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Volatile compounds in whole meal bread crust: The effects of yeast level and fermentation temperature

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ABSTRACT

The influence of fermentation temperatures (8 °C, 16 °C, and 32 °C) and yeast levels (2%, 4%, and 6% of the flour) on the formation of volatile compounds in the crust of whole meal wheat bread was investigated. The fermentation times were regulated to optimum bread height for each treatment. The volatile compounds were extracted by dynamic headspace extraction and analyzed by gas chromatography-mass spectrometry. The results were evaluated using multivariate data analysis and ANOVA. In all crust samples 28 volatile compounds out of 58 compounds were identified and the other 30 compounds were tentatively identified. Higher fermentation temperatures promoted the formation of Maillard reaction products 3-methyl-1-butanol, pyrazine, 2-ethylpyrazine, 2-ethyl-3-methylpyrazine, 2-vinylpyrazine, 3-hydroxy-2-butanone, 3-(methylsulfanyl)-propanal, and 5-methyl-2-furancarboxaldehyde whereas at lower temperature (8 °C) the formation of 2- and 3-methylbutanal was favored. Higher levels of yeast promoted the formation of 3-methyl-1-butanol, 2-methyl-1-propanol and 3-(methylsulfanyl)-propanal, whereas hexanal was promoted in the crust fermented with lower yeast level.

1. Introduction

Recently, higher demand for whole grain products is observed worldwide due to their high nutritional values and protective effects against several chronic diseases (Slavin, Tucker, Harriman, & Jonnalagadda, 2013). Whole meal wheat bread is one of the most consumed whole grain products, however, it has lower consumer acceptability compared to wheat bread made from refined flours (Bakke & Vickers, 2007).

Crust is commonly defined as the drier, darker and harder surface of the bread and distinguishable from the crumb (Lind & Rask, 1991; Westurlund, Theander, & Aman, 1989). The thickness and darkness of the crust are proportional to the time and temperature of baking (Mondal & Datta, 2008; Purlis, 2011; Zanoni, Peri, & Pierucci, 1993). The formation of color and flavor in the crust of bread during baking is mainly a result of Maillard reactions, the non-enzymatic chemical reactions occurring between free amino acids and carbonyl groups of reducing sugars (Hodge, 1953). The reactions depend on the water content, baking temperature, pH, and the content of reducing sugars and free amino acids. The moisture content on the surface of the loaf is usually less than 20% due to the baking process (Vanin, Lucas, & Trystram, 2009) and increases the surface temperature to more than 100 °C which provides suitable conditions for the formation of Maillard reaction products and in turn leads to crust formation (Zanoni et al., 1993). Thus higher content of aldehydes, ketones, furans, pyrazine derivatives, and some other compounds generated from Maillard reactions, were observed in the whole meal bread crust and wheat bread crust compared to the crumb (Bianchi, Careri, Chiavaro, Musci, & Vittadini, 2008; Chang, Seitz, & Chambers, 1995; Jensen, Oestdal, Skibsted, Larsen, & Thybo, 2011; Moskowitz, Bin, Elias, & Peterson, 2012; Seitz, Chung, & Rengarajan, 1998).

The flavor of the bread crust is expected to be more intense than the bread crumb as the crust is exposed to higher temperature than the crumb (Zehentbauer & Grosch, 1998a). Schieberle and Grosch (1991) found 2-acetyl-tetrahydropyridine and 2-acetyl-1-pyrroline with their roasty notes as the most important flavor compounds of wheat bread crust, and they found a higher content of these compounds in the crust compared to the crumb of the same bread. Also, the components 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 2-methylpropanal, 2,3-butanedione, 3-(methylsulfanyl)-propanal,

Abbreviations: OT, Odor Threshold; dPLS, Discriminant Partial Least Squares; GC-MS, Gas Chromatography-Mass Spectrometry; DHE, Dynamic Headspace Extraction.

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2- and 3-methylbutanal, and (E)-2-nonenal were characterized as important odorants in wheat bread crust (Grosch & Schieberle, 1997; Schieberle & Grosch, 1991; Zehentbauer & Grosch, 1998a; Zehentbauer & Grosch, 1998b). The review by Cho and Peterson (2010) also reported that (Z)-2-nonenal, (Z)-4-heptenal, 1-(Z)-5-octadien-3-one, phenylacetaldehyde, (E,Z)-2,6-nonadienal, (E,E)-2,4-decadienal and (E,E)-2,4-nonadienal were other key odorants of wheat bread crust. The contents of Maillard reaction products were higher in fully baked wheat bread compared to the partially baked wheat bread (Poinot et al., 2008).

Zehentbauer and Grosch (1998a, 1998b) evaluated the crust aroma of wheat baguette prepared from two different recipes; conventional and artisan. They found that baguette prepared according to a conventional recipe with short process time (26 g yeast/kg flour, fermented for 160 min at 26 °C) had higher content of 2-acetyl-1-pyrroline, 2-ethyl-3,5-dimethylpyrazine, hexanal, (E)-2-nonenal, and 2,3-butanedione in the crust compared to the crust from an artisan recipe made with low yeast amount (15 g yeast/kg flour), addition of pre-fermented dough and final fermentation in long time at low temperature (18 h at 4 °C). On the other hand, the crust of baguette made due to the artisan recipe had higher content of the aldehydes 3-(methylsulfanyl)-propanal, 2- and 3-methylbutanal, 2-methylpropanal, 2,4-decadienal, and 2-methoxy-4-vinylphenol. The crust of baguette made by conventional recipe had higher intensity of roasty attribute, meanwhile, the crust made by artisan recipe had higher intensity of malty attribute. The attributes of buttery and sour however, were perceived as being similar in intensity in the bread crust made from both recipes.

Information is not available about the influence of fermentation conditions towards the formation of important volatile compounds in the crust of whole meal wheat bread. Therefore, the aim of this work is to investigate to which degree the formation of volatile compounds in the crust of whole meal wheat bread is influenced by fermentation conditions such as yeast amount and fermentation temperature.

2. Materials and methods

2.1. Experimental design

Bread was made from wheat whole meal flour with three levels of yeast corresponding to 2%, 4% and 6% of the flour. The bread dough was fermented at three different temperatures 8 °C, 16 °C and 32 °C. The combination of different yeast levels and fermentation temperatures add up to 9 treatments. Bread with the same treatment was made twice (duplicate) on two consecutive days which made up 18 bread samples in total. The analysis of volatile compounds was carried out in triplicate with a total of 54 samples. The bread samples were named as follow: yeast level (2, 4, or 6); fermentation temperature (8, 16 or 32); treatment duplicate (a or b) and analysis triplicate (1, 2, or 3).

2.2. Wheat grain and whole meal flour

Wheat grains of the variety Øland (organically grown) were purchased from Aurion A/S Milling and Baking Company (Denmark). Protein content was 14 g/100 g, lipid 2.8 g/100 g and ash 1.5 g/100 g. The grains were milled on a Brabender Quadrumat junior laboratory roller mill the day before baking. The particle size of the whole meal was as follows: 27% of the flour had particle size less than 75 μm; 37% between 75 μm and 160 μm; 16% between 160 μm and 250 μm; 8% between 250 μm and 500 μm; 8% between 500 μm and 1000 μm, and only 4% had a particle size bigger than 1000 μm.

The moisture content of the flour was measured on the day of baking (HOH-express, Pfeuffer) and varied from 10.7 to 10.9%. The wet gluten content in the sifted flour with particle size < 160 μm was 27.9% (Glutomatic 2100, Perten) (Method no. 38-12, AACC, 1995). The falling number of whole meal flour was 230 s (Falling Number 1500, Perten) (Method no 107/1, ICC, 1995).

