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Phospholipids in homemade bread

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Abstract

The paper presents a study on the variation of phospholipidic profile in homemade bread containing exo-phospholipases. The bread samples were obtained in laboratory from whole meal flour (Carani, Timiş County, Romania) with addition of specific enzymes (LysoMax, Enzyme and Derivatives Ingredients). The enzyme concentration was varied in the range of 0-600 mg/100 g flour, which corresponds to a range of 0-3000 enzyme units/kg of flour). The profile of the principal compounds from phospholipids class (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidic acid) was determined by high-pressure liquid chromatography (HPLC); the samples analyzed by HPLC were obtained by successive extraction of bread samples, first with a hydrophobic solvent (petroleum ether) and second with a less hydrophobic solvent (dichloromethane-methanol).

The concentration of phosphatidylcholine in bread was in the range of 2.8-6 mg/g and lower in the case of phosphatidylethanol and phosphatidylinositol (0.1-0.6 mg/g and 0.4-1.2 mg/g, respectively). The variation of the concentration of phospholipids in bread samples has an optimum at 300-400 mg/100 g sample, this variation having a parabolic shape.

Keywords: homemade bread, phospholipase, phospholipids, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol

1. Introduction

Cereals were used in the human diet from ancient times, having high nutritional potential and very good organoleptic properties; they can also be easily stored [1]. The products obtained from grounded grains were still known from 2700 B.C., when the Egyptians obtain bread from flour, by using yeasts. The industrialization in this field was started in the eighteenth century, when the mechanical equipments for obtaining flour products from cereals and industrial installations appear [2].

The wheat represents the most important cereal used in the obtaining of bread and bakery products. Some wheat species are grown in the world, but in Europe only two are the most important: *Triticum aestivum*, known as the wheat for bread, and *Triticum durum*, known as the wheat for pasta [3].

The wheat flour contains especially starch, proteins, and lipids. Wheat flour contains also iron with a concentration of 3-4 mg/100 g of flour (17-20% of the daily requirement) Starch is the most concentrated material, the mean being 70% (to dry material basis). The starch (amylose and

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amylopectin) is found in the endosperm as microscopic granules or particles (lenticular and spherical shapes, with dimensions between 10 µm and 25 µm) [4].

The protein content of wheat flour varies in the range of 6-18%, according to the environmental and genetic factors. Generally, a higher content of proteins conduct to a decrease of the starch content, while approximate 1% of protein are lost in the milling process. Cereal proteins are classified according to their solubility in various solvents: water soluble proteins (albumins), proteins soluble in diluted sodium chloride aqueous solutions (globulins), in alcohol-water solution (prolamins), and in diluted acidic or alkaline solutions (glutelins) [1].

Wheat is less concentrated in lipids (~2%), which are not uniformly distributed in the grain. Wheat germs are more concentrated in lipids (~28%), while the endosperm presents the lowest concentration (~1.5%). The non-polar (triglycerides, fatty acids, sterols and corresponding esters) and polar lipids are equally distributed in wheat. The polar lipids consist of phospholipids and glycolipids. These polar lipids have surface tension properties and play an important role for obtaining baked goods [1,5,6].

Wheat contain also non-starch polysaccharides (such as pentosans), which absorb higher quantities of water, stabilize the formation of protein foam, and play an important role in the fermentation process [2,7-9].

In the obtaining of baked goods and bakery products enzymes are used besides the common ingredients (yeast, fats, sugar, milk, emulsifiers, salts, water etc.) [10,11]. The specific enzymes used in these processes are α-amylases, xylanases, lipoxygenases, gluco-oxydases, transglutaminases, proteolytic enzymes, and lipases). Enzymes are used in bread and bakery products production in order to hydrolyze the triglycerides, polar lipids (such as glycolipids), and phospholipids. The polar group of phospholipids is important in the overall surface tension and the splitting of one fatty acid group from the phospholipid structure (25-75% of phospholipids are hydrolyzed to lysophospholipids, Figure 1) with increases of this surface tension.

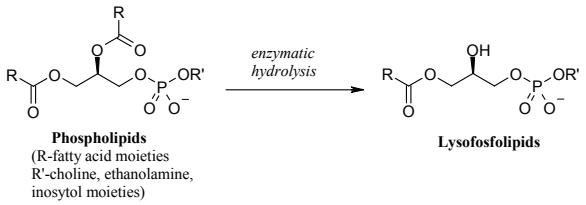

Figure 1. Hydrolysis of phospholipids

The concentration of enzymes is up to 10000 units/kg of flour (generally in the range of 1-100 units/kg of flour for the most active enzymes) [11].

In this study the variation of the concentration of polar lipids in the homemade bread by using phospholipases as enzyme additive.

2. Material and methods

2.1. Materials. Whole meal flour (Carani, Timiş County, Romania) was used as the main constituent for homemade bread production. Dry yeast, salt, and sugar were also used. The enzyme used in this study was from phospholipase class (LysoMax from Enzyme and Derivatives Ingredients). Petroleum ether (boiling interval of 30-60ºC, reagent grade, Merck&Co., Inc., New Jersey), methanol (HPLC grade, Merck), acetonitrile (HPLC grade, Merck), dichloromethane (HPLC grade, Merck), and anhydrous calcium dichloride (analysis grade, Chimopar, Bucharest) were used for separation and analysis of phospholipids.

