ASPECTS OF USING PROTEOLYTIC ENZYMES IN THE BAKERY INDUSTRY

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Abstract: The papers presents theoretical and aspects concerning the usage of proteolityc enzymes in bakery industry. It shows the technological role of proteolityc enzymes in the baking industry, enzymes sources and their actions on some bakery products' quality.
Generally, enzymes, particularly combinations, offer more freedom for optimizing the process and end product. Proteolityc enzymes soften the dough, improve flavour and colour, and help reduce costs.

Keywords: proteolytic activity, bakery products, technological role

1. INTRODUCTION

The proteolytic activity of healthy ungerminated wheat is, normally, low. Proteolytic enzymes are unevenly distributed in the wheat grain, in spite of this wheat flour has both proteasic activity and a peptidasic one. The proteasic activity is concentrated in the pericarp, testa husk, aleurone layer, germs and is presents in small quantities in the endosperm, while the peptidazic activity is concentrated in endosperm and lower in the rest of the grain. The uneven distribution of enzymes in the grain makes the flours of different extractions have different proteolityc activity. The richest are flours of small extractions proceeded from the endosperm, thus the peptidazic activity of a black flour is three times larger than that of white flour obtained from the same wheat [1].

Many proteases of cereals are papain like which means they become active (like α-amylase) in the presence of compounds which reduce disulfuric bounds. Proteases native from flour have a small importance in the quality of bread. Although it is known the fact that proteolytic enzymes, which are capable of hydrolyzing glutenic proteins, can have a drastic effect over the bakery qualities. The controlled addition of proteolitycl enzymes can be used, also with obvious advantages. The dough's viscosity and the optimum time for kneading decrease, having as a result the improvement of dough's viscoelastic properties. Both the dough's processability and the bread's core have improved. The influence of proteolytic enzymes native in cooked rye bread is even less obvious, because of the fact that, in this case, the proteins play a smaller role on account of the high pentosani content of rye flour [2,3].

The addition of proteolytic enzymes is more and more used at obtaining sweet biscuits and crackers in order to control the dough's viscoelastic properties. The proteases have been used, on a large scale, in order to replace bisulfite, which was used beforehand to control the dough's consistency by reducing disulphuric bounds, while the proteolytic activity degrades peptidic bounds. In both cases though, the gluten's structure is weakened in the same manner. Proteases hydrolyze the peptidic bound between the amino group of an amino acid and the carboxyl group of the next amino acid from the protein. In the dough, this leads to the establishment of the gluten's network. This can influence the dough in two ways, depending on the moment in which the proteases is added. If the protease is added at the beginning of the process – for example in the starter faze – it reduces the kneading time needed for making dough. Adding the proteases at the beginning of the process though, will produce a weakening of the gluten structure, having as a result an irregular core structure [3].

In spite of these, the proteases should be added to the dough later on, in the kneading faze. In these conditions, the kneading time won't decrease, because the enzyme doesn't have enough time to hydrolyze more gluten. If hydrolysis takes place during moulding and fermentation, the proteases will improve the dough's processability.

Another usage of proteases is in replacing sodium metabisulfites in crackers' dough. Crackers' dough contain water and fats in small quantities, which make then dense and thus, difficult to mould. Metabisulfites hydrolyze disulphuric bounds from the gluten's molecule, decreasing its resistance to expansion and having as a result a more plastic dough.

2. PROTEASES SOURCES

For a long time now, malt has been added to wheat flour as a source of exogenous proteases. Fungal proteases are obtained by growing a mold, generally *Aspergillus oryzae*, in a sterile fluid inoculated with spores. The enzymes are obtained in the stable form by precipitation with alcohol and low temperature drying. Proteases of bacterial origin can be isolated from *Streptomyces* and purified with for example ammonium sulfate.

Papain is a vegetal protease obtained from Carica papaya which is limitedly used because it produces an advanced hydrolysis over gluten. The vegetable proteases ficin, bromelain, and papain are found in the latex of trees of the genus Ficus, in fresh pineapple juice, and in papayas, respectively. The proteases are used less in the baking industries than enzymes of fungal and bacterial origin. Knowledge of their action on gluten allows the technologist to make discerning choice, within the limits imposed by national legislation, with bases on the flour used and the obtainment of the desired product [4].

Some microbial proteases were studied, by using them in modifying flour's proteins. These studies are shown in the following table, which being by far complete, shows the wide aspect of the proteases studied, as process helpers (for example Woods and others, 1980) and studies in which purified enzymes were used to modify isolated proteins from gluten (table 1).

Table 1

Proteases sources

Sources	Proteases type/microorganism sp.	References
Plants	Papain, ficin, bromelain	Shimada, *et al.* (1982), Kruger, *et al.* (1987), Yoshinaka and Ikeda (1989)
Fungal origin	*Aspergillus oryzae* *Aspergillus sp.*	Gabor, *at.al.* (1982) Kruger, *et al.* (1987)
Bacterial origin	*Bacillus subtilis* *Bacillus sp.* *Streptomyces sp.*	Gabor, *at.al.* (1982) Wootton, *et al.* (1982) Kruger, *et al.* (1987)
Yeast	*Saccharomyces carlsbergensis*	Woods (1980)
Animal origin	Pepsin, chimotripsin, tripsin	Ram and Nigam (1986), Mason, *et al.* (1986) Danno and Natake (1980)

Different types of proteases have different catalytic mechanisms. Firstly, different mechanism actions indicate the way in which the enzyme acts in different pH conditions. The proteases used are of many types:

- **acid proteases –** can be found in flour and have a low optimum action pH; they are used for gluten steeping during long fermentations, at a low crackers' dough's pH;

