem amylazy lub mąki zaparzonej przygotowano metodą BFP. Po wypieku i schłodzeniu zmierzono objętość chleba i jego właściwości mechaniczne, używając analizatora tekstury. Badania tekstury powtarzano po 1, 3, 5 i 7 dniach. Uzyskane wyniki wskazują, że istnieje związek pomiędzy dawką stosowanego enzymu lub mąki zaparzonej a objętością pieczywa oraz szybkością jego twardnienia. Źródło enzymu jest istotne, ponieważ  $\alpha$ -amylaza bakteryjna obniża jakość chleba, podczas gdy enzym grzybowy przedłuża trwałość pieczywa, nie powodując niekorzystnych skutków. Zamiana 2-6% mąki na mąkę zaparzoną wpływa na wzrost objętości pieczywa i opóźnia jego twardnienie. Mąka zaparzana wpływa na jakość chleba podobnie jak  $\alpha$ -amylaza grzybowa. Uzyskane wyniki dowodzą, że jest możliwe zastąpienie  $\alpha$ -amylazy grzybowej dodatkiem mąki zaparzonej. Taka zamiana jest celowa ponieważ  $\alpha$ -amylaza pochodząca z *Aspergillus oryzae* może wywoływać astmę u osób zatrudnionych w piekarniach.

**Key words:** mąka zaparzana,  $\alpha$ -amylaza, astma piekarzy, starzenie się pieczywa

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