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The influence of phospholipase on volatile compounds in bread loaf

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Abstract:

Certain phosphatidylcholine acylhydrolases, known as phospholipases, have started to be used for improving the quality of the baked products. The aim of this study was quantification of some volatile compounds from bread loaves, by using recipes with addition of different doses of a commercial available phospholipase, named Belpan FTG. The working methodology consisted of baking tests, by using different doses of phospholipase, like improver. Through experiments, volatile compounds of bread were investigated by steaming extraction and analysis by gas chromatography coupled with mass spectrometry. The results indicated an increasing trend for concentration of the volatile compounds such as alcohols, aldehydes, esters, pyrazine and ether in respect of augmentation of the enzyme dosage. The use of a combination of phospholipases into an enzymatic mixture as bread improver may represent a valuable alternative to chemical compounds for improvement of the bread aroma as well. In fact, it is the first information available on bread aroma improved with exogenous phospholipases.

Keywords: bread, exogenous phospholipase, volatile compound, baking improver

Introduction

The wheat flour contains around 2 to 3% lipids classified into polar lipids (mainly phospholipids and glycolipids) and nonpolar or neutral lipids (preponderant triglycerides) in approximately equal amounts [1,2,3]. Even though the role of the natural lipids from flour was a controversial issue, it has been shown that many of them participate in physical, chemical as well as biochemical processes in flour and dough and finally influence the baking performances, such as loaf volume and bread crumb properties [4,5,6,7,8].

Phospholipids are relatively minor wheat constituents, mainly derived from the endosperm and represent ca. 10…20% of the total lipid content of wheat flour [2,9]. Speaking from biochemical point of view, the phospholipids are esters of polyalcohols with fatty acids and contain a phosphate group; beside the above mentioned components, phospholipids may contain a nitrogen base attached to the phosphate group, too. Phosphatidyl Cholines also called lecithins are glycerophospholipids containing the choline as nitrogen base. Although phospholipids are in very small amounts in wheat flour, previous studies showed they can

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influence the rheology of dough and loaf volume [10]. Degradation of phospholipids occurs in several hydrolysis stages catalysed by phospholipases and contributes to the metabolism of simple lipids, solubilisation of sterols and release of the unsaturated fatty acids. However, it is suggested that high lysophosphatidylcholine content in wheat endosperm is given by starch lipids and is not from degradation of phosphatidylcholine [7].

As it is known, the phospholipases is a group of enzymes (esterases) that hydrolyse phospholipids and release different compounds depending on the site of hydrolysis (for example lysophospholipids, free fatty acids, diacylglycerols, choline phosphate and phosphatidates). The most widely studied phospholipases are phosphatidylcholine 2-acylhydrolases named also phospholipases A2 (EC 3.1.1.4), which facilitate the conversion of lecithins into lysolecithins through the split of unsaturated fatty acid from C-2 position of lecithins [3,11,12]. As Ramrakhiani and Chand (2011) mentioned, the phospholipases A2 (PLA2) have industrial applications especially for enzymatic degumming of edible oils and synthesis of triglycerides enriched in polyunsaturated fatty acids [13]. However, in baking industry PLA2s are used to act on the phospholipids already present in the ingredients in order to produce emulsifier-like molecules (e.g. lysolecithin and monoglycerides) [3]. Regarding the studies on the effect of exogenous phospholipases used as baking improver, the researches focused mainly on their ability to act as dough conditioner agent and therefore to increase loaf volume and improve bread crumb softness [2,3,13,14,15,16]. Nevertheless, Sirbu and Paslaru (2008) observed a light improvement of bread flavour in relationship with exogenous phospholipases used.

In fact, one of the most important factors that determine the acceptance of bread for the consumers is aroma. The characteristic aroma of bread crumb is due to the volatile compounds formed during the bread-making process as the result of enzymes, fermentation or thermal reactions [17,18]. There are several stages in which these aroma compounds can be produced: through enzymatic activity during the dough processing (kneading – fermentation – baking); metabolism of yeast or dough fermentation by using different cultures of microorganisms (yeast, lactic acid bacteria etc.); lipid oxidation reactions and thermal reactions taking place during baking, mainly by Maillard and caramelisation reactions [17,18,19,20,21,22]. It seems that heat treatment in baking has the most significant effect on the generation of flavouring compounds in bread [23,24,25]. Lipases can produce short chain fatty acids with flavour implications, too. However, no studies had been reported in relation to effects of exogenous phospholipases used on bread aroma.

Although the volatile fraction of bread consists of a variety of compounds, only a relatively limited number is important for its aroma [24,26,27]. Alcohols, carbonyl compounds, acids, esters, lactones, pyrazines, furans and hydrocarbons are the main volatile compounds of fresh bread that are responsible for its flavour [25,27].

The present work was aimed at verifying the potential effect of exogenous phospholipases (PLAs) on bread aroma. For this, the assessment of certain volatile compounds from bread loaves, by using recipes with addition of different doses of a commercial available phospholipase, named Belpan FTG was done.

Materials and methods

The working methodology consists of two steps:

1. It was performed baking tests, by using Belpan FTG like improver (see working scheme in table 1); Belpan FTG is a commercial improver, supplied by Enzymes & Derivates Romania (617140 Costişa, Neamţ), and represents a fungal non - GM strain of lecithinase

PLA2 (from *Aspergillus niger)* produced by Lyven (Cagny, France); Belpan FTG is a light brown powder with an activity of 2000 U.E./10^{-3} kg.

2. The volatile compounds test was achieved through steaming extraction and analysis by gas chromatography coupled with mass spectrometry (GC-MS). Their identification was made by comparison of mass spectra according with National Institute of Science and Technology - US (NIST) database, and volatile content was assessed on the basis of GC retention times for reference compounds and mass spectrometry (MS).

The analyses were made in duplicate.

Bread-making:

Baking tests were performed by a recipe, which comprised whole wheat flour, water (56.5%), salt (2%), dry yeast (1.6%), Belpan FTG (%, basis 100 kg flour). The Pakmaya dry yeast (ROMPAK SRL Paşcani, Romania) and wheat flour retailed on the local market (Romania) were used in the study. The flour quality assessment was performed according with the Romanian standards, and the results indicated an average quality of flour, which corresponds to flour used in the Romanian industry for baking the regular breads.

Dough samples for bread-making were prepared by mixing all ingredients and following the working scheme introduced in table 1. A standard baking test was used in laboratory conditions. The dough samples were kneaded for 10 min and then they had risen for 30 min at 30^0C. Proofing was carried out at 35^0C for 45 min. The loaves were then baked at 250^0C for 45 min.

Table 1. Working scheme for baking test

Crt. No.	Code	Wheat flour (10^{-3} kg)	Water (10^{-6} m^3)	Salt (10^{-3} kg)	Dry yeast (10^{-3} kg)	Belpan FTG (10^{-5} kg)
1	FTG0 (blank)	437	247	8.8	7	0
2	FTG1					0.437
3	FTG2					0.874
4	FTG3					1.311
5	FTG4					1.748
6	FTG5					2.185
7	FTG6					2.622
8	FTG7					3.059

Analytical assays:

Volatile compounds were separated by steaming extraction from bread samples, and then were analyzed by gas chromatography coupled with mass spectrometry (GC-MS).

Finished breads were allowed to cool at room temperature and then the crumb was separated from the crust. Volatile compounds were extracted by steaming extraction from bread samples. A solid phase extraction sampling method was used. Specifically, 100 10^{-3} kg of sample and 500 10^{-6} m^3 of water were transferred into a 1000 10^{-6} m^3 sealed glass vial of an assembled distillation installation. After the steam distillation, the volatile compounds were captured in 10 10^{-6} m^3 hexane (Merck, Romania) that was recovered and dried on

anhydrous sodium sulphate. The analysis of volatiles in bread crumb samples was carried out by gas chromatography coupled with mass spectrometry (GC–MS).

Separation of volatiles was performed on a HP 6890 Gas Chromatograph connected with a GCMS-HP 5973 Mass Spectrometer by following working conditions:

- capillary column - 0.25 10^{-3}m internal diameter, and length 30 m; 0.25 10^{-6}m film thickness of stationary phase;

- mobile phase - helium, temperature program of 50-250°C with heating rate of 6°C/60s; temperature injector / detector - 250°C; injected volume – 1 $10^{-9}m^3$.

Volatile compounds detected in bread were identified by comparison of retention times and mass spectra data with those of reference compounds and data obtained from NIST libraries. The volatile content was assessed on the basis of ratio peak area (see formula (1):

(1): $Area_{GC\text{-}MS}=Area_{\text{volatile compound peak}}/Area_{\text{peaks per total}}\ x100$

Results and discussion

Through experiments a sum of volatile compounds were identified in wheat bread crumb. By using bread-making recipes with addition of different doses of an exogenous phospholipase – PLA2 commercially available, seven alcohols, nine aldehydes, five esters, a lactone, ether and a pyrazine were analysed. As it observes in table 2, the determination revealed 24 volatiles in comparison with more than 28 odorants detected in similar studies [17,22,25]. But then, the aim and objectives of our work were different from Schieberle and Grosch (1991) that main goal was to study the potent odorants of the wheat bread crumb focused on the differences to the crust and the effect of longer dough fermentation [17]. In the case of Birch & al (2013) a total of 46 aroma compounds were identified in the bread crumb, of which 45 compounds were confirmed, but the scope of that study was to investigate how aroma in wheat bread crumb is influenced by different fermentation conditions (amount of yeast and fermentation temperature) [22]. But Cho and Peterson (2010) reviewed the presence of more than 540 volatile compounds that form the bread aroma, from which around 28 compounds were reported as key odorants generating the flavours in bread [25].

Volatile compounds composition from bread through GC-MS analysis is introduced in table 2. Some of analysed compounds were not achieved by GC-MS, although it was expecting. Like alcohols, 1-pentanol, 2-methylbutanol, 4-pentanol, 3-methyl-pentanol, 1-hexen-3-ol, phenyl ethanol and 1-octanol were investigated. Hexanal, pentanal, heptanal, 2-heptenal, benzaldehyde, 2-octanal, nonanal, 2-nonenal and (*E,E*)-2,4-decadienal were determined from aldehydes compounds. Five esters of fatty acids, namely ethyl myristate, ethyl palmitate, ethyl linoleat, ethyl oleate and ethyl stearate, as well as 3,6-dimethyl-hexa-hydrobenzofuran, 5-hidroxy-methyl-dihydrofuran-2-one and 2-ethyl-6-methyl-pyrazine were assessed, too.

Table 2. Volatile compounds composition from bread through GC-MS analysis

	Retention time	FTG1	FTG2	FTG3	FTG4	FTG5	FTG6	FTG7
Alcohols								
1-pentanol	2.98	-	+	+	+	+	+	+
2-methyl-butanol	3.02	-	+	+	+	+	+	+
4-pentanol	4.06	-	+	+	+	+	+	+
3-methyl-pentanol	5.27	+	+	+	+	+	+	+
1-Hexen-3-ol	8.34	-	-	+	-	-	-	+
phenyl-ethanol	12.84	+	+	-	-	+	+	-
1-Octanol	14.46	-	-	-	-	-	-	-
Aldehydes								
hexanal	3.91	-	+	+	+	+	+	+
pentanal	4.97	-	-	-	-	-	-	-
heptanal	6.11	-	-	+	-	-	-	-
2-heptenal	7.68	+	+	+	+	+	+	+
benzaldehyde	7.91	+	+	+	+	+	+	+
2-octanal	10.95	-	+	+	+	+	+	+
nonanal	12.52	-	+	+	-	-	-	-
2-nonenal	14.46	-	-	-	-	-	-	-
2,4-decadienal	19.9	+	+	+	+	+	+	+
Esters								
ethyl myristate	33.82	+	+	+	+	+	+	+
ethyl palmitate	38.81	+	+	+	+	+	+	+
ethyl linoleat	42.61	+	+	+	+	+	+	+
ethyl oleate	42.76	+	+	+	+	+	+	+
ethyl stearate	43.37	-	-	-	-	-	-	-
Ethers								
3,6-dimethyl-hexa-hydrobenzofuran	15.44	+	+	+	+	+	+	+
Lactone								
5-hidroxy-methyl-dihydrofuran-2-one	21.27	-	-	+	-	-	-	-
Pyrazine								
2-ethyl-6-methyl-pyrazine	9.12	+	+	+	+	+	+	+

The concentration of volatile substances varied with dose of Belpan FTG added into bread recipe. A general increasing trend was observed for both alcohols and aldehydes, like the volatile compounds of loaf bread related to the Belpan FTG dose increment, but with different significance level for any substances.

Among the alcohols, higher concentration was achieved for 1-pentanol, and 1-octanol was not detected. As it is shown in figure 1, the concentration of 1-pentanol increased together with Belpan FTG dose. For example, in case of sample no. 7 (FTG6), the concentration increment of 1-pentanol was from 0.65% to 20.8% and a similar increasing, but less significant, was observed for all identified alcohols.

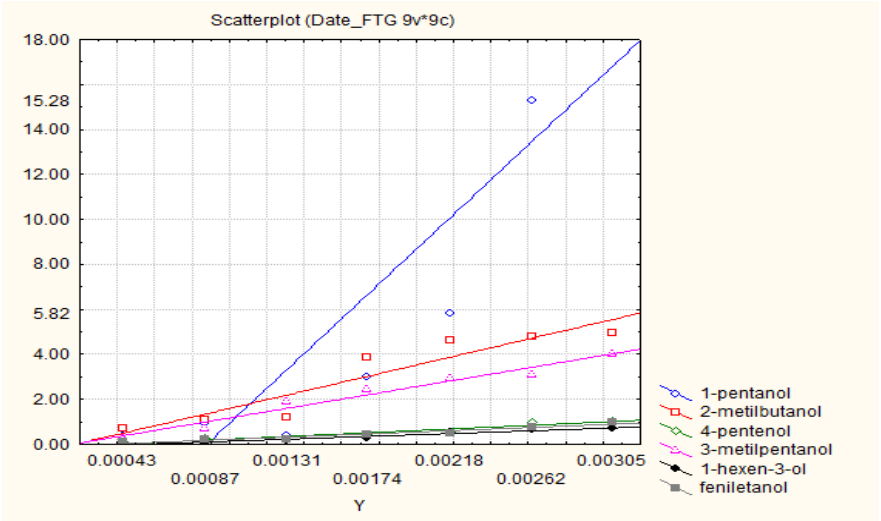

Figure 1. Variation of alcohols concentration

Concerning carbonyl compounds, best results were obtained for hexanal (see figure 2); but 2-nonenal, pentanal, and (E,E)-2,4-nonadienal in the wheat bread crumb were not detected, although are included as key odorants according with Cho and Peterson (2010) [25].

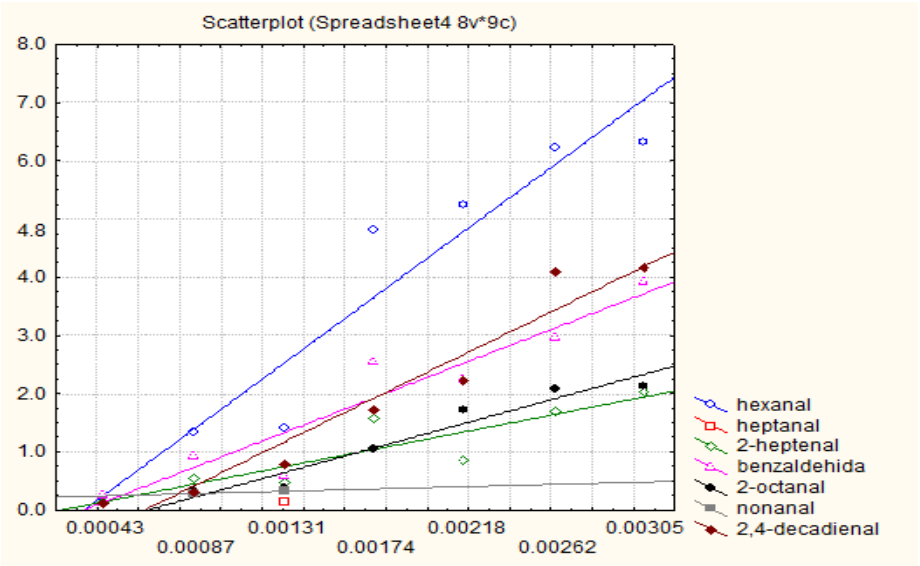

Figure 2. Variation of aldehydes content

From five investigated esters, there were identified four volatile compounds in all samples, and their variation in relationship with lecithinase dose is presented in figure 3.

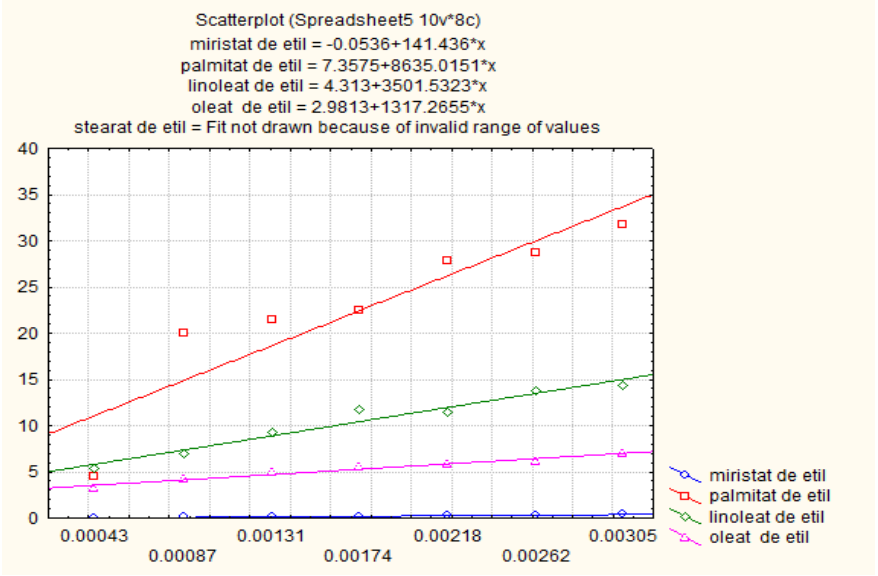

Figure 3. Variation of esters concentration

5-hidroxy-methyl-dihidrofuran-2-one was found out only for FTG3 sample, while pyrazine and ether concentrations described similar rate of curves depending on enzyme dose after a polynomial regression.

Conclusions

This is the first information available on flavour content of bread improved with exogenous phospholipases.

Steaming extraction and analysis by GC-MS showed a quantitative variation in bread flavours. GC-MS analysis of volatile content of improved breads has showed a mixture of 35% alcohols, 42% aldehydes, 20% esters, as for the rest lactones, pyrazines and ethers. Variation in volatile compounds composition was observed among bread loaves samples improved with exogenous phospholipases.

By using Belpan FTG as baking improver, bread loaves acquired an increasing trend for concentration of the volatile compounds such as alcohols, aldehydes, esters, pyrazine and ether in respect of augmentation of enzyme dosage. Increasing exogenous phospholipases usage in bread-making determined an improved flavour of the baked products.

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