2.2. Bread production. The homemade bread samples were obtained by using a Moulinex breadmaker with the following conditions and ingredient composition: a flour:water ratio of 64:36, salt 2%, dry yeast 1.6%; the enzyme concentration was in the range of 0-0.6 g/100 of flour, with a linear increasing of this concentration (0.1 increments, six samples with enzyme and one blank sample).

2.3. Extraction of non-polar and polar lipids. Non-polar lipids from bread samples were extracted in a semi-continuous solid-liquid extractor by using 25-50 g of finely grounded bread core and 100 mL petroleum ether. The extraction flask was heated at 60-65ºC and six extraction cycles were performed. The etheric extract containing non-polar lipids was dried over anhydrous calcium chloride and analyzed.

Polar lipids (phospholipids) were extracted in another solid-liquid extractor (discontinuous extractor) where the sample residue resulted from non-polar lipid extraction, which was mixed with 30 mL solvent (dichloromethane-methanol at a volume ratio of 2:1), at room temperature for 30 minutes. The extract was filtered and the residue was extracted again with another 30 mL solvent for 30 minutes. The final extract was filtered again, washed with 10 mL of the same solvent. The extracts were combined and analyzed by HPLC.

2.4. High pressure liquid chromatography (HPLC) analysis of polar lipids. The main phospholipids from bread samples (phosphatidylcholine – PC, phosphatidylethanolamine – PE, and phosphatidyl-inositol – PI) were identified and quantified by HPLC, using lecithin phospholipids as standard compounds. Thus, PC was identified at a retention time of 6.8 min, PE at 3.2 min, and PI at 5.7 min. An Agilent 1100 RP-HPLC apparatus was used in the following conditions: Zorbax SB-C18 column (250 x 4.6 mm x mm, 5 µm particle diameter), wavelength of 205 nm, mobile phase of acetonitrile:water at a ratio of 4:1, temperature of 25ºC, and a flow rate of 0.8 mL/min. The injected sample was 20 µL.

3. Results and discussion

The most important phospholipids were identified and quantified according to the lecithin standard (containing 35% PC, 25% PE, 15% PI, and other unidentified constituents), where PC was the most concentrated and was identified at a retention time of 6.8 min. The following calibration curve for PC was obtained from HPLC analysis of a series of lecithin standard solutions (concentration range of 1-10 mg/mL) (Figure 2).

$$Area_{(PC)}\,(\text{mV}\cdot\text{min}) = 2350 + 3586\cdot c_{PC}\,(\text{mg/mL})$$
$$n = 5,\ r^2 = 0.992$$

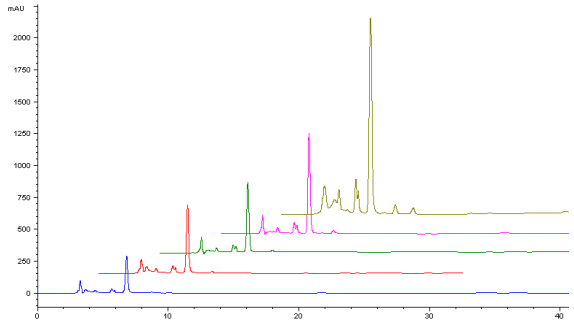

Figure 2. Superimposed HPLC chromatograms for lecithin standard solutions

PE and PI have higher concentrations and were identified at 3.2 min and 5.7 min, respectively. The calibration curves for these phospholipids were the following:

$$Area_{(PE)}\,(\text{mV}\cdot\text{min}) = 448 + 3621\cdot c_{PE}\,(\text{mg/mL})$$
$$n = 5,\ r^2 = 0.973$$

$$Area_{(PI)}\,(\text{mV}\cdot\text{min}) = 28 + 5570\cdot c_{PI}\,(\text{mg/mL})$$
$$n = 5,\ r^2 = 0.998$$

In the case of homemade bread samples the variation of the main phospholipids has a parabolic shape. Thus, phosphatidylcholine was identified in all samples (Figure 3 for HPLC analysis) and the concentration of this compound has a parabolic dependence with the concentration of added enzyme (Figure 4). This can be explained by the significant activity of enzyme at higher concentrations (more than 300 mg/100 g of flour) in the bread making mixture. The concentration of PC increases with the concentration of enzyme, and this concentration decrease for enzyme content more than 400 mg/100 g of flour. Probably, the phospholipids are further hydrolyzed to other compounds (such as lysophospholipids) at higher enzyme concentrations and the PC concentration decreases. The maximum PC concentration was 5.8 mg/g of bread sample, this optimum being obtained at an enzyme concentration of 300 mg/100 g of flour.

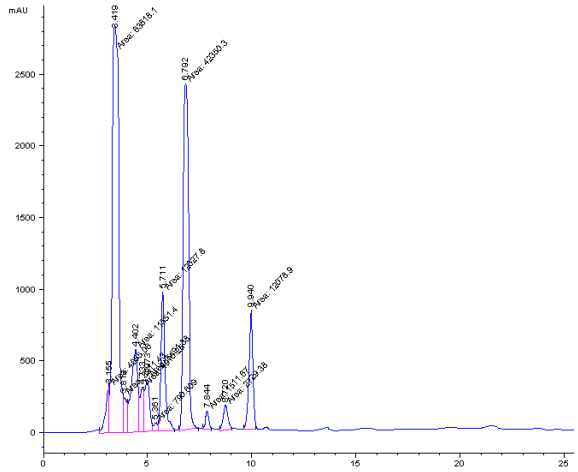

Figure 3. HPLC chromatogram for the bread polar lipid extract with optimum enzyme addition

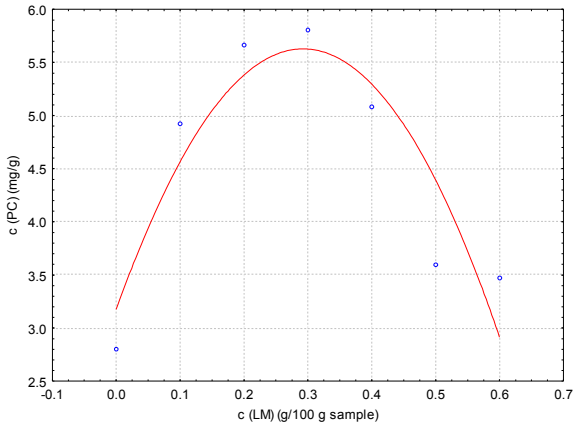

Figure 4. The variation of the concentration of phosphatidylcholine ($c_{(PC)}$, mg/g) with the concentration of the added enzyme to the bread samples

In the case of phosphatidylethanolamine, the concentration varies from 0.14 mg/g to 0.57 mg/g, but without a clear dependence with the enzyme contents in the initial mixture. However, the maximum concentration of PE (0.57 g/g of bread sample) was identified at a concentration of enzyme of 400 mg/100 g of flour. The variation is not clearly a parabolic one, but a similarity with the variation of PC concentration exists (Figure 5).

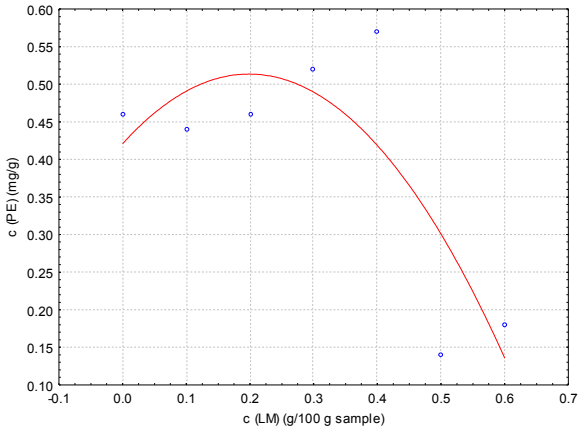

Figure 5. The variation of the concentration of phosphatidylethanolamine ($c_{(PE)}$, mg/g) with the concentration of the added enzyme to the bread samples

This parabolic correlation is more significant in the case of phosphatidylinositol, which increase to 1.2 mg/g of bread sample and decreases at lower values than 1.1 mg/g for concentrations of enzymes higher than 400 mg/100 g of flour (Figure 6).

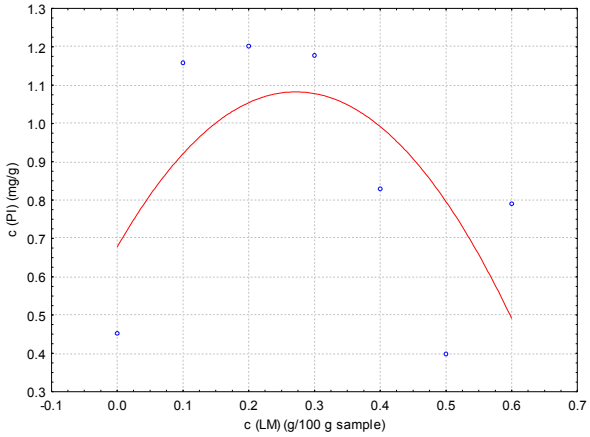

Figure 6. The variation of the concentration of phosphatidylinositol ($c_{(PI)}$, mg/g) with the concentration of the added enzyme to the bread samples

4. Conclusion

The following conclusions can be drawn among the influence of adding of phospholipases in the bread making process: (1) the overall concentration of phospholipids in the final bread samples increases with the increase of the enzyme concentration in the bread mixture ingredients;

(2) the variation of phospholipid concentration with the enzyme concentration has a parabolic shape, with an optimum at 300 mg of enzyme/100 g of flour; (3) the most concentrated phospholipids in the homemade bread samples was phosphatidylcholine, with a maximum concentration of 5.8 mg/g of bread sample. The overall conclusion is that the concentration of phospholipase enzymes must be use in a concentration up to 300-400 mg/100 g of flour in order to have a maximum concentration of phospholipids.

Acknowledgements

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