EFFECTS OF A2 PHOSPHOLIPASE ON DOUGH RHEOLOGICAL PROPERTIES AND BREAD CHARACTERISTICS

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Abstract: *Flour lipids, though representing 2% of flour mass, play an important technological role because they interact with proteins and starch in dough, influencing the rheological properties of dough, bread quality and its freshness.*
The properties of lipids and particularly of phospholipids are given by the structural and functional particularities of molecules. It is considered that phospholipids are amphipathic substances because there is no polarity in the core of molecule, and because the extremities have opposite poles.
The paper presents some experimental results obtained regarding the effects of exogenous phospholipase (A2) used in different quantities on the rheological properties of dough and bread quality. The rheological property of flours were determined on Mixolab, as well as by using a Chopin Alveograph and the effect upon the bread quality was determined by baking tests. The results obtained on mixolab indicate an increase of dough stability, a clear reduction of C1 value and a greater difference of the points C5-C4 with the addition of A2 phospholipase. From the alveographic point of view an increase of dough strength was noted along with higher phospholipase A2 content. From the technological point of view, the best results have been obtained for a dose of 3000 U/100 kg flour added to flour.

Keywords: *phospholipase, Mixolab, Alveograph, baking test*

Introduction

It has been demonstrated that lipid fractions may be involved in the glutenice complex either through hydrophilic bonds or hydrophobic interactions. As far as starch-lipid interactions are concerned, the lipid fractions appear as inclusions in the matrix of amylase polyglucans or are chemically tied to carbohydrates. This is why it is considered that, the hydrophilic-lipophilic balance (HLB) and the content of fat acids are important criteria for establishing the probability of appearance of interactions such as lipids-proteins, lipids-starch while preparing flours in the process of bread making [1].
A phosholipase is an enzyme that converts phospholipids into fatty acids and other lipophilic substances [2]. It is known that phospholipids contain in their molecules glycerol, fatty acids, phosphoric acid and preferential nitrogenous bases, reason for which they are amphophilic properties [3]. Depending on the type of polyalcohol in their structure - glycerol, inositol or sphingosine, phospholipids have been classified as follows: glycerophospholipids, inosiphospholipids and sphingphospholipids. As fatty acids, phospholipids contain in their molecules both saturated and unsaturated fatty acids: palmitic, stearic, lignoceric, nervonic, linoleic, arachidonic etc. Usually, in the phospholipid molecule we can only find a single rest of phosphoric acid, scarcely two. Colin, ethanolamine and serine are predominant as nitrogenous bases in phospholipids [4].
The extremity that contains the rest of phosphoric acid and the nitrogenous base forms the hydrophilic part of the molecule

due to the existent polar groups, whereas the acyl groups resulted from the fatty acids form the hydrophobic part.
Due to the bipolar molecular structure - Table 1, the phospholipids participate equally in hydrophobic interactions and in forming hydrogen bonds [5]

Table 1.
Fosfolipids polarity, on HLB base [5]

	Phospholipids	HLB
Non-polar lipids	Phosphatidyl choline	6
	Phosphatidyl ethanolamine	7
	Lysophosphatidyl choline	7
	Lysophosphatidyl ethanolamine	8
Polar lipids	Phosphatidyl inozitol	11
	Phosphatidyl seryne	12

Depending on the phospholipases' action on phospholipids (see figure 2), these enzymes are divided into four groups as follows:
- phospholipases A1 (PLA1) which specifically eliminate fatty acid from the terminal α position of phospholipids;
- phospholipases A2 (PLA2) which specifically eliminate fatty acid from the central β position of phospholipids thus forming the lysophospholipids;
- phospholipases B (PLB) which eliminate two fatty acids from the phospholipids' molecule in positions α and β corresponding to phospholipase A1 and A2; it is actually thought that phospholipase B is a mixture of phospholipases A1 and A2;
- phospholipases C (PLC) also named lecithinases C or glycerophosphatase, hydrolyze the esteric bond between glycerol and phosphoric acid from phospholipids;
- phospholipases D (PLD) also named lecithinases D, hydrolyze the esteric bond between the phosphoric acid and the nitrogenous base (coline, ethanol-amine etc.) from phospholipids;

Depending on the nitrogenous base from phospholipids, phospholipases D are also named colinophosphatases.
Phospholipase A2 facilitates conversion of lecithines into lysolecithines (the main reaction components) through unbinding the unsaturated fatty acid in position C-2 of lecithines and the growth of content of free fatty acids, mainly of linoleic acid [6].

