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AROMA OF WHEAT BREAD CRUMB

Effect of fermentation temperature and baker's yeast

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PhD thesis by

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PREFACE

This thesis is based on research done to fulfil the requirements of obtaining a PhD degree at the Faculty of Science (Science) at the University of Copenhagen (KU). The experimental work has been carried out in the Quality and Technology section (Q&T) at Science, KU. The PhD project was financed by Lantmännen-Unibake Research Foundation, Food Research School Denmark and Science, KU, and the financial support is greatly acknowledged.

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Thanks to friends and family for your support during my project. Special thanks to my fantastic mother† and father. Finally, thank you René, Victor, Oscar and August each one of you being so very important to me.

ABSTRACT

Understanding how the dough fermentation conditions influence the wheat bread production time and the bread aroma is important for the bread industry. The overall purpose of this PhD project is to investigate the effects of commercial baker's yeast (level and type) and fermentation temperature on dough expansion and aroma in bread crumb.

In Paper I the effects of commercial baker's yeast (level and type) and fermentation temperature on dough expansion were investigated. Wheat doughs were fermented by seven commercial baker's yeasts (baker's yeast I to VII) at different yeast concentrations ($2.88 \cdot 10^{11}$, $5.76 \cdot 10^{11}$ and $8.64 \cdot 10^{11}$ CFU/kg flour, corresponding to 20-40, 40-80 and 60-120 g/kg flour) and fermentation temperatures (5, 15, 25 and 35°C). Dough expansion was investigated by monitoring the dough height and the expansion profile was found to be described well by a first order kinetic model. The highest kinetic rate constants corresponding to the shortest fermentation times were found for doughs fermented at 25°C and the highest yeast concentration. Doughs fermented with commercial baker's yeast I, II, III and V had shorter fermentation times compared to fermentation with baker's yeast IV, VI and VII. The longest fermentation times were generally found for doughs fermented with all baker's yeasts at 5°C and the lowest yeast concentration ($2.88 \cdot 10^{11}$ CFU/kg flour).

In Paper II, III and V wheat breads were produced for volatile analysis. The dough samples were fermented to equal height and baked and the volatile compounds from the bread crumb were extracted by dynamic headspace sampling and analysed by gas chromatography mass spectrometry. A wide range of volatile compounds was identified in bread crumb, mainly originating from the activity of yeast and from oxidation of flour lipids. The dominating fermentation compounds were alcohols, aldehydes as well as 2,3-butanedione (diacetyl), 3-hydroxy-2-butanone (acetoin), esters and acids. Furthermore, oxidation of flour lipids was generating primarily aldehydes and ketones.

Quantification of the volatile compounds in bread crumb was performed by multiple headspace extraction (Paper II, III and V). The compounds were evaluated according to their odour activity value (OAV). The most aroma active compounds (OAV > 6) identified in bread crumb were; (E)-2-nonanal (green, tallow), 3-methylbutanal (malty), 3-methyl-1-butanol (balsamic, alcoholic), nonanal (citrus), hexanal (green), 2,3-butanedione (buttery, caramel), 1-octen-3-ol (mushroom) and phenylacetaldehyde (honey-like). Esters were also identified in bread crumb (e.g. ethyl acetate, ethyl hexanoate and ethyl octanoate) and they are of interest because of their fruity and pleasant odours, however the OAV's of the esters were generally low (0.1 to 0.5).

In Paper II the effects of yeast level (20, 40 and 60 g baker's yeast VII/kg flour) and fermentation temperature (5, 15 and 35ºC) on aroma in bread crumb were investigated. Fermentation with the highest yeast concentration (60 g/kg flour) resulted in bread containing the highest concentration of the majority of the compounds formed from yeast activity (e.g. 2,3-butanedione and phenylacetaldehyde), compared to doughs fermented at lower yeast concentrations (20 and 40 g/kg flour). A fermentation temperature at 5°C resulted in breads with the highest concentration of the three esters; ethyl acetate, ethyl hexanoate and ethyl octanoate, compared to breads fermented at higher temperatures (15 and 35°C). Fermentation at 15 and 35°C resulted in breads with the highest con-

centration of many lipid oxidation compounds (e.g. heptanal and hexanal) compared to breads fermented at 5°C.