2.3. Yeast

Commercial pressed fresh baker's yeast *Saccharomyses cerevisiae*, SKÆRTOFT MØLLE, an organically produced baker's yeast from Agrano, Germany was used. The yeast was taken from the same batch for all bakings.

2.4. Bread making

The whole meal bread was made according to Nor Qhairul Izzreen, Petersen, and Hansen (2016). 300 g of wheat whole meal (adjusted to 14% moisture content), 192 mL water (30 °C), 4.2 g saccharose, 4.2 g NaCl and 6 g, 12 g or 18 g yeast (corresponding to 2%, 4% and 6% of the flour), respectively were mixed in a bread maker machine (XBM 5, FOVEA A/S, Denmark) and set to knead for 19 min. After kneading, 400 g dough from each batch was transferred to a baking tin and then placed in an incubator at 8 °C, 16 °C or 32 °C, respectively for fermentation. The fermentations were terminated when the doughs reached 8 cm in height. Preliminary tests were carried out to find the optimal fermentation times for each combination of yeast level and fermentation temperature by monitoring the dough height by a web-camera during the fermentations. The optimal fermentation time was the time when each dough reached 8 cm which was the maximum height of dough just before it collapsed (Table 1). The dough was baked at 200 °C for 17 min to a center temperature of 99 °C in a convection oven (Conmatic line, Houno, Brønnum). After baking, the loaves were cooled at ambient temperature for 15 min, then taken out from the baking tin and further cooled on a grate at the same temperature for another hour. The top 0.5 cm of the bread loaf was cut off and taken as the crust sample. The samples were then packed in aluminum foil in a plastic bag and kept frozen at −18 °C for two weeks until analysis.

2.5. Fermentation temperature

The fermentation temperatures of 32 °C, 16 °C and 8 °C were chosen, as these temperatures are realistic in commercial bread production. Most dough fermentations are made within the range of 25-35 °C which is the optimal temperature interval for baker's yeasts (Torija, Rozès, Poblet, Guillamón, & Mas, 2003). In addition, low fermentation temperature (5 to 10 °C) is used in dough retarding (Cauvain, 2007).

2.6. Dynamic headspace extraction

A dry bread crust sample weighing 8 g was cut into pieces (0.5 cm × 0.5 cm × 0.5 cm) and transferred to a 500 mL purge glass

Table 1
Fermentation times for doughs to reach optimal height at combination of fermentation temperatures and yeast concentrations.

Fermentation temperature (°C)	Yeast concentration (% of flour)		
	2	4	6
8	20 h 15 min	8 h 45 min	4 h 50 min
16	7 h 30 min	4 h	3 h
32	2 h	1 h 40 min	1 h 10 min

flask (7.5 cm diameter) for dynamic head space extraction (DHE). The purge flask containing the sample was closed by a purge head connected to a Tenax trap (250 mg Tenax TA, 60/80 mesh, Buchem BV, Apeldoorn, Netherlands). The conditions for the extraction were done according to Nor Qhairul Izzreen et al. (2016).

2.7. Gas chromatography-mass spectrometry (GC-MS)

The trapped volatiles were desorbed using an automatic thermal desorption unit (ATD 350; Perkin Elmer, Waltham. MA). Primary desorption was carried out by heating the trap to 250 °C with a hydrogen flow (50 mL/min) for 15 min. The stripped volatiles were re-trapped in a Tenax-TA cold trap (30 mg, 5 °C) which was subsequently heated at 300 °C for 4 min (secondary desorption, outlet split 1:10) which allowed a rapid transfer of volatiles to the GC-MS (7890A GC-system interfaced with a 5975C VL MSD with triple-axis detector from Agilent Technologies, Palo Alto, CA) through a heated (225 °C) transfer line with hydrogen as carrier gas (1 mL/min). Separation of volatiles was carried out on a DB-Wax capillary column (30 m, 0.25 mm, 0.50 μm; J&W Scientific). The column temperature was kept at 40 °C for 10 min, increased at 8 °C/min to 240 °C and kept isothermal for 5 min. The ionization energy for the mass spectrometer was 70 eV and mass-to-charge ratios between 15–300 *m*/*z* respectively. The transfer line, source and quadruple temperatures were set to 220, 230 and 150 °C respectively.

2.8. Compound identification

Volatile compounds were identified by matching their mass spectra (MS) with those of a commercial database (Wiley275.L, G1035A, Agilent Technologies) and with mass spectra of reference standards. The software program, GCD Plus ChemStation G1701EA (version E.01.00.237, Agilent Technologies) was used for the comparisons of the library MS spectra with the MS samples. Retention Indices (RIs) of all identified volatiles were calculated using a series of *n*-alkanes (C_6–C_{22}) (Hewlett Packard, Avondale, PA) and compared to the RI of reference compounds (obtained using the same method). The RIs for compounds when compared with commercial database and/or RI reported previously in published literature (Giri, Osako, Okamoto, & Ohshima, 2010) and/or online databases (http://www.chemspider.com; http://www.flavornet.com) are claimed to be tentatively identified. The odor characteristics and odor threshold values (OT) of each volatile compound were also referred from the online databases unless stated otherwise (Table 2).

2.9. Data analysis

Discriminant Partial Least Squares regressions (dPLS) were computed using LATENTIX (version 2.00, Latent5) by correlating peak areas of volatile compounds (*X-matrix*) to a dummy matrix (*Y-matrix*) describing the experimental design. All data was autoscaled and models were validated using full cross-validation. The statistical analyses were done on the peak areas of the volatile compounds. One-way and two-way analyses of variance (ANOVA) were computed using JMP software (version 7.0, SAS Institute Inc.) with fermentation temperature and yeast concentration as model effects. When ANOVA showed significant differences, Tukey's test was computed for the volatile compounds (95% significance level) to describe the effects of fermentation temperature and yeast concentration.

3. Result and discussion

3.1. Volatile compounds in the crust of whole meal bread

In the crust samples of whole meal bread, 28 volatile compounds were identified and additionally 30 volatile compounds were tentatively identified (Table 2). The volatile compounds comprise 12 alcohols, 11 aldehydes, 11 pyrazines, 8 ketones, 6 furans, 4 esters, and 6 other compounds.

Fig. 1A is a PLS score plot which describes the influence of fermentation temperature and Fig. 2A describes the influence of yeast level on the relative level of the volatile compounds. Out of the 54 samples, five samples with the codes 68a2, 616a3, 616b1, 632a2, and 216b3 were considered as outliers based on the Residual variance and Hotellings T2, and therefore were not included in data analysis. For most of the remaining samples, the duplicates of fermentation treatments and the analysis triplicates are generally placed close to each other, which indicate good reproducibility of the fermentation treatments and analytical method. The Relative Standard Deviation (RSD) was calculated based on two replicates of bread produced in two different days, and the three analysis triplicates of crust pieces of the same bread for each treatment. The RSD was calculated separately within the variation of the baking and the analysis with the values less than 20% respectively for all compounds. Our RSD is higher than the value reported by Bianchi et al. (2008) as their RSD was ⩽10%. In this work, bread crust pieces were used while Bianchi et al. (2008) used powdered crust. Therefore, the samples used in this study had a more heterogeneous nature, which might affect the volatilization from the food matrix. The effects of temperature and yeast amount were, however, strong enough to give significant effects using this analytical approach.

Based on two-way ANOVA, the mean peak areas of 18 compounds were significantly influenced by yeast level and/or fermentation temperature with a non-significant interaction between the two fermentation parameters (Table 3). The mean peak areas of nonanal, heptanol, hexanol and 2-butanone were neither influenced by yeast levels nor fermentation temperatures. The remaining 36 compounds show significant interaction between the two factors, therefore one-way ANOVA was calculated to analyze differences resulting from the combined effects of the two factors, and this showed that there were significant differences in the mean peak areas for all 36 compounds (Table 3).

3.2. Effect of fermentation time

All bread was fermented until the optimal height of the dough was obtained. No statistical correlation was, however, found between fermentation time and the peak areas of the volatile compounds identified (results not shown), and fermentation time will therefore not be discussed further.

3.3. Effect of fermentation temperature

The score plot in Fig. 1A shows a systematic effect of fermentation temperature, since samples fermented at 8 °C, 16 °C and 32 °C are placed in separate groups. Samples fermented at 8 °C with 2% yeast, do, however, tend to form a separate cluster at the upper left corner of the plot. The loading plot shows that most of the compounds are placed to the right of the plot which corresponds to the samples fermented at higher temperature. The PLS-plot explains a variance of 43% (Fig. 1A and B). Furthermore, the two-way ANOVA analysis shows that the mean peak areas of 12 compounds were significantly influenced by fermentation temperature (Table 3). The level of hexanal and most other lipid oxidation

Table 2
List of volatile aroma compounds identified and tentatively identified in the crust of whole meal wheat bread by DHS and GC–MS.

Chemical group	Volatile compounds	Calculated RI	Kovats RI of reference compounds	Kovats RI (wax) from literature[a,b,c]	Origin[*]	Odor description[b,c,d,e,g]	Odor threshold (OT) in water (μg/L)[c,d,f]
Aldehydes	Hexanal	1089	1089	1084	LO	Green, rancid	4.5
	Heptanal	1192	1192	1174	LO	Dry fish	8
	Nonanal	1403	1404	1402	LO	Green, fatty	1.1
	Pentanal	980	983	986	LO	Malt, green	NA
	2-Methylbutanal	914	913	912	M,F	Malt	3
	3-Methylbutanal	917	916	912	M,F	Malt	0.2
	2-Methypropanal	813		814	M	Nut	1.5
	(E)-2-Nonenal	1553	1551	1558	LO	Green	0.08
	Benzaldehyde	1538	1541	1521	LO,M,F	Almond	750
	3-(Methylsulfanyl)propanal	1467	1468	1450	M	Boiled potato	0.2
	Phenylacetaldehyde	1662		1623	M,F	Honey	4
Alcohols	*Propanol*	1055		1037	F	Plastic, musty	6600
	Pentanol	1273	1274	1251	LO	Fruity	4000
	Hexanol	1371	1372	1360	LO	Green, grassy	5.6
	Butanol	1165	1166	1147	NA	Solvent-like	459
	Heptanol	1471		1451	LO	Fresh, nutty	5.4
	1-Penten-3-ol	1178	1177	1181	LO	Burnt, meaty	358.1
	(Z)-3-Hexenol	1398	1397	1378	NA	Grassy	70
	1-Octen-3-ol	1464	1464	1445	LO	Fishy, grassy	1.5
	2-Furanmethanol	1675		1685	M	Burnt sugar	4500
	2-Phenylethanol	1935	1936	1925	F	Honey, yeasty	564
	3-Methyl-1-butanol	1226	1222	1205	F	Balsamic	4
	2-Methyl-1-propanol	1112	1110	1084	F	Solvent-like	6505
Pyrazines	*Pyrazine*	1218		1194	M	Pungent-sweet	18×10^4
	2-Methylpyrazine	1280		1249	M	Roasted, popcorn	6×10^4
	2-Ethylpyrazine	1349		1354	M	Nut, roasted	6×10^3
	2,5-Dimethylpyrazine	1338	1335	1348	M	Roasted	1700
	2,6-Dimethylpyrazine	1344		1308	M	Nut, roasted	1500
	2,3-Dimethylpyrazine	1361		1377	M	Nut, green	2500
	2-Ethyl-6-methylpyrazine	1397		1375	M	Potato, nut	6×10^3
	2-Ethyl-5-methylpyrazine	1402		1386	M	Nut, roasted	100
	2-Ethyl-3-methylpyrazine	1417		1384	M	Nut, roasted	130
	2-Vinylpyrazine	1450		1429	M	burnt, nut	700
	2-Ethyl-3,5-dimethylpyrazine	1460		1441	M	Peanut, nut, caramel	0.04
Esters	*Ethyl acetate*	897		885	F	Fruity, orange	5
	Ethyl hexanoate	1251		1229	F	Fruity, orange	2.2
	Ethyl octanoate	1448	1445	1427	F	Fruity, orange	19.4
	Ethyl benzoate	1690	1683	1675	F	Flowery, musty	55.6
Ketones	2,3-Butanedione	986	985	955	F,M	Butter, caramel	0.06
	3-Hydroxy-2-butanone	1301		1278	F,M	Butterscotch	8×10^3
	1-Hydroxy-2-propanone	1313		1295	M	Caramel	10^5
	2,3-Pentanedione	1074	1073	1054	M	Cream, butter	20
	2-Heptanone	1192	1190	1170	LO	Soap	14×10^4
	2-Butanone	905	906	900	NA	Ether-like	35,400
	6-Methyl-5-hepten-2-one	1354		1345	NA	Sweet, fruity	68
	Dihydro-2-methyl-3(2H)-furanone	1278		1304	M	Roasted	NA
Furans	*2-Ethylfuran*	952		960	LO	Rubber-like	2.3
	2-Pentylfuran	1247		1231	LO, M,	Green bean	5.8
	2-Methylfuran	871		877	M	Ether-like	3.5
	2-Furancarboxaldehyde	1478	1476	1457	M	Bread, almond	3×10^3
	1-(2-furyl)ethanone	1522	1520	1490	M	Balsamic	8×10^4
	5-Methyl-2-furancarboxaldehyde	1588	1586	1567	M	Almond, spicy	6.0
Other compounds	*1H-Pyrrole*	1531		1507	M	Nut	49,600
	1-(1H-pyrrol-2-yl)ethanone	1961		1975	M	Herbal, nut	58,585
	1,3-Thiazole	1262		1249	M	Nut	NA
	αβ-Farnesene	1679		1648	NA	Citrus, green	NA
	2-Methoxy-4-vinylphenol	2095		2033	M	Wood, amber	12
	3-Ethyl-2-methyl-1,3-hexadiene	1430		1421	NA	NA	NA

RI from literature were selected base on the range of values obtained on the same chromatographic phase.
RI of reference compounds obtained by GC separation on DB-wax column.
Full identification obtained by comparison to LRI and mass spectrum of reference compound. Tentative identification obtained by comparison with LRI from the literature and mass spectra from spectra library. Tentatively identified volatiles are written in *Italic*.

[*] F – yeast fermentation, LO – lipid oxidation, M – Maillard reaction, NA – not available.
[a] www.Chemspider.com.
[b] www.flavornet.org.
[c] Giri et al. (2010).
[d] Buttery, Orts, Takeoka, and Nam (1999).
[e] Yang, Lee, Jeong, Kim, and Kays (2008).
[f] Leffingwell.com.
[g] Fors (1983).

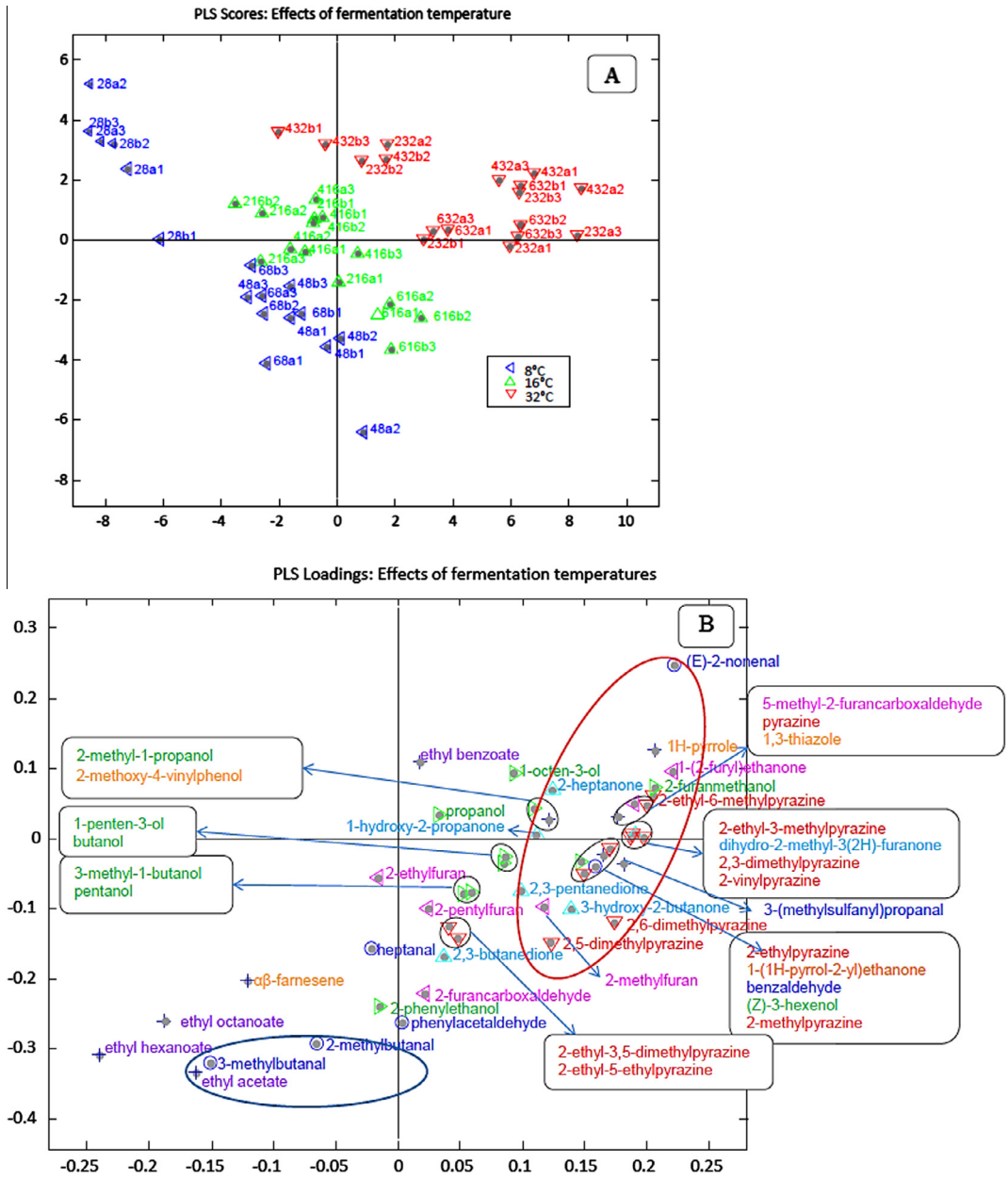

Fig. 1. PLS score plot (A) and loading plot (B) showing the effects of fermentation temperatures on the formations of volatile compounds in the crust of whole meal bread. Only compounds with significant values are shown. The first two principal components explained 43% of the total variance. Blue and red triangles show samples fermented at low (8 °C) and high (32 °C) fermentation temperatures respectively and their correspond compounds in the loading plot. Blue and red ellipses in the loading plot show volatile compounds in the bread crust fermented at low and high fermentation temperatures respectively. Sample code: yeast level, fermentation temperature, treatment duplicate, analysis triplicate. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

products (nonanal, pentanal, pentanol, hexanol, heptanol) were not influenced by fermentation temperature.

3.3.1. High fermentation temperature

Based on two-way ANOVA (Table 3), fermentation at 32 °C contributes to significantly higher peak areas of 3-methyl-1-butanol, pyrazine, 2-ethylpyrazine, 2-ethyl-3-methylpyrazine, 2-vinylpyrazine, 3-hydroxy-2-butanone, 3-(methylsulfanyl)-propanal, and 5-methyl-2-furancarboxaldehyde in the bread crust compared to lower fermentation temperatures. Additionally, the one-way ANOVA also shows relatively higher peak areas for the remaining pyrazine derivatives, (E)-2-nonenal, dihydro-2-methyl-3(2H)-furanone, 2,3-pentanedione, 1,3-thiazole, 1H-pyrrole, 1-(1H-pyrrol-2-yl)-ethanone, 1-(2-furyl)-ethanone and 2-furanmethanol (Table 3). All these compounds are located close to or within the red ellipse in Fig. 1B, which corresponds to samples fermented at high temperature and most of them, are generated through Maillard reactions.

(E)-2-nonenal and 3-(methylsulfanyl)-propanal were reported as important compounds for wheat bread crust (Cho & Peterson, 2010; Grosch & Schieberle, 1997; Schieberle & Grosch, 1991). Due to the relatively higher level of these compounds in bread fermented at high temperature in the present work, and as the OT-value for each compound is very low (Table 2), they are probably also important for the sensed odor of the crust. Zehentbauer and Grosch (1998a) found the content of (E)-2-nonenal and 3-(methylsulfanyl)-propanal were influenced by fermentation conditions as they found higher content of (E)-2-nonenal in bread made

Table 3
Mean peak area (x10^6) of compounds identified in the crust of whole meal wheat bread influenced by yeast concentrations and fermentation temperatures. The results of two-way ANOVA are shown for the two factors; yeast concentration (yc) and fermentation temperature (ft) and their possible interaction (yc * ft).[a]