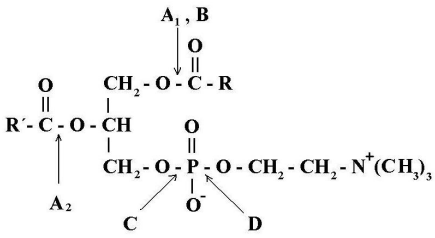

Figure 1. Phospholipases acts on phospholipids [6]

The lysolecithines' molecules are more polar (higher HLB) than the molecules of initial lecithines, having a better ability to interact with certain flour components in dough while preparing semi-manufactured goods. It also appears that lysolecithin is an emulsifier with great capacity of acting on the rheological properties of dough with effects in terms of increasing loaf volume [7]. Besides forming lysolecithine, phospholipases (A_1, A_2, and B) lead to an increase of content of free fatty acids, mainly linoleic acid. Unsaturated fatty acids formed this way can become substratum for enzymatic oxidation,

catalyzed by lipoxygenase or for the chemical oxidation, with positive effects on flour growth.

Materials and methods

The study was carried out on flours for bread making of good quality. Commercial white flour 650 type, obtained from the last (2007) crops' milling was used – abbreviated M. As flour improvers Lysomax in different doses (doses of 1000 U/100 kg – P1 abbreviated, 2000U/100kg – P2 abbreviated, 3000U/kg- P3 abbreviated and 4000 U/kg – P4 abbreviated flour added to the flour) was used.

Lysomax is a commercial product supplied by Enzymes & Derivates Romania Ltd. which comprised true phospholipase A2 from bacterial non-GM strain of *Streptomyces violaceoruber* with an enzymatic activity of 500 PLA2 units/grams (minimum).

Control flour was analyzed by performing Romanian standards methods: ash content (SR EN ISO 2171:2002), wet gluten content (SR EN ISO 21415-1:2007), gluten deformation (SR 90:2007) and falling number (SR EN ISO 3093:2007). The determined values for physico-chemical properties are mentioned as follows: ash content 0.65 %, wet gluten content 26 %, gluten deformation 4 mm, and falling number 342 s. The rheological properties of flours were determined on Mixolab, as well as by using a Chopin Alveograph according to SR ISO 5530-4:2005. Bread quality characteristics are determined after the baking tests have been made (SR 91:2007 ref.).

Results and Discussion

Dough rheological behaviour, prepared according to the working scheme, is appreciated on the basis of determinations made using Mixolab device (Table 2) and Chopin Alveograph (Table 3). All values shown are the means of three times analyses ± standard deviation ($\bar{x}$±S.D.).

Table 2.
The parameters resulted on the Mixolab for dough obtained from control flour supplemented with different doses of Lysomax

Characteristics/Samples	M	P1	P2	P3	P4
Water absorption (%)	58%				
Maximum consistency during phase 1 (C1, N·m)	1.12± 0.02	1.09± 0.02	1.05± 0.01	1.09± 0.02	1.11± 0.01
Maximum consistency during phase 2 (C2, N·m)	0.35± 0.01	0.34± 0.02	0.31± 0.02	0.30± 0.01	0.29± 0.02
Maximum consistency during phase 3 (C3, N·m)	1.52± 0.02	1.54± 0.02	1.57± 0.02	1.60± 0.02	1.63± 0.02
Maximum consistency during phase 4 (C4, N·m)	1.08± 0.02	1.10± 0.01	1.14± 0.02	1.18± 0.02	1.22± 0.01
Maximum consistency during phase 5 (C5, N·m)	1.37± 0.02	1.44± 0.01	1.54± 0.02	1.64± 0.02	1.73± 0.01
Stability (min)	6.54± 0.04	6.63± 0.05	7.04± 0.04	7.44± 0.04	7.53± 0.03
Difference of the points (C5 - C4, N·m)	0.29	0.34	0.4	0.46	0.51

Taking into consideration the rheological behaviour of different Lysomax dose addition tests, comparatively to control sample, expressed on the basis of Mixolab results, a decrease of maximum consistency during phase 1 (C1, N·m) was noticed. Dough stability increase was registered as well while kneading the samples where a smaller quantity of Lysomax was added in.

Table 3.
The parameters resulted on the Alveograph for dough obtained from control flour supplemented with different doses of Lysomax

Samples	Maximum pressure (mm H_2O)	Extensibility (mm)	Deformation energy (10^{-4} J)	Alveograph ratio P/L
M	100± 2	72± 2	268± 2	1.38
P1	104± 2	74± 2	282± 2	1.40
P2	112± 2	77± 2	310± 2	1.45
P3	110± 1	79± 1	315± 2	1.39
P4	110± 1	81± 1	325± 2	1.35

Also maximum consistency during phase 2 (C2, N·m) has decreased with 17.14% by Lysomax addition comparatively to control sample.

This is understandable, because through the action of phospholipase A2 on the phospholipid substrate, they help to change the polarity of molecules, facilitating the transformation of lecithins into lizolecitines, as well the increase in free fatty acids content. The reaction compounds formed by their high content of hydrophilic groups and lipophilic groups can form cross-links between the granular starch with hydrophilic surface and gluten, also between gliadin and glutenin, forming true lipoproteic complexes between starch, gluten and other hydrophobic compounds. Depending on the complexity of bonds, they can form cross-linked components, which compete in the compactness and stability of dough, formation of gas penetration-resistant film.

Regarding the parameters of phases 3, 4 and 5, we observed that, by adding some Lysomax, a clear change of dough behaviour in the gelling process. Lizolecitines form complexes with amylose and amylopectin, slowing the post-baking starch recrystallisation. This slowdown is reflected by a decrease of degree of bread ageing, showed by a C4 - C5 difference with 75.8 % for one dose of 4000U/100 kg Lysomax flour added.

From the alveographic point of view, though the control sample has higher extensibility, by Lysomax addition, the effect upon gluten strengthening is obvious from the dough resistance increase. Lysomax showed also an increase of dough tenacity with 10% for P4 sample, with increasing effect of mechanical effect, but without significant modification in the ratio of alveograph curve configuration. The increase of baking strength (energy of deformation) is explainable, because in the process of kneading, due to the absorption of oxygen, free fatty acid oxidation resulted from the action of phospolypases A2 on phospolypides is promoted. This influences positively the enzymatic oxidation of dough rheological properties, which means higher energy consumption in kneading, but also a dough that gives bread higher volume after the process of baking.

The technological effects of Lysomax improver were obtained by baking tests assessment. The variation registered for the loaf volume values are shown in Figure 4.

According to the samples shown in Figure 4, all improved flour samples, regardless of additive type and dose used, lead to the obtaining of higher volume breads than the blank sample. All samples showed also an improvement of crumb characteristics such as crust aspect, crumb texture, porosity and elasticity as well as freshness.

The effects of Lysomax on dough rheological properties were obvious, in relation to the method of analysis used.

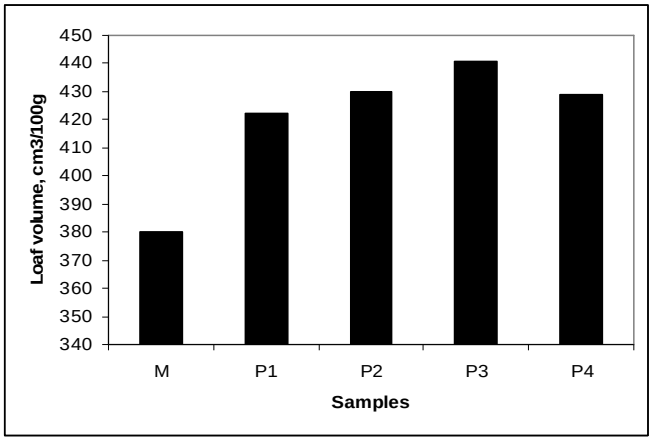

Figure 4. Loaf volume values of the samples analyzed

Conclusion

In flours of good breadmaking potential, the addition of phospolipase A2 promotes a substantial improvement in therms of physical specific feature of bread (significant increase of volume by 16 %) for an added dose of 3000U/Lysomax in the baking process.

These improvements are a consequence of improving rheological properties of dough, to strengthen the glutenice network, resulting in higher dough strength on demand and increasing the P parameter. At Mixolab, the addition of Lysomax into flour led mainly to a decrease in maximum consistency during phase 2 and a slight increase in its stability. An increase in the difference of points C4-C5, which shows a reduction of starch gelling can be seen as well.

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