In Paper III the effect of the type of commercial baker's yeast (baker's yeast I to VII, $2.88 \bullet 10^{11}$ yeast CFU/kg flour) on bread aroma was investigated. Breads fermented by commercial baker's yeast I to IV had the significantly highest concentration of 2,3-butanedione and 1-propanol compared to breads fermented by the other yeasts. Furthermore, 3-methylbutanal, 2-methyl-1-propanol and ethyl acetate were found in significantly highest concentration in breads fermented by baker's yeast I and II. Phenylacetaldehyde and 2-phenylethanol were found in the significantly highest concentration in breads fermented by baker's yeast V and VI. The fact that dough fermentation time and bread aroma was significantly influenced by the type of commercial yeast indicates that some of the seven baker´s yeasts might differentiate in yeast strain.

Commercial baker's yeast (level and type) and fermentation temperature are concluded to significantly influence the fermentation time and the aroma profile of bread crumb. Sensory analysis and analysis of acids and non-volatile compounds in breads fermented by the same conditions as in this PhD thesis could be relevant in future research studies.

SAMMENDRAG

Viden om hvordan forskellige fermenteringsbetingelser påvirker produktionstiden og aromaen i hvedebrød er vigtig for brødindustrien. Det overordnede formål med dette PhD projekt er at undersøge effekterne af kommercielle bage gær (niveau og type) og fermenteringstemperatur på dejekspansion og brødaroma.

I Paper I undersøges effekterne af kommercielle bage gær (niveau og type) og fermenteringstemperatur på dejekspansion. Hvededejene blev fermenteret med syv kommercielle gær (bage gær I til VII) ved forskellige gærkoncentrationer ($2.88 \cdot 10^{11}$, $5.76 \cdot 10^{11}$ og $8.64 \cdot 10^{11}$ CFU/kg mel, svarende til 20-40, 40-80 og 60-120 g/kg mel) og fermenteringstemperaturer (5, 15, 25 og 35°C). Dejekspansion blev undersøgt ved at monitorere dejhøjden, og det blev fundet, at ekspansionen af dej kunne beskrives ud fra en første ordens kinetisk model. Den højeste kinetiske hastighedskonstant, svarende til den korteste fermenteringstid, blev fundet for deje fermenteret ved 25°C og den højeste gærkoncentration. Deje fermenteret med de kommercielle bage gær I, II, III og V havde kortere fermenteringstider sammenlignet med deje fermenteret med bage gær IV, VI og VII. De længste fermenteringstider blev generelt fundet for deje fermenteret med alle bage gær ved 5°C og den laveste gærkoncentration ($2.88 \cdot 10^{11}$ CFU/kg mel).

I Paper II, III og V blev der produceret hvedebrød til analyse af flygtige stoffer. Dejene blev fermenteret til den samme højde og bagt, og de flygtige stoffer blev opsamlet og analyseret ved hjælp af dynamisk headspace opsamling og gas kromatografi masse spektrometri. En stor variation af flygtige stoffer blev identificeret i brødkrummen, og de stammer hovedsageligt fra gæraktivitet og oxidation af melets lipider. De dominerende fermenterings stoffer er alkoholer, aldehyder, 2,3-butanedion (diacetyl), 3-hydroxy-2-butanon (acetoin), estrer og syrer. Derudover dannes især aldehyder og ketoner ud fra oxidation af melets lipider.

Kvantificering af de flygtige stoffer i brødkrummen blev foretaget ved hjælp af multiple headspace ekstraktion (Paper II, III og V). Aroma aktiviteten af hvert stof blev evalueret ud fra deres duft aktivitets værdi (odour activity value; OAV). De mest aroma aktive stoffer (OAV > 6), der blev identificeret i brødkrummen, var; (E)-2-nonanal (grøn, talg), 3-methylbutanal (malt), 3-methyl-1-butanol (balsamisk, alkoholisk), nonanal (citrus), hexanal (grøn), 2,3-butanedion (smøragtig, karamel), 1-octen-3-ol (champignon) og phenylacetaldehyd (honning). Der blev også identificeret estrer i brødkrummen (f.eks. ethyl acetat, ethyl hexanoat og ethyl octanoat), og de er interessante på grund af deres frugtagtige og behagelige duft, men OAV'erne af estrene var dog generelt lave (0.1 til 0.5).