- **sulfhidril proteases -** are found in many cereal based ingredients, like flour and malt; also, they are extracted from pineapple strings (bromelina) and papaya (papain); sulfhidril proteases have an optimum pH domain between 3.5 – 8.5;
- **serin proteases –** are often named alkaline proteases, because their activity is optimum at a pH of 7.5;
- **neutral proteases-** are the most often found in commerce; the optimum pH is around 7;

2. THE EFFECTS OF PROTEASES IN BAKERY

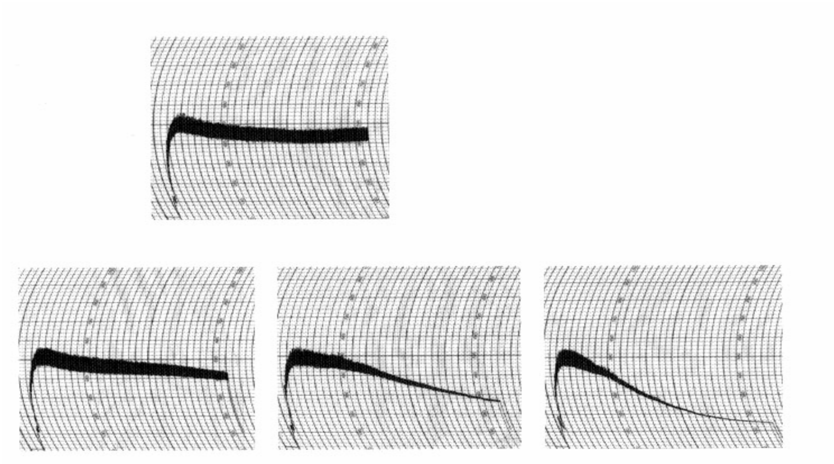
The proteases added in the dough improve its processability properties as the gluten's elasticity and texture, thus helping at the substantial increase in volume. The standards allow usage of fungic proteases, bromelin and papain.

Fungic proteases are much easier to use, by using two types, one with negligible amilazyc activity and another with moderate amilazyc activity. It is known that proteases native in the flour donot participate in the manufacturing of bread, and that's why proteolytic supplements must be made from other sources. Newer methods of bread manufacturing need a strict control of kneading tim, in this faze the proteolytic enzymes having a substantial influence,

The technological effect of adding proteolytic enzymes depends on: the enzyme source, the quantity of enzymes, the quality of flour and the salt content from the bread manufacturing recipe. Adding proteases in the dough becomes necessary in the case of strong flours which form gluten characterized by high resistance and elasticity and small extensibility, poor in enzymes or processed with short kneading times. When an appropriate protease level is used the dough's processability properties improve. The high quantity of enzymes will give too weak doughs, soft and extensible, unwillingly sticky.

Trial runs with the farinograph show in the presence of proteases a fast decrease in consistency during overmixing (high steeping, low stability) (fig.1.). Moreover, the alveograph shows that for suitable enzyme concentrations, plastic properties are improved, which facilitates dough handling.

A



B C D

Fig.1. **Farinograph of flours with proteolytic enzymes: A - reference; B – flour for liquid wafer batters; C – flour for biscuit and cracker battters; D – flour for fast breakdown of gluten [5].**

The effect of addition proteolytic bacterial enzymes is presented in table 2. In table 3 are presented results about papain addition influence upon dough rheological characteristics.

3. USING PROTEOLYTIC ENZYMES AT MANUFACTURING BISCUITS

At manufacturing biscuits, the addition of proteolytic enzymes determines a series of important advantages:

- improving taste by eliminating the taste determined by using other reducing substances;
- improving soften properties makes the dough piece be more easily and more uniformly stretched;
- improving flavour and colour because of Maillard reaction intensification [5].

Table 2

Influence of proteolityc enzymes addition upon the quality flour

Sample	Gluten content [%]	Analysis results in alveograph						Analysis results in farinograph					
		W	P	P_{200}	L	Ie	P/L	Hydrat. Capacity [%]	Development [min.]	Stability [min]	Elasticity [U.B.]	Steeping [U.B.]	P
Flour 650 – control sample	28,8	111,8	60,8	20	58,7	0,33	1,03	52,7	1,50	7,00	160	70	52
Control sample +0,01% protease	28,8	102,0	62,4	18,7	55,1	0,30	1,13	53,0	1,25	7,50	140	60	56

Table 3

Influence of papain addition upon the quality flour

Sample	Gluten content [%]	Analysis results in alveograph						Analysis results in farinograph					
		W	P	P_{200}	L	Ie	P/L	Hydrat. Capacity [%]	Development [min.]	Stability [min]	Elasticity [U.B.]	Steeping [U.B.]	P
Flour 650 – control sample	23,6	275,0	93,5	53,9	60,8	0,57	1,53	53,0	1,10	9,80	150	50	63
Control sample +0,001% papain	23,5	146,5	64,0	27,5	68,0	0,43	0,90	52,6	1,25	6,00	160	80	58
Control sample +0,005% papain	23,4	66,7	46,5	12,1	51,3	0,26	0,90	-	-	-	-	-	-

4. USING PROTEOLYTIC ENZYMES AT MANUFACTURING PASTES

Using proteolytic enzymes in a suitable quantity at manufacturing pastes leads to an improvement of rheological properties of the dough, regular shapes being obtained at pressing. Another effect of enzyme usage is facilitating the humidity transfer in the product which leads in the end to a decrease of drying durations (the acceleration of drying).

5. CONCLUSIONS

Using proteolytic enzymes must be made with caution because it is difficult to control their action in order to obtain a favourable result. Protease improve the texture and organoleptice properties of bakery products but a high dosage is detrimental to volume as well as other product characteristics.

The effect of adding proteolytic enzymes is:

- decreasing the kneading duration;
- dough steeping
- flavour and colour improvement
- cost reduction

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