DETECTION OF VOLATILE COMPOUNDS DURING WHEAT DOUGH FERMENTATION

Martins Sabovics, Evita Straumite, Ruta Galoburda
Latvia University of Agriculture
martins.sabovics@inbox.lv

Abstract

Taste, smell and the flavour are the most important attributes determining the quality of bread or baked cereal products in general. Bread flavour is composed of hundreds of volatile and non-volatile compounds, i.e. many alcohols, ketones, aldehydes, acids, esters and other compounds. Many researchers have been studying volatile compounds in different breads worldwide, but in Latvia only few studies are conducted on volatile compounds in bread and its production stages. The aim of this research was to analyse the composition of volatile compounds during wheat dough fermentation. Experiments were done in 2011 and carried out in the Laboratory of Bread Technology and Laboratory of Packing Material Investigations at the Department of Food Technology in the Latvia University of Agriculture. An investigation of volatile compounds was done using solid-phase microextraction (SPME) and gas-chromatography mass-spectrometry (GC-MS). Volatile compounds were analyzed on the 10th, 20th and 30th minutes of wheat dough fermentation. In a fermentation process of wheat dough totally 15 volatile compounds were detected. Eight of them were alcohols, two aldehydes, two ketones, one ester, one acid and one terpene. Three volatile compounds – 1-octanol, caryophyllene and acetophenone, were detected in the dough samples only after 30 minutes of fermentation – those were not detected at the earlier stages of fermentation. The peak areas of 11 volatile compounds increased, but peak area of one volatile compound decreased along the fermentation time. The study proved that solid-phase microextraction can be used for detection of volatile compounds in wheat dough fermentation process.

Key words: wheat dough, volatile compounds, solid-phase microextraction, gas-chromatography.

Introduction

Wheat (*Triticum aestivum*) is a raw material for various staple foods in many parts of the world. It is grown throughout the world and is adaptable to the wide range of environmental conditions. Wheat based foods are major source of nutrients such as carbohydrates, protein, vitamins and minerals in many regions of the world. It is extensively used in many parts of the world for the preparation of different types of bread and many other products (Kent and Evers, 1994). In many European regions for wheat and rye bread making is used sourdough. In Latvia sourdough is typically used for rye bread production, but yeast is used for wheat bread production. Sourdough is a key element in traditional rye bread baking, where it contributes significantly to the process ability, flavour and texture (Poutanen et al., 2009).

Taste, smell and the flavour are undoubtedly the most important attributes determining the quality of bread or baked cereals in general and one of the most important attributes influencing the acceptance of the consumer (Schieberle, 1996; Hansen and Schieberle, 2005). Flavour is usually the result of the presence, within complex matrices, of many volatile and non-volatile components possessing diverse chemical and physicochemical properties whereas the non-volatile compounds contribute mainly to the taste, the volatile ones influence both taste and aroma. A vast array of compounds may be responsible for the aroma of the food products, such as alcohols, aldehydes, esters, dicarbonyls, short to medium-chain free fatty acids, methyl ketones, lactones, phenolic compounds and sulphur compounds (Gatfield, 1988; Urbach, 1997).

The compounds which contribute to the aroma of food are usually present in trace to ultra-trace amounts comprising a diverse range of classes of chemical compounds. This chemical diversity may be further enhanced by subsequent processing. The physical properties of these compounds are equally diverse extending from that of the permanent gases to substances with boiling points exceeding several hundred degrees. This facilitates separations but complicates simultaneous recovery of the full range of aroma compounds. Methods used to assess the aroma compounds contributing to flavour in cereals are reviewed (Zhou et al., 1999). The ingredients and various technological processes (dough fermentation and the baking process) play a vital role in the development of characteristic flavour of the bread. The formation of volatile compounds in dough is started during lactic acid and alcoholic fermentation (Hansen and Hansen, 1994; Hansen and Schieberle, 2005). Aroma compounds in typical wheat bread are 3-methylbutanal, 2,3-butandione, 3-methylbutanol, acetic acid,

1-octen-3-one, 1-hexanol, 1-propanol, 2-methyl-1-butanol, 2-phenylethanol, ethyl acetate etc. (Schieberle, 1996; Poinot et al., 2008), but in bread made by sourdough composition of aroma compounds are propanol, pentanol, ethyl acetate, heptanal, acetic acid, ethanol, 2-pentylfurane, acetaldehyde, 3-methyl-1-butanal etc. (Damiani et al., 1996; Hansen and Schieberle, 2005; Guerzoni et al., 2007)

Many researchers have been studying volatile compounds in different breads worldwide, but only few studies are conducted on formation of volatile compounds in wheat bread and rye bread made by scald and sourdough technologies used in Latvia. Solid-phase microextraction (SPME) can be used for investigation of volatile compounds in wheat dough fermentation process.

Solid-phase microextraction is a modern, fast, sensitive, solvent-free and economical sample preparation technique before analysis through gas chromatography (Arthur and Pawliszyn, 1990; Hook et al., 2002). This technique has been developed to combine sampling and sample preparation in one step to analyse volatile compounds in bread crumb. Three different types of fibres can be used to determine the volatile compounds. Carboxen/Polydimethylsiloxane showed the best extraction efficiency for volatile analysis (Ruiz et al., 2003).

The aim of this research was to analyze the composition of volatile compounds during wheat dough fermentation.

Materials and Methods

Experiments were carried out in the Laboratory of Bread Technology and Laboratory of Packing Material Investigations at the Department of Food Technology in the Latvia University of Agriculture in 2011.

For this research wheat flour (550th type of flour), purchased from joint stock company 'Rigas Dzirnavnieks' (Latvia) was used. Wheat flour (550th type) is especially strong flour which is suitable for baking. Properties of 550th type of flour: ash content 0.62 g 100 g^{-1}, Falling Number 280 s, quantity of protein – 29 g 100g^{-1}, stability of protein – 5 min, water absorption – 58%. Other raw materials – sugar (Danisco Sugar 'Dansukker', Finland), salt ('Artimsol', Ukraine) and dried yeast (S.I.Lesaffre 'San-instant', France) – were purchased from a local retail store. The recipe for experimental bread making is presented in Table 1.

Table 1

Wheat dough recipe per 250 g of flour amount

Raw material	Amount
Wheat flour, 550th type, g	250.0±1
Salt, g	3.0±1
Sugar, g	3.5±1
Dried yeast, g	3.5±1
Water, mL	160.0±1

The research structure is presented in Fig. 1. Water temperature for dough mixing was adjusted to 37.5 ± 0.3 °C. Dough mixing time 2 minutes (the first step – slow) and 8 minutes (the second step - fast). The dough samples were analyzed after 10, 20 and 30 minutes of yeast fermentation in triplicates.

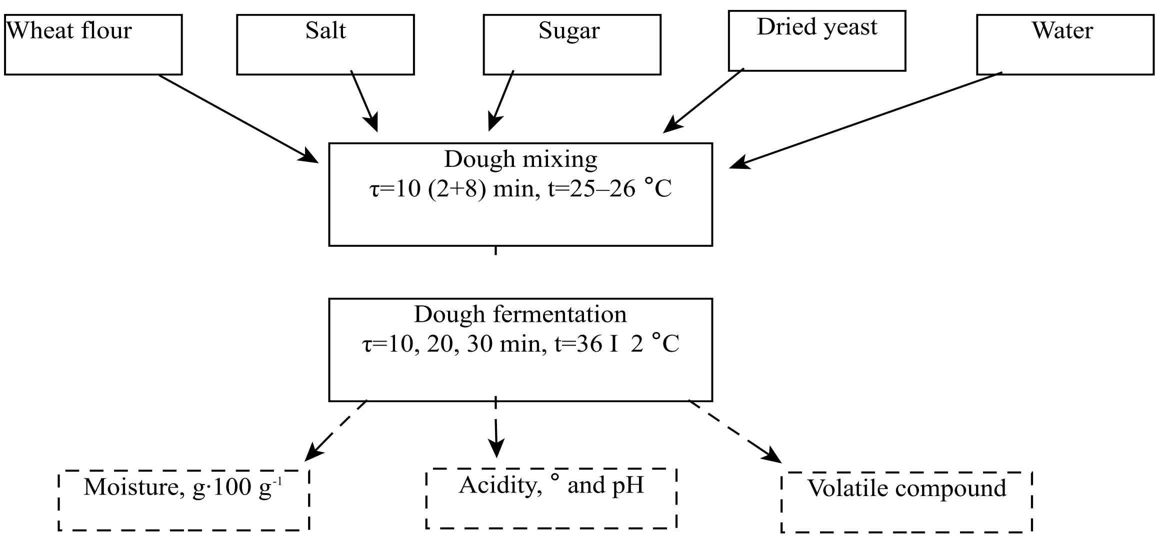

Figure 1. Preparation and analysis of dough samples.

Digital pH meter 'Jenway 3510' (Barloworld Scientific Ltd., UK) was used for pH measurement of dough. For pH measurement 5.00 ± 0.07 g of a sample was mixed with 50 mL of distilled water and stirred for two minutes in a 100 mL beaker (Standard method: AACC 02-52). Acidity was measured by titration against standardized 0.1 N NaOH and 1% phenolphthalein alcohol solution presence until pink colour (AACC 02-31). Moisture content was analysed drying sample in a moisture scale 'Precisa XM 105' (Precisa Instruments AG, Switzerland) at the temperature of +110 ± 1 °C until constant weight (Express method).

For detection of volatile compounds 50.0 ± 0.1 g of mixed wheat dough was used, the sample was weight into a 250 mL glass container equipped with aluminium lid with 1 mm diameter hole in the middle for solid-phase microextraction (SPME) fibre. The container was placed in a water bath 'Clifton Food Range' (Nickel-electro Ltd, UK) at water temperature 36 ± 2 °C for fermentation process (Figure 2).

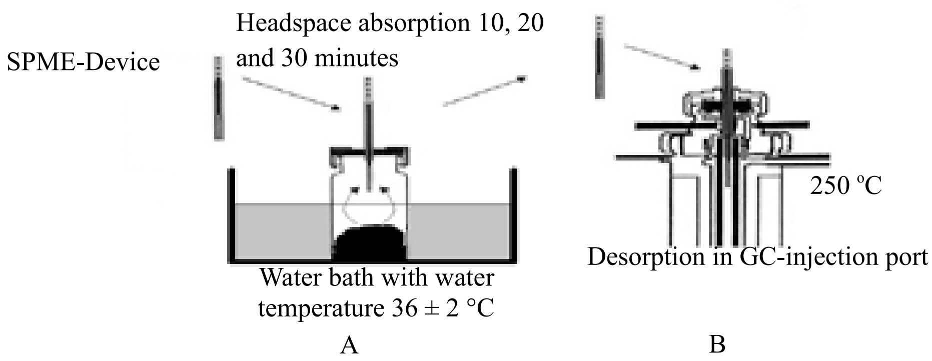

Figure 2. Volatile compound absorption (A) and desorption (B) process (Drawn by Sporkert and Pragst, 2000).

Volatile compounds were extracted from wheat dough samples using SPME fibre in a combination with gas chromatography/mass spectrometry. The SPME fibre itself is a fused-silica optical fibre, coated with a thin polymer film – Carboxen/Polydimethylsiloxane (CAR/PDMS). The SPME device is a modified syringe (Figure 3) consisting of manual fibre holder and a fibre assembly, which includes a 1 – 2 cm long retractable SPME fibre (Vas and Vékey, 2004).

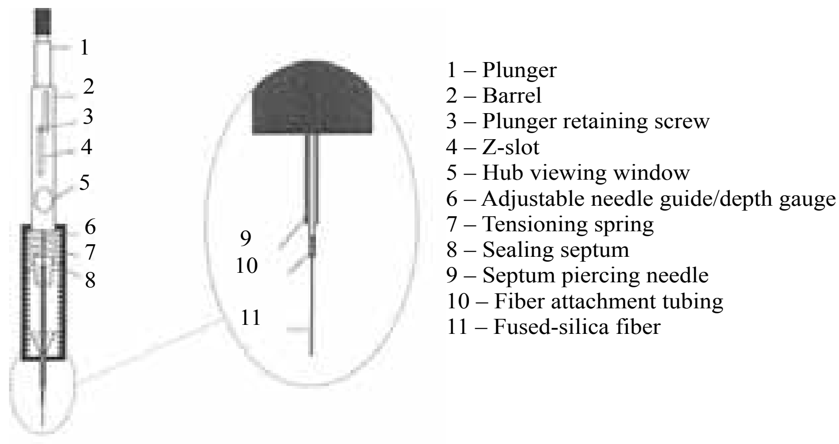

Figure 3. Schematic diagram of a commercial SPME device (Reprinted by Vas and Vékey, 2004).

The film thickness is 85 μm with biopolar polarity (Supelco, Inc., USA). Volatile compounds from fibre were thermally desorbed in the injector of a gas chromatograph-mass spectrometer 'Clarus 500 GC/MS '(PerkinElmer, Inc., USA). Before usage, the fibers were conditioned according to the manufacturer's instructions (30 min at 250 °C). The SPME extraction time was done for different duration of dough fermentation: the first - extraction time is 10 min at 36±2 °C without pre-incubation, the second – 20 min and the third – 30 min.

Volatile compounds from fiber were thermally desorbed in the injector of gas chromatograph/mass spectrometer. Separation of volatiles was carried out on the Elite-Wax (PerkinElmer, Inc., USA) capillary column (60 m × 0.25 mm i.d., DF 0.25 μm). The details of the program used in GC–MS analysis are as follows: the initial temperature was 40 °C, held for 7 min, then ramped from 40 °C to 160 °C at a rate of 6 °C min^{-1} and from 160 °C to 210 °C at a rate of 10 °C min^{-1} with a hold time for 15 min. The total run time was 47 min for a sample. Mass spectrometer in Electron impact Ionization mode was set on 70 eV as the electron energies, while the ion source temperature was set to 250 °C and inlet line temperature 250 °C. Injections were performed in splitless mode and helium (He) was used as carrier gas at a constant flow of 1 mL min^{-1}. Acquisition parameters in full scan mode: scanned m/z 40-300. Compounds were identified by comparison of their mass spectra with mass spectral library Nist98. Gas chromatograph-mass spectrometer 'Clarus 500 GC/MS' was used for the qualitative analysis of volatile compounds in fermented wheat dough and in the results there are shown peak areas of volatile compounds.

Statistical analysis - means, standard deviation of the means were derived with Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, WA).

Results and Discussion

Water plays a very significant role in bread making. In quantity, water ranks second as an ingredient in bread dough. The amount of added water determines dough consistency and it is also essential for the gelatinization of starch and for the heat-setting of proteins. The wheat flour moisture was 11.6 ± 0.15 g 100 g^{-1}. In dough making process water in amount of 64% from wheat flour mass was added. Flour moisture and added water amount formed dough moisture to 29.60 ± 0.13 g 100 g^{-1}. Dough moisture is summarized in Figure 4.

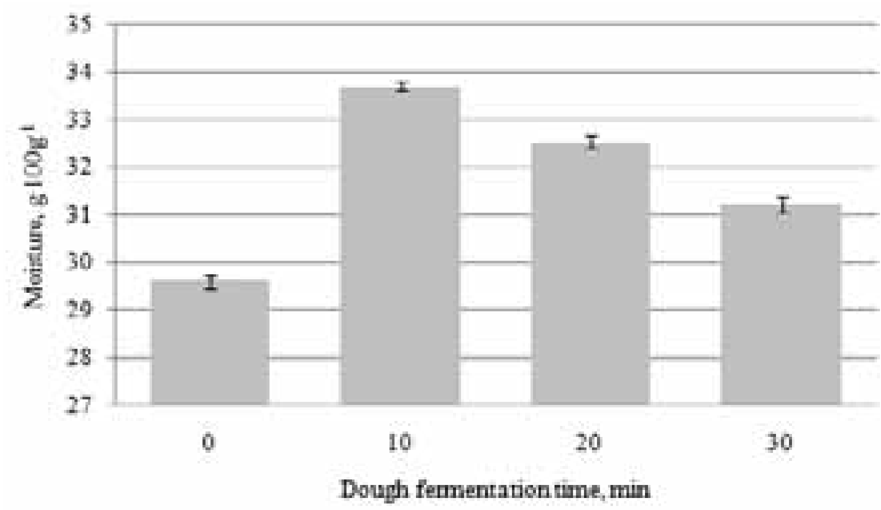

Figure 4. Moisture content in fermented wheat dough.