I Paper II blev effekterne af gærniveau (20, 40 og 60 g bage gær VII/kg mel) og fermenteringstemperatur (5, 15 og 35ºC) på aroma i brødkrumme undersøgt. Fermentering med den højeste gærkoncentration resulterede i brød indeholdende den højeste koncentration af de fleste fermenteringsstoffer (f.eks. 2,3-butandion og phenylacetaldehyd) sammenlignet med brød fermenteret ved lavere gærkoncentrationer (20 and 40 g/kg mel). En fermenteringstemperatur på 5°C resulterede i brød med den højeste koncentration af de tre estrer; ethyl acetat, ethyl hexanoat og ethyl octanoat, sammenlignet med brød fermenteret ved højere temperaturer (15 og 35°C). Fermentering ved 15 og

35°C resulterede derimod i brød med den højeste koncentration af mange lipid oxidationsprodukter (f.eks. heptanal and hexanal) sammenlignet med brød fermenteret ved 5°C.

I Paper III undersøgtes effekten af kommercielle bage gær (bage gær I til VII, $2.88 \bullet 10^{11}$ gær CFU/kg mel) på brødaroma. Brød fermenteret med bage gær I til IV havde den signifikant højeste koncentration af 2,3-butanedion og 1-propanol sammenlignet med brød fermenteret med bage gær V til VII. Derudover var koncentrationen af 3-methylbutanal, 2-methyl-1-propanol og ethyl acetat signifikant højest i brød fermenteret med bage gær I og II. Koncentrationen af phenylacetaldehyd og 2-phenylethanol var derimod signifikant højest i brød fermenteret med bage gær V og VI. Den kendsgerning, at både fermenteringstiden og brødaromaen var signifikant påvirket af typen af kommerciel bage gær, indikerer at nogle af de syv kommercielle bage gær måske indeholder forskellige gær stammer.

Type og niveau af kommercielle bage gær samt fermenteringstemperatur er tre vigtige faktorer, der signifikant påvirker både fermenteringstiden af dej og aromaprofilen i brødkrummen. Sensoriske analyser og analyser af syrer og ikke-flygtige stoffer i brødkrumme fermenteret under samme betingelser som undersøgt i denne PhD afhandling, kunne være relevant i fremtidige forskningsstudier.

LIST OF PUBLICATIONS

PAPER I

Birch, A. N., van den Berg, F. W. J. and Hansen, Å. S. Expansion profiles of wheat doughs fermented by seven commercial baker's yeasts. Accepted for publication in the *Journal of Cereal Science*.

PAPER II

Birch, A. N., Petersen, M. A. and Hansen, Å. S. The aroma profile of wheat bread crumb influenced by yeast concentration and fermentation temperature. *LWT - Food Science and Technology,* 50, pp. 480-488, 2013.

PAPER III

Birch, A. N., Petersen, M. A., Arneborg, N. and Hansen, Å. S. Influence of commercial baker's yeasts on bread aroma profiles. *Food Research International,* 52 pp. 160-166, 2013.

PAPER IV

Birch, A. N., Petersen, M. A. and Hansen, Å. S. Aroma of wheat bread crumb. Review. Submitted to *Cereal Chemistry*. The outline of the review was pre-approved by *Cereal Chemistry*.

PAPER V

Birch, A. N., Hansen, Å. S. and Petersen, M. A. Multiple headspace extraction - an effective method to quantify aroma compounds in bread crumb. Accepted for publication in Proceedings of the 13th Weurman Flavour Research Symposium, Elsevier. In press.