Aroma compound	Aroma compound peak area value in bread crust samples (mean value)									Two way ANOVA								
	2%			4%			6%			yc * ft	yc (%)				Ft (°C)			
	8 °C	16 °C	32 °C	8 °C	16 °C	32 °C	8 °C	16 °C	32 °C	p-value	p-value	2	4	6	p-value	8	16	32
Aldehydes																		
Hexanal	7.64	8.61	8.27	6.82	6.94	5.63	6.75	7.01	6.53	ns	0.0004	a	b	b	ns			
Heptanal	0.59bc	0.65ab	0.75a	0.63ab	0.51d	0.51d	0.66ab	0.64ab	0.59bc	<0.0001								
Nonanal	0.63	0.65	0.70	0.60	0.60	0.72	0.61	0.71	0.80	ns	ns				ns			
Pentanal	1.04	1.25	1.37	1.01	0.93	0.97	0.97	1.10	1.08	ns	0.0005	a	b	ab	ns			
2-Methylbutanal	23.8ab	22.3ab	21.4b	23.6ab	17.4 cd	13.3e	25.9a	19.2c	15.3d	0.0017								
3-Methylbutanal	21.2a	19.1a	16.6ab	22.3a	15.7ab	8.5c	23.0a	15.4ab	11.2bc	<0.0001								
2-Methypropanal	1.72	3.08	2.52	2.29	1.63	1.58	3.74	2.86	2.36	ns	0.0191	ab	b	a	ns			
(E)-2-Nonenal	0.17d	0.27ab	0.31a	0.19 cd	0.17d	0.32a	0.21bcd	0.26ac	0.31a	<0.0001								
Benzaldehyde	1.71e	3.03bc	3.37ab	3.08bc	2.71 cd	3.22ab	2.53d	3.74a	3.50ab	<0.0001								
Phenylacetaldehyde	2.58d	5.01bc	5.79bc	7.15a	5.90bc	4.40c	5.06bc	5.14bc	4.60bc	<0.0001								
3-(Methylsulfanyl)propanal	0.26	0.42	0.76	0.31	0.34	0.64	0.51	0.62	0.72	ns	<0.0001	b	b	a	<0.0001	b	b	a
Alcohols																		
Propanol	3.19bc	2.90 cd	2.39 cd	2.14d	2.52 cd	2.37 cd	1.92d	5.15a	4.18ab	<0.0001								
Pentanol	1.69b	2.38a	2.20a	2.24a	1.81b	2.00ab	1.97ab	2.37a	2.28a	0.0070								
Hexanol	8.51	11.1	11.3	10.0	10.2	10.1	8.47	10.2	10.4	ns	ns				ns			
Butanol	0.32e	0.70 cd	0.73 cd	0.78bc	0.70 cd	0.75bc	1.12b	1.13b	1.53a	<0.0001								
Heptanol	0.22	0.34	0.32	0.31	0.32	0.31	0.28	0.32	0.33	ns	ns				ns			
1-Penten-3-ol	3.28 cd	4.52a	4.54a	4.22b	4.31ab	3.60bc	2.94d	4.57a	4.54a	0.0068								
(Z)-3-Hexenol	0.18e	0.28 cd	0.31bc	0.30bc	0.31bc	0.32abc	0.23de	0.38a	0.37ab	<0.0001								
1-Octen-3-ol	0.93ef	1.67a	1.47ab	1.36bc	1.0def	1.15cde	0.80ef	1.32bcd	1.38abc	<0.0001								
2-Furanmethanol	1.09d	3.82 cd	16.1a	7.97bc	8.67b	12.1ab	3.09d	9.38b	9.69b	<0.0001								
2-Phenylethanol	3.23c	3.19c	4.14c	9.71a	8.71b	7.15b	8.51ab	9.94a	9.50a	<0.0001								
3-Methyl-1-butanol	68.3	70.4	71.9	78.8	82.3	82.5	83.8	97.6	97.2	ns	<0.0001	c	b	a	0.0210	c	b	a
2-Methyl-1-propanol	22.7	29.7	28.5	27.9	36.4	32.0	29.9	40.5	48.8	ns	<0.0001	b	b	a	<0.0001	b	a	a
Pyrazines																		
Pyrazine	0.37	1.28	3.86	1.72	2.56	3.76	1.79	3.48	3.61	ns	0.0404	b	a	a	<0.0001	c	b	a
2-Methylpyrazine	2.81d	11.4c	24.6ab	22.4ab	22.4ab	23.9ab	17.6bc	25.4ab	26.6a	<0.0001								
2-Ethylpyrazine	0.33	1.74	3.98	3.67	3.97	4.84	2.39	4.35	5.85	ns	0.0002	b	a	a	0.0068	c	b	a
2,5-Dimethylpyrazine	1.36d	4.08c	7.01b	8.06ab	8.14ab	8.64ab	6.41bc	8.92ab	9.85a	<0.0001								
2,6-Dimethylpyrazine	0.77c	3.24b	7.01a	6.88a	7.17a	7.91a	4.63b	7.62a	8.49a	<0.0001								
2-Ethyl-6-methylpyrazine	0.06d	0.75 cd	2.85a	1.55bc	1.51bc	2.46ab	1.07c	2.30ab	2.71a	<0.0001								
2,3-Dimethylpyrazine	0.13e	0.82de	2.94a	1.72bcd	1.71bcd	2.47ab	1.08cde	2.20abc	2.49ab	<0.0001								
2-Ethyl-5-methylpyrazine	0.63e	0.65de	0.67de	1.36b	0.55e	0.94 cd	0.99c	2.11a	2.22a	<0.0001								
2-Ethyl-3-methylpyrazine	0.02	0.73	1.91	1.29	1.24	1.95	0.75	1.87	2.43	ns	0.0034	b	a	a	<0.0001	c	b	a
2-Vinylpyrazine	0.05	0.27	0.54	0.41	0.42	0.65	0.38	0.66	0.67	ns	<0.0001	b	a	a	<0.0001	c	b	a
2-Ethyl-3,5-dimethylpyrazine	0.29	0.36	0.36	0.44	0.51	0.53	0.56	0.53	0.59	ns	<0.0001	b	a	a	ns			
Esters																		
Ethyl acetate	6.88b	6.62c	6.28d	6.90b	6.61c	4.52e	10.6a	10.5a	4.60e	<0.0001								
Ethyl hexanoate	0.23a	0.16 cd	0.07e	0.20ab	0.12d	0.08e	0.13d	0.17bc	0.08e	<0.0001								
Ethyl octanoate	0.23b	0.21b	0.11d	0.25ab	0.17c	0.20bc	0.24b	0.30a	0.20bc	<0.0001								
Ethyl benzoate	0.44a	0.46a	0.42a	0.40ab	0.31bc	0.40ab	0.26c	0.32bc	0.31bc	<0.0001								
Ketones																		
2,3-Butanedione	14.6e	21.3d	22.5 cd	26.9bc	23.5 cd	21.0d	21.8 cd	32.4a	30.7b	<0.0001								
3-Hydroxy-2-butanone	27.2	29.0	35.5	42.2	40.0	43.4	39.4	44.1	46.3	ns	<0.0001	b	a	a	0.0002	b	b	a
2,3-Pentanedione	1.26e	3.06d	5.69a	3.33 cd	3.23 cd	4.38bcd	3.75bcd	4.53abc	4.78ab	<0.0001								
1-Hydroxy-2-propanone	8.61	2.03	1.44	10.8	4.84	1.36	7.25	10.5	12.9	ns	ns				0.0068	ab	b	a
2-Heptanone	0.49c	0.73b	1.01a	0.71b	0.70b	0.70b	0.74b	0.8b	0.78b	<0.0001								
2-Butanone	0.56	0.93	0.82	0.63	0.70	0.66	1.00	0.78	0.67	ns	ns				ns			
6-Methyl-5-hepten-2-one	0.29	0.36	0.45	0.22	0.22	0.30	0.31	0.31	0.32	ns	0.0125	a	b	ab	ns			

(*continued on next page*)

Table 3 (*continued*)

Aroma compound	Aroma compound peak area value in bread crust samples (mean value)												Two way ANOVA								
	2%			4%				6%					yc * ft	yc (%)				Ft (°C)			
	8 °C	16 °C	32 °C	8 °C	16 °C	32 °C	8 °C	16 °C	32 °C	p-value	p-value	2	4	6	p-value	8	16	32
Dihydro-2-methyl-3(2H)-furanone	0.57e	1.50de	4.71a	2.57bcd	2.22 cd	3.77ab	2.36bcd	3.81ab	3.84ab	<0.0001								
Furans																		
2-Ethylfuran	0.38 cd	0.50a	0.47ab	0.35d	0.41bcd	0.32d	0.46abc	0.37bcd	0.35bcd	0.0055								
2-Pentylfuran	3.03b	4.23a	4.11a	3.35b	3.18b	2.91b	3.24b	3.47ab	3.02b	0.0013								
2-Methylfuran	0.06	0.12	0.17	0.11	0.12	0.16	0.15	0.15	0.14	ns	ns				0.0447	b	ab	a
2-Furancarboxaldehyde	1.27c	1.52ab	1.55ab	1.68a	1.40bc	1.43bc	1.43bc	1.51ab	1.49b	0.0034								
5-Methyl-2-furancarboxaldehyde	0.13	0.70	3.01	1.20	1.29	4.26	0.76	3.76	3.91	ns	0.0589	b	ab	a	<0.0001	b	b	a
1-(2-furyl)ethanone	1.21e	6.63de	20.3a	9.01d	9.25 cd	16.0ab	6.83de	11.3bcd	15.4abc	<0.0001								
Other compounds																		
1H-Pyrrole	0.16c	0.59ab	0.61ab	0.26c	0.28c	0.65a	0.46b	0.49ab	0.66a	<0.0001								
1,3-Thiazole	0.16d	0.37ab	0.41a	0.29c	0.31bc	0.36b	0.32bc	0.35bc	0.37ab	<0.0001								
1-(1H-pyrrol-2-yl)ethanone	0.19c	0.21bc	0.68a	0.56a	0.53a	0.57a	0.34b	0.55a	0.63a	<0.0001								
α/β-Farnesene	1.57	1.67	1.23	1.56	1.46	1.21	1.48	1.51	1.18	ns	ns				0.0243	a	ab	b
2-Methoxy-4-vinylphenol	0.26 cd	0.25 cd	0.22d	0.62a	0.48abc	0.55ab	0.33bcd	0.37abcd	0.56abc	0.0259								
3-Ethyl-2-methyl-1,3-hexadiene	0.34	0.45	0.37	0.35	0.30	0.31	0.41	0.37	0.38	ns	0.0330	a	b	ab	ns			

Different letters in the same row indicate significant differences (significant different 95%). When the interaction between yc * ft is significant, the p-value are values for the combination effects and the letters are shown for each concentration.

All compounds were identified based on comparing mass spectra and RI using series of hydrocarbons and comparing with those reported previously in the published literatures and several authentic databases. Tentatively identified volatiles are written in *Italic*.

[a] If a significant interaction effect was found between yc * ft, the two-way ANOVA is not performed on the main effects; yeast concentration and fermentation temperature separately but one-way ANOVA on the combination factor of both effects. Hence the interaction effect is then the most important effect.

with conventional bread making procedure and higher content of 3-(methylsulfanyl)-propanal in bread made according to artisan recipe. 3-(Methylsulfanyl)-propanal can be formed from the amino acid methionine via the Strecker degradation and by yeast activity via Ehrlich pathway which involves the transamination into α-keto-γ-(methylthio)butyrate (α-KMBA), decarboxylation into 3-(methylsulfanyl)-propanal and reduction into methionol (Hazelwood, Daran, van Maris, Pronk, & Dickinson, 2008). Fermentation temperature of 32 °C is within the range of where the activity of yeast is at optimum and therefore may enhanced the formation of volatile compounds generated via Ehrlich pathway. Besides, the formation of 3-(methylsulfanyl)-propanal is accelerated at high temperature, in this case during baking, when the amino acid is available (Stadler & Lineback, 2009). Meanwhile, (E)-2-nonenal with a green-like odor can be generated by oxidation of linoleic acid by the activity of endogenous flour lipoxygenases during the storage of flours and during dough mixing and fermentation (Belitz, Grosch, & Schieberle, 2009; Poinot et al., 2008).

Large numbers of Maillard reaction products for example most of pyrazine derivatives, furan derivatives and pyrroles were present in the samples in this study, however, the OT-values of most Maillard reaction products are relatively high, and each of them might be sensory less important. Although the compounds with high OT-values, especially most pyrazines and some furans derivatives, are less important than compounds with low OT-values (Table 2), the combination of several compounds with high OT-values might make them important for the crust aroma of the present work (Table 2). Meanwhile, some of the Maillard reaction products such as 2-ethyl-3,5-dimethylpyrazine, 3-(methylsulfanyl)-propanal, 2-ethylfuran, 2-methylfuran, 2-pentylfuran, and 5-methyl-2-furfural have all low OT-values (Table 2), and should be considered as important compounds in the present work. As mentioned by El-dash and Johnson (1970), higher temperature during the initial state of baking lysed the yeast cells and released amino acids to the dough, and this could enhance the formation of Maillard reaction products especially in the crust being directly exposed to the heat during baking.

3.3.2. Low fermentation temperature

The crust of bread fermented at low temperature had lower relative mean peak areas of all identified compounds except for 2- and 3-methylbutanal and ethyl acetate (Fig. 1B; Table 3). The highest peak areas of 2- and 3-methylbutanal, which have malty odors (Table 2), were found in all bread fermented at 8 °C (Fig. 1B, *blue ellipse*). According to Zehentbauer and Grosch (1998a), 2- and 3-methylbutanal were regarded as key aroma compounds in baguette crust, and they also found higher content of 2- and 3-methylbutanal in baguette crust made from artisan recipe with low yeast amount, with the addition of pre-fermented dough and final fermentation at low temperature in long time. The sensory importance of 2- and 3-methylbutanal was confirmed by their low OT-values (Table 2). They can be formed by via Strecker degradation as part of the Maillard reactions by amino acid leucine during baking (Pozo-Bayón, Guichard, & Cayot, 2006). The Strecker degradation reaction results in the formation of unstable Schiff's bases which decarboxylate to form enamines that subsequently undergo hydrolysis to form an aldehyde. 2- and 3-methylbutanal can also be formed during fermentation of dough via the Ehrlich pathways (Perpe'te & Collin, 2000). Samples fermented at low temperatures (8 °C and 16 °C) resulted in higher peak areas of the esters ethyl acetate, ethyl octanoate and ethyl hexanoate compared to samples fermented at high temperature (32 °C) (Table 3; Fig. 1B). These esters with a fruity and sweet odor (Table 2) can be formed enzymatically by yeast during dough fermentation (Hazelwood et al., 2008).

3.4. Effect of yeast concentration

The PLS plots shown in Fig. 2A demonstrate a clear effect of yeast content on the level of volatile compounds. The first two principal components explained 45% of the total variance (Fig. 2A and B). Samples fermented with 2% yeast are placed to the left of the score plot, while samples with higher yeast levels are placed further to the right. However, samples fermented with 6% yeast at 8 °C are clustered close to the samples fermented with 4% yeast and they are placed close to '0' line of PC1. Samples fermented with the combination of low yeast level (2%) and low temperature (8 °C) seems to be separated from other samples (Fig. 2A). PLS loading plot shows that with higher amount of yeast, relatively higher levels of most of the compounds found in the crust were observed. Additionally, ANOVA showed that 15 volatile compounds were significantly influenced by yeast level (Table 3).

3.4.1. High yeast level

Bread fermented with high yeast level (6%) was characterized by having significantly higher peak areas of 3-methyl-1-butanol, 2-methyl-1-propanol and 3-(methylsulfanyl)-propanal, and lower peak area of hexanal compared to bread made with lower yeast level (Table 3, Fig. 2A). 3-Methyl-1-butanol and 2-methyl-1-propanol are often found in bread crust (Bianchi et al., 2008; Jensen et al., 2011), and 3-methyl-1-butanol (OT-value 4 μg/L) is expected to be sensory more important than 2-methyl-1-propanol (OT-value 6505 μg/L) due to the much lower OT values (Table 2).

The alcohols 2-phenylethanol, 3-methyl-1-butanol and 2-methyl-1-propanol found in this present work are fermentation products from the Ehrlich pathways, and these compounds were also found by Frasse et al. (1992) in dough fermented with yeast compared to dough without the addition of yeast. Schieberle and Grosch (1991) found that 2-phenylethanol with honey and yeast-like odor was an important compound in bread crumb but not in the crust. Higher level of 2-phenylethanol was also found in the crumb of the same whole meal bread as analyzed in this study when it was fermented with high yeast level at high temperature (Nor Qhairul Izzreen et al., 2016). Birch, Petersen, and Hansen (2013) also found higher level of 2-phenylethanol in wheat bread crumb when fermented with combination of high yeast level and fermentation temperature. 3-(Methylsulfanyl)-propanal, 2- and 3-methylbutanal, 3-methyl-1-butanol, 2-methyl-1-propanol, 2-phenylethanol, ethyl acetate, ethyl octanoate and 2,3-butanedione can be formed during dough fermentation, which is an anaerobic process where the decarboxylation of α-keto acid produces aldehydes that can be reduced into fusel alcohols via the Ehrlich pathway from the catabolism of amino acids (Hazelwood et al., 2008). 2,3-Butanedione can be formed from decarboxylation of 2-acetolactate via the same pathway. In general, the formation of compounds during yeast fermentation was through the Ehrlich pathways and the formation was elevated when higher amount of yeast was used for the fermentation and contributed to their higher levels in the crust of this bread.

Also, high relative peak areas of the Maillard reaction products pyrazine, 2-ethylpyrazine, 2-ethyl-3-methylpyrazine, 2-vinylpyrazine, 2-ethyl-3,5-dimethylpyrazine and 3-hydroxy-2-butanone were found in bread fermented with 4% and 6% yeast compared to low yeast level. Interaction between yeast level and temperature were seen for several compounds, and based on one-way ANOVA, the mean peak areas of propanol, butanol, 2-phenylethanol, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2-ethyl-5-methylpyrazine, ethyl acetate, ethyl octanoate and 2,3-butanedione were higher in bread fermented with high yeast levels, especially at 16 and 32 °C (Table 3). Among those compounds, 2,3-butanedione have been noted as an important compound in the crust of wheat baguette (Zehentbauer & Grosch, 1998a), and 3-(methylsulfanyl)-propanal, 2-ethyl-3,5-dimethylpyrazine and 2-ethyl-3 and 5-methylpyrazine were found to be important compounds in wheat and rye bread crust (Grosch & Schieberle, 1997; Zehentbauer & Grosch, 1998a, 1998b). High peak areas of those compounds together with the esters ethyl acetate and ethyl octanoate might be sensorily important for the aroma of the bread crust due to their low OT-values (Table 2). The higher level of several Maillard reaction products in the present bread fermented with high yeast level compared to low yeast level can be explained by higher content of some free amino acids as Maillard precursors in yeast fermented dough (El-dash & Johnson, 1970). El-dash and Johnson (1970) found that the addition of yeast to the dough increased the total content of amino acids in the dough which were measured after the yeast cells were lysed. The total amount of free amino acids was then decreased at the end of fermentation as yeast used free amino acid for the metabolism during fermentation, however, the concentration increased for the following amino acid methionine, glycine, lysine and proline. This is supported by Collar, Mascaros, and Debarber (1992) as they observed a decrease of total amino acids in yeast fermented dough compared to a bacteria fermented dough. El-dash and Johnson (1970) also found that 95% of the total free amino acid in fermented dough was used for crust formation during baking.

3.4.2. Low yeast level

Hexanal was the only compound that had significantly higher peak area in the crust of bread fermented with low yeast amount compared to the other bread (Table 3). This can also be seen by comparing the PLS score and loading plots in Fig. 2A and B where hexanal is placed corresponding to the samples made with low yeast amount. This is in accordance with Frasse et al. (1992) who reported that the aldehydes were decreased when yeast was added to the dough compared to un-yeasted dough.

Hexanal is basically a product of lipid oxidation, and has low OT-value, which might be important for the bread crust in the present work. Also, the level of ethyl benzoate was higher in the crust of bread fermented with low yeast level compared to higher yeast level in this study (Table 3; Fig. 2B, blue ellipse), however, this compound might not contribute much to the crust odor due to its high OT-value (Table 2). For the samples fermented with the combination of low yeast level and low temperature, the peak areas of most of the compounds were relatively low compared to other samples (Table 3; Fig. 2A and B). Dough fermented with low yeast level has limited source of amino acids contributed by the flour and yeast. As expected dough fermented with low yeast level had relatively lower levels of the compounds formed by yeast activity as reported by Pico, Bernal, and Gomez (2015).

3.5. General discussion of compounds identified

Most of the compounds found in the crust of the whole meal bread in this study were similar to the compounds found in the crust of Altamura bread (Bianchi et al., 2008), whole meal wheat bread (Jensen et al., 2011) and in whole meal wheat bread made from hard white and red winter wheat (Chang et al., 1995). The compounds 2-acetyl-1-pyrroline, (E,E)-2,4-decadienal, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, (Z)-2-nonenal, (Z)-4-heptenal, 1-(Z)-5-octadien-3-one, (E,Z)-2,6-nonadienal, 1-octen-3-one, (E,E)-2,4-nonadienal and 2-acetyl-tetrahydropyridine have earlier been reported as important components in bread crust aroma (Cho & Peterson, 2010; Grosch & Schieberle, 1997; Moskowitz et al., 2012; Raffo, Carcea, Castagna, & Magri, 2015; Schieberle & Grosch, 1991; Zehentbauer & Grosch, 1998a), but were not found in the crust of this present bread. This is probably due to different extraction methods used, since most of these studies used solvent extraction, which is known to give a volatile profile that is very dif-

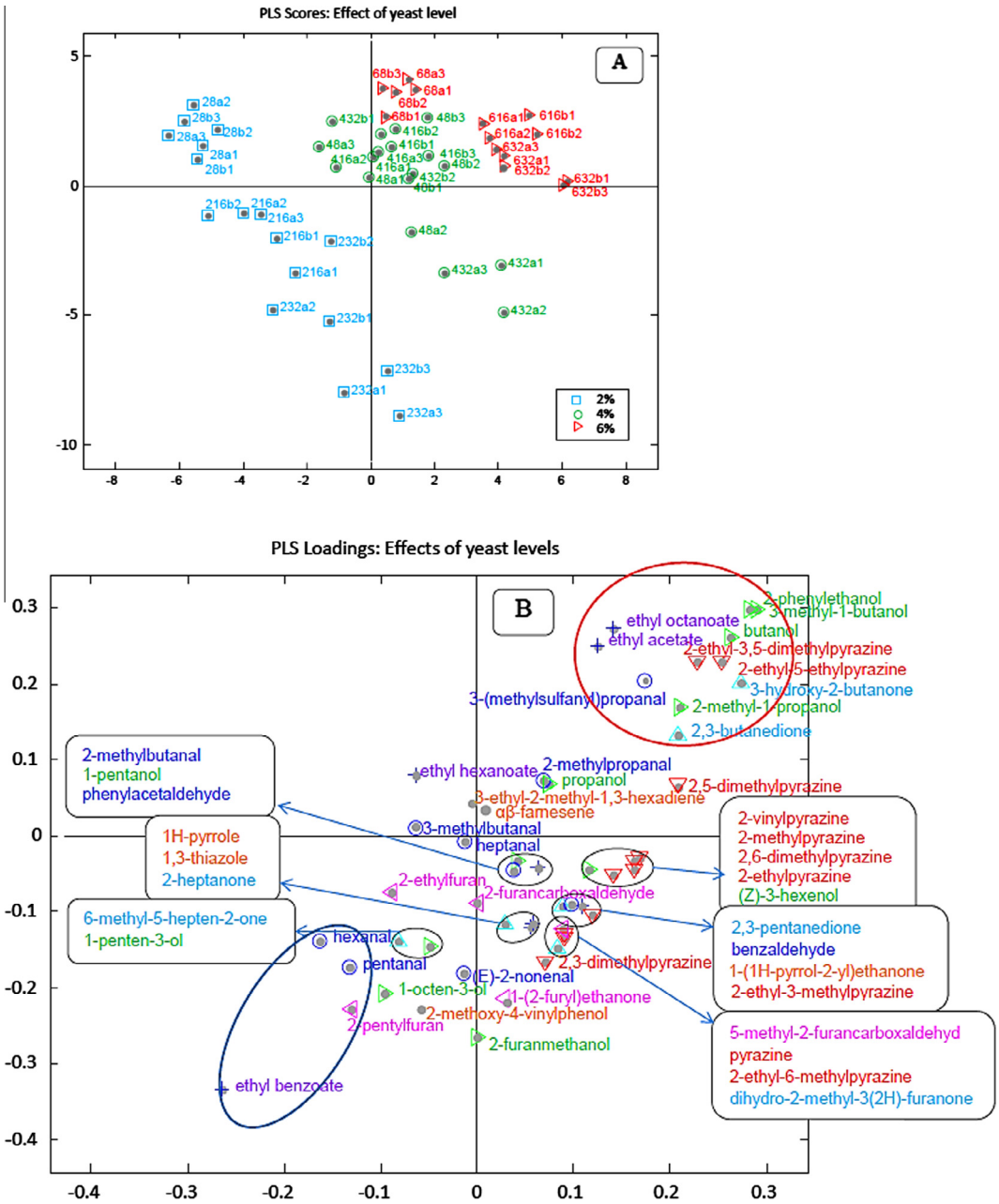

Fig. 2. PLS score plot (A) and loading plot (B) showing the effects of yeast levels on the formation of volatile compounds in the crust of whole meal bread. Only compounds with significant values are shown. The first two principal components explained 45% of the total variance. Blue and red triangles show samples fermented at low (2%) and high yeast levels (6%) respectively and their correspond compounds in the loading plot. Blue and red ellipses in the loading plot show volatile compounds in the bread crust fermented at low and high fermentation temperatures respectively. Sample code: yeast level, fermentation temperature, treatment duplicate, analysis triplicate. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ferent from headspace extraction methods. However, some compounds (Z)-4-heptenal, 1-octen-3-one, (E,E)-2,4-nonadienal, (E,E)-2,4-decadienal, (E,Z)-2,6-nonadienal have been identified in bread crust by headspace SPME technique by other researchers which were not dependant on solvent extraction (Paraskevopoulou, Chrysanthou, & Koutidou, 2012; Raffo et al., 2015). Furthermore, (E,E)-2,4-decadienal has been identified by us in the crumb of the same bread as used in this study (Nor Qhairul Izzreen et al., 2016). On the other hand, the important bread crust volatiles 2-acetyl-1-pyrroline and 4-hydroxy-2,5-dimethyl-3(2H)-furanone have very low odor threshold value and are poorly released to the headspace at room temperature (Blank, 2002).

3.6. Comparison of volatile compounds in crumb and crust

The influence of fermentation conditions has also been investigated on the relative levels of volatile compounds in the crumb of the same bread as used in this study (Nor Qhairul Izzreen et al., 2016). Contrary to the volatiles in the bread crumb that were dominated by dough fermentation products, the volatile compounds in the crust are highly influenced by thermal reactions occurring during baking via the Maillard reactions followed by lipid oxidation products and least of fermentation products. Therefore, higher number of aldehydes, furans, pyrazines and ketones were observed in the crust compared to the crumb of the same bread.

Most of the pyrazines derivatives, 5-methyl-2-furancarb oxaldehyde, 2-furanmethanol, 2-methoxy-4-vinylphenol, 2-acetylpyrrole, 3-(methylsulfanyl)-propanal, 1,3-thiazole, 1-hydro xy-2-propanone and dihydro-2-methyl-3(2H)-furanone were found only in the crust of the present work. In particular, 2-ethyl-3,5-dimethylpyrazine (OT-value 0.04 μg/L) might be sensorily important for bread crust since it has the lowest OT-value of the pyrazine derivatives found in the crust samples (Table 2). Paraskevopoulou et al. (2012) found higher content of 5-methyl-2-furancarboxaldehyde, 2-furanmethanol, 2-methoxy-4-vinylp henol and 1-(1H-pyrrol-2-yl)ethanone in the crust of wheat bread than in the crumb. Meanwhile, Jensen et al. (2011) found the presence of 1-hydroxy-2-propanone only in the crumb of whole meal wheat bread, but in the present work, the level of this compound is similar in the crumb as in the crust of the same bread.

2-Methoxy-4-vinylphenol can be formed by thermal degradation of ferulic acid (Belitz et al., 2009), and it is expected to be found in higher content in whole meal bread compared to bread made from refined flour as bran is the major source of ferulic acid (Moskowitz et al., 2012). Even at low levels it can contribute to the clove-like, woody and spicy odor (http://www.chemspider.com).

The highest peak area of the lipid oxidation product hexanal was observed in both crust and crumb of whole meal bread fermented with low yeast level compared to bread made with higher yeast level when the results from this study is compared to the work done by Nor Qhairul Izzreen et al. (2016). However, the level of hexanal is much lower in the crust of the present work than in crumb of the same bread, and this might be due to the high volatility of hexanal and evaporation during baking.

4. Conclusion

The fermentation condition as temperature and yeast level influenced the formation of most of the volatile compounds formed in the crust of whole meal wheat bread. The level of 18 volatile compounds was significantly influenced by yeast level and/or fermentation temperature with a non-significant interaction between the two fermentation parameters. Additionally 36 compounds show significant interaction between fermentation temperature and yeast level.

In general, higher formation of Maillard reaction products and fermentation products was observed in the crust of bread that was fermented with higher amount of yeast and higher temperature. Specifically, high fermentation temperature (32 °C) resulted in relatively higher peak areas of most of the Maillard reaction products and (E)-2-nonenal, compared to lower fermentation temperatures at 8 °C and 16 °C. The crust of bread fermented with high yeast amount had higher levels of the important aroma compounds 3-(methylsulfanyl)-propanal, 2-ethyl-3,5-dimethylpyrazine, ethyl octanoate, 3-hydroxy-2-butanone, 2,3-butanedione and 2-phenylethanol. Bread fermented at low temperature had high levels of ethyl acetate and 2- and 3-methylbutanal. Bread fermented with the combination of low yeast level (2%) at low temperature (8 °C) resulted in lower peak areas of most of the compounds identified in the bread crust.

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