The results show that the highest moisture content (33.71 ± 0.10 g 100 g^{-1}) was in dough after 10 minutes of fermentation, but in the further process (after 20 and 30 min) moisture of dough decreased to 32.52 ± 0.14 g 100 g^{-1} and 31.22 ± 0.15 g 100 g^{-1}, respectively. This could be explained by the wheat flour ability to bind water at the beginning of fermentation, but in the next minutes moisture was released from dough when temperature was rising to 36 ± 2 °C.

Fresh dough had an initial pH 5.833 ± 0.049. Dough pH, acidity and volatile compounds total peak area are summarized in Table 2.

Table 2

pH, acidity and total peak area of volatile compounds in fermented dough

Fermentation time, min	pH	Acidity, °	Total peak area (×10^6) of volatile compounds
10	5.645±0.042	1.6±0.1	122.19
20	5.575±0.021	1.8±0.1	200.89
30	5.399±0.038	2.0±0.1	316.73

Flour and yeast contained small amounts of bacteria, which can produce acetic and lactic acids as their main metabolic products (Sluimer, 2005). Because of acid formation in a dough, fermentation pH value decreased. Freshly prepared dough acidity was 1.0 ± 0.1°, but after 30 minutes of fermentation the dough acidity increased to 2.0 ± 0.1°. Comparing the pH value in dough after 10 minutes or 30 minutes of fermentation, the pH decrease from 5.645 ± 0.042 to 5.399 ± 0.038 was observed. Wheat dough acidity possibly increased due to the formation of various acids in a fermentation process.

The volatile profile of different bread types has been widely investigated during the past years. These studies demonstrated that bread flavour is composed by different volatile compounds, belonging to several chemical classes, mainly heterocyclic compounds, alcohols, aldehydes, ketones, etc. Depending on the characteristic of each kind of bread, volatile compounds are present in well defined ratios (Grosch and Schieberle, 1997; Rehman et al., 2006; Bianchi et al., 2008).

Analysing the volatile compounds after 10, 20 and 30 minutes of wheat dough fermentation overall was identified

8 alcohols, 2 aldehydes, 2 ketones, 1 terpene, 1 ester and 1 acid. In wheat dough after 10 minutes of fermentation 11 volatile compounds were detected, after 20 minutes – 12, but after 30 minutes – 15 volatile compounds. The volatile compounds that were found in wheat dough fermentation time are presented in Table 3 as ($\times10^6$) of peak area.

The peak areas of detected 11 volatile compounds increased in dough fermentation process, but peak area of acetic acid decreased. The highest peak area of acetic acid was detected after 10 minutes (3.84) of wheat dough fermentation, but the lowest after 30 minutes of fermentation (3.03). This can be explained that in the wheat dough fermentation process, alcohols and acids which further can be transformed to ethyl acetate detected after 20 and 30 minutes of fermentation can be formed from yeast. Ethanol was not detected in wheat dough after 30 minutes of fermentation as a volatile compound, but it can be formed from sugars in the fermentation process. Ethyl acetate peak area increased by 2.91 during fermentation between 20 and 30 minutes. After 30 minutes of fermentation 3 volatile compounds (1-octanol, caryophyllene and acetophenone) which were not detected after 10 and 20 minutes of fermentation were identified.

Table 3

Volatile compounds in wheat dough at different stages of fermentation

Compounds	Retention time (min)	Peak area ($\times10^6$) depending on dough fermentation time		
		10 min	20 min	30 min
4-amino-1-pentanol	4.77	11.64	15.53	23.77
2-ethyl-butanal	6.09	0.79	0.86	1.63
Ethyl acetate	7.80	n.d.	1.92	4.83
4-penten-2-ol	9.25	76.00	129.91	211.80
2-methyl-1-propanol	14.60	1.46	2.90	3.80
1-ethoxy-2-propanol	16.89	10.48	11.16	19.23
3-methyl-1-butanol	18.09	13.73	27.69	36.68
1-pentanol	19.28	0.41	0.72	1.03
3-hydroxy-2-butanone	20.54	0.57	0.90	1.59
1-hexanol	21.94	2.03	4.22	6.91
Nonanal	23.14	0.51	0.61	1.17
Acetic acid	24.52	3.84	3.16	3.03
1-octanol	26.58	n.d.	n.d.	0.79
Caryophyllene	27.81	n.d.	n.d.	1.04
Acetophenone	28.96	n.d.	n.d.	1.00

n.d. – not detected

Researchers describe that the volatile compounds in bakery products are formed in mixing, fermentation (yeast) and baking process. Added raw materials and their amount has great effect on product`s aroma formation (Martínez-Anaya, 1996; Thiele et al., 2002; Sabovics et al., 2010). The highest peak area value (211.80) among all detected volatile compounds had 4-penten-2-ol (alcohol) indentified after 30 minutes of fermentation, but the lowest peak area value (0.41) was detected for 1-pentanol (alcohol) after 10 minutes of fermentation. From 3 volatile compounds detected only after 30 minutes of fermentation, the highest peak area value (1.04) showed caryophyllene (terpene), but the lowest (0.79) – 1-octanol (alcohol).

In all dough samples 11 common volatile compounds (Table 3) were found: alcohols (4-amino-1-pentanol, 4-penten-2-ol, 2-methyl-1-propanol, 1-etoxy-2-propanol, 3-methyl-1-butanol, 1-pentanol, 1-hexanol), aldehydes (2-ethylbutanal, nonanal), ketone (3-hydroxy-2-butanone) and acid (acetic acid). Alcohols can be formed in alcoholic fermentation when yeast can produce long-chain and complex alcohols, otherwise aldehydes and ketones can be formed from alcohols. Acetic acid is formed in dough fermentation process from yeast. Yeast secondary metabolism can form 3-methyl-1-butanol, which gives malty flavour to dough. The amount of flavour compounds formed in dough can be affected by yeast amount and activity, fermentation time and fermentation temperature. All of the detected volatile compounds can produce dough aroma: 2-methyl-1-propanol produce whiskey odour, 4-penten-2-ol – fruity, 1-pentanol – sweet, 3-hydroxy-2-butanone – like butter or yogurt, 1-hexanol – freshly mown grass and nonanal - strong fruity or floral odour (Schieberle, 1996; Zhou et al., 1999; Kulp et al., 2003).

Volatile compounds detected after 30 minutes of dough fermentation adds more specific aroma to dough, because caryophyllene give woody spicy and hay odour, acetophenone - spicy (almond, nuts), fruity (cherry, strawberry), but 1-octanol can give to yeast fermented wheat dough fresh like orange-rose, waxy and sweet odour (Schieberle, 1996; Zhou et al., 1999; Kulp et al., 2003).

Conclusion

1. The highest moisture content 33.71 ± 0.10 g 100 g^{-1} was in dough after 10 minutes of fermentation, but in the further process (after 20 and 30 min) moisture of dough decreased to 32.52 ± 0.14 g 100 g^{-1} and 31.22 ± 0.15 g 100 g^{-1}.
2. Solid-phase microextrection in combination with GC/MS can be used for detection of volatile compounds in fermented wheat dough.
3. After 30 minutes of fermentation three compounds – 1-octanol, caryophyllene and acetophenone, which were not present in samples tested after 10 or 20 minutes of fermentation were detected.
4. In all dough samples (after 10, 20 or 30 minutes of fermentation) totally 15 volatile compounds out of which 11 volatile compounds were common: alcohols (4-amino-1-pentanol, 4-penten-2-ol, 2-methyl-1-propanol, 1-etoxy-2-propanol, 3-methyl-1-butanol, 1-pentanol, 1-hexanol), aldehydes (2-ethylbutanal, nonanal), ketone (3-hydroxy-2-butanone) and acid (acetic acid) were detected.

Acknowledgment

This research has been prepared within the framework of the ESF Project „Formation of the Research Group in Food Science", Contract No. 2009/0232/1DP/1.1.1.2.0/09/APIA/ VIAA/122.

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