LIST OF ABBREVIATIONS

AAT	Acetyltransferase
AEDA	Aroma Extract Dilution Analysis
ATF	Acyltransferase
Bf	Complete sample elution volume
Bs	Safe sample volume
Bv	Breakthrough volume
CoA	Coenzym A
DHS	Dynamic Headspace Sampling
DP	Degree of Polymerisation
EHT	Ethanol Hexanoyltransferase
FD	Flavour Dilution
GC	Gas Chromatograph
GC-MS	GC Mass Spectrometry
GC-O	GC Olfactometry
HPLC	High Performance Liquid Chromatography
LAB	Lactic Acid Bacteria
MHE	Multiple Headspace Extraction
OAV	Odour Activity Value
PARAFAC	PARAllel FACtor analysis
PCA	Principal Component Analysis
SPME	Solid Phase Micro Extraction
TIC	Total Ion Chromatogram

TABLE OF CONTENTS

1. INTRODUCTION

Flour, water, salt and yeast are the four basic ingredients that by mixing, fermentation and baking can undergo the unique transformation to aromatic bread. Bread making has been known for over 6000 years (Jacob, 1944) and bread is still a very important part of the diet for people in most parts of the world.

The quality of bread is often described by the texture, the volume and the aroma. For decades research has mainly been focussing on improving bread texture and increasing volume (Young, 2012). However, recently focus from both the consumers and the bread industry has been directed more towards aroma as a quality criterion.

The aroma of wheat bread crumb is mainly formed from the fermentative activity of yeast and from oxidation of flour lipids (Schieberle and Grosch, 1991; Frasse et al., 1992), while the aroma of bread crust is mainly formed by Maillard reactions during baking (Purlis, 2010). Changes in the fermentation conditions therefore mainly influence the aroma formation in the crumb. Today, industrially produced wheat bread is often made from dough with a high concentration of yeast (40-60 g/kg flour) and the dough is fermented at high temperatures (30 to 35°C) in order to decrease the production time (Cauvain, 1998). A short production time (1-2 hrs) results in an economical benefit and a continuous flow on the production line. However, it is generally believed that a longer fermentation time results in bread with a more pleasant aroma (Decock and Cappelle, 2005), although only few studies have been done within this area (Schieberle and Grosch, 1991; Maeda et al., 2009). Fermentation temperature has been found to influence aroma formation during wine fermentation (Beltran et al., 2008), however knowledge within the influence of temperature on volatile formation during dough fermentation is sparse (Gassenmeier and Schieberle, 1995).

Furthermore, little attention has generally been paid towards the influence of the yeast strain in bread making, however research within alcoholic fermentation shows that the strain of yeast is very important for the aroma profile of the final fermented beverage (Procopio et al., 2011; Suárez-Lepe and Morata, 2012). Different commercial baker's yeasts may potentially consist of different strains of *Saccharomyces cerevisiae*.

Different yeast strains might also have different fermentative activities influencing the dough expansion profile and hence the dough fermentation time. Furthermore, changes within yeast level and fermentation temperature will most likely alter the fermentation time of the dough. Thorough investigation of these correlations will provide important information for the baking industry, as dough fermentation time is an important aspect.

Bread staling is an important issue for the bread industry as the shelf-life of wheat bread is markedly decreased by bread firming during storage. A possible influence of the fermentation conditions on bread staling is relevant to investigate, as it is assumed that a long fermentation time might decrease the staling rate, caused by an increased time for starch degradation by α-amylases naturally present in the flour.

1.1 Overall purpose

The overall purpose of this PhD project was to investigate the influence of commercial baker's yeast (level and type) and fermentation temperature on dough expansion and aroma in bread crumb.

1.2 Overview of the research work

An overview of the research work can be seen in **Figure 1** and the purposes of Paper I-V are presented in section 1.2.1 and 1.2.2.

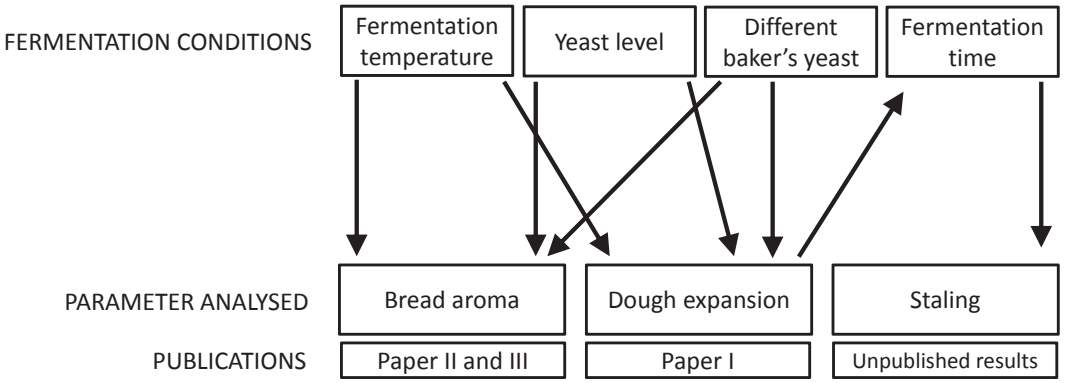

Figure 1. Overview of the research work carried out in this PhD thesis.

1.2.1 Dough expansion

Paper I

The purpose was to investigate the expansion of doughs fermented by seven commercial compressed baker's yeasts by development of a theoretical kinetic model. Simultaneously, the effects of temperature (5, 15, 25 and 35°C) and yeast concentration ($2.88 \cdot 10^{11}$, $5.76 \cdot 10^{11}$ and $8.64 \cdot 10^{11}$ CFU/kg flour) on dough expansion were investigated.

1.2.2 Bread aroma

Paper II

The purpose was to investigate how different yeast concentrations (20, 40 and 60 g Malteserkors/kg flour) and dough fermentation temperatures (5, 15 and 35°C) influence the aroma profile of wheat bread crumb.

Paper III

The purpose was to investigate the influence of seven commercial baker´s yeasts on the formation of bread aroma by comparing the aroma profile of bread fermented at 25°C by seven commercial compressed baker's yeasts ($2.88 \cdot 10^{11}$ CFU/kg flour).

Paper IV

The purpose was to present the compounds identified in wheat bread crumb from 11 research studies from 1979 to 2013 with focus on the most aroma-active compounds. Furthermore, the purpose was to provide an overview of the complex reactions involved in formation of the volatile compounds during dough fermentation. Crust aroma compounds might diffuse into the crumb, therefore an overview of the most aroma-active crust aroma compounds formed during baking were also presented.

Paper V

The purpose was to investigate the possibility of using multiple headspace extraction (MHE) combined with dynamic headspace sampling followed by gas chromatography-mass spectrometry in order to quantify the aroma compounds in bread crumb. The sample set consisted of bread fermented with 40 g Malteserkors/kg flour at 15°C.

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2. BREAD MAKING

The expansion of dough and formation of bread aroma compounds are highly influenced by the ingredients as well as the bread making process. In this section ingredients and processes used in Paper I-III and V are described. Furthermore, the influence of the fermentation conditions on dough expansion is discussed.

2.1 Ingredients

As for all processed food products the quality and the choice of ingredients used in bread making are very important factors for the overall quality of the final bread product. A list of ingredients in the wheat bread produced in Paper I-III and V is presented in **Table 1**.

Table 1. Ingredients in wheat bread produced for Paper I-III and V.

Ingredients	**% of Flour, w/w**
Wheat flour (adjusted to 14% moisture content)	100
Water[a]	61.6 (Paper I and III) and 63.3 (Paper II and V)
Baker's yeast	2-6 (Paper II), 4 (Paper V) and 2-12[b] (Paper I and III)
Salt	1.3
Sugar	1.3

[a]The amount of water was chosen from knowledge of the water uptake of the flour and pre-trials with baking of doughs containing different amounts of water. [b]In Paper I and III the amount (w/w) of baker's yeast varies according to the amount of colony forming units (CFU) in the seven commercial baker's yeasts, three different concentrations were used: Paper I ($2.88 \cdot 10^{11}$, 5, $76 \cdot 10^{11}$ and $8.64 \cdot 10^{11}$ CFU/kg flour) and Paper III ($2.88 \cdot 10^{11}$ CFU/kg flour) .

The amounts of yeast in doughs investigated in Paper II and V were based on the weight of baker's yeast (**Table 1**), as the study was based on one type of commercial yeast (Maltserkors). While in Paper I and III the amount of added yeast to the dough was based on the number of colony forming units (CFU) of yeast cells in seven commercial bakers yeast's (**Table 1**).

2.1.1 Wheat flour

Wheat flour is the main ingredient in typical wheat bread and the main constituents of wheat flour are given in **Table 2**.

Table 2. Constituents of wheat flour (Pedersen and Eggum, 1983; Goesaert et al., 2005).

Wheat flour	**%**
Starch	70-75
Water	14
Proteins (mainly gluten)	10-12
Dietary fibres	2-3
Lipids	2
Minerals	0.7
Sugars	0.5

The gluten proteins (gliadins and glutenins) are highly responsible for formation of the strong, cohesive and viscoelastic network formed during mixing (Li et al., 2004; Barak et al., 2013). The gliadins primarily form intra-chain disulphide bonds with cysteine residues (Shewry and Tatham, 1997) and they have been found to provide extensibility to the dough, allowing the dough to rise during fermentation (Khatkar et al., 1995). The glutenins also form intra-chain disulphide bonds with cysteine residues, but they also form inter-chain disulpide bonds with cysteine residues to other glutenins (Shewry and Tatham, 1997). Glutenins provide elasticity and strength to the dough, preventing the dough from being over-extended and collapse during fermentation or baking (Khatkar et al., 1995). The gluten proteins bind water during mixing (Bot and de Bruijne, 2003), and a high content of gluten and a high gluten quality are generally correlated to a high water uptake of the flour. The gluten content in wheat flour is primarily influenced by the method of cultivation of wheat but also by the variety, while the gluten quality is primarily genetically determined (Shewry et al., 1995). The composition of high molecular weight (HMW) subunits of glutenin has been found to be the dominating factor determining the gluten strength (Popineau et al., 2001). Flours from different wheat varieties might therefore exhibit differences in dough volume capacities, since the gluten content and quality might differ (Khatkar et al., 1995; Barak et al., 2013).

Starch originates from the endosperm of the wheat kernel and starch consists of approximately 25% amylose and 75% amylopectin (Goesaert et al., 2005). Amylose is an essentially linear molecule, consisting of α-(1,4)-linked D-glucopyranosyl units with a degree of polymerisation (DP) in the range of 500–6000 glucose units. In contrast, amylopectin is a very large, highly branched polysaccharide with a DP ranging from $3 \cdot 10^5$ to $3 \cdot 10^6$ glucose units. It is composed of chains of α-(1,4)-linked D-glucopyranosyl residues which are interlinked by α-(1,6)-bonds (Goesaert et al., 2005) .

The level of α-amylases naturally present in wheat flour is important, as α-amylases are responsible for converting hydrated starch molecules into dextrins during baking. α-Amylases has its maximum activity between 60 and 70°C and the α-amylase is inactivated at 85°C (van der Maarel et al., 2002). A high α-amylase activity (Falling number <180 sec) increases the rate of starch degradation and hence the risk of sticky bread (Every and Ross, 1996). Very low α-amylase activity (Falling number > 300 sec) result on the other hand in starch molecules being so large that it complicates the starch gelatinisation, making the bread dry and crumbling. The enzyme activity is mainly influenced by the weather conditions at the time of harvesting (Dornez et al., 2008).

Wheat flour should have a certain quality, before it is accepted for commercial wheat bread making, primarily regarding gluten content, gluten quality, α-amylase activity, and water uptake. The acceptable levels of these parameters in wheat flour for bread making and the standard methods used for testing the flour used in Paper I-III are given in **Table 3**.

Table 3. Acceptable gluten content (% wet gluten), gluten quality (gluten wash), α-amylase activity (Falling number), dough development time (calculated from a farinogram) and water uptake (calculated from a farinogram) in wheat flour for bread making.

Quality test	Acceptable level	Method used in Paper I-III
% Wet gluten	> 26[a]	AACC method no. 38-12 (AACC, 1995b)
Gluten wash	Elastic and coherent[b]	AACC method no. 38-12 (AACC, 1995b)
Falling number	220-250 sec[a]	ICC standard method no. 107/1 (ICC, 1995)
Farinograph dough parameters:		
Water uptake	> 60[c]	AACC method no. 54-21 (AACC, 1995a)

[a]Erekul and Kôhn (2006). [b]Shewry et al.(1995). [c]Cattarall (1998).

Wheat variety has been found to influence the volatile profile of bread, though very few studies have treated this subject (Chang et al., 1995; Brabant et al., 2007). The milling procedure has also been found to affect the aroma profile with significantly higher concentrations of some aroma compounds in whole-meal flour when compared to milled white flour (Czerny and Schieberle, 2002). In the research work of this PhD thesis milled wheat flour from the same batch was used for the experiments for Paper I-III, respectively. The influence of wheat variety and milling procedure on aroma formation in wheat bread was therefore not investigated.

2.1.2 Baker's yeast

Baker's yeast, which consists of the yeast species *Saccharomyces cerevisiae* (**Figure 2**), is the typical leaving agent used in wheat bread making, and it is also the most common yeast species identified on cereals (Poitrenaud, 2004). Commercially produced yeast for bread making was introduced in the late 19th century (Weibiao and Nantawan, 2012). Today, baker's yeast is produced in aseptic systems in which the suitable strain is selected and propagated under sterile conditions. Yeast cells are grown in specific media for cultivation, and substrate availability, pH, aeration rate, and temperature are all important factors influencing yeast growth (Weibiao and Nantawan, 2012).

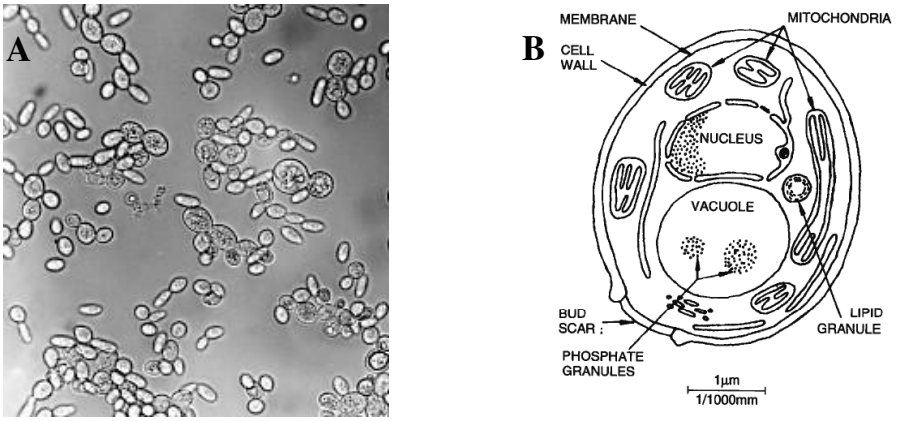

Figure 2. A: Microscopy of *Saccharomyces cerevisiae* cells (Users.rcn.com, 2013). B: The yeast cell (Williams and Pullen, 1998).

The cells of baker's yeast vary in size from 6 to 8 µm and are round to oval in shape (**Figure 2**). One g of compressed baker's yeast with a 30% dry matter content contains about $1*10^{10}$ Colony Forming Units (CFU) (Paper I and III). Yeast is a unicellular organism. In the nucleus the cell's genetic information is stored as DNA (**Figure 2B**) and a yeast cell has around 6000 different genes (Williams and Pullen, 1998). The yeast reproduces by budding and, in ideal conditions, a cell can reproduce itself in 20 min (Williams and Pullen, 1998).

The following types of compressed commercial baker's yeast were included in the research work in Paper I and III: MALTESERKORS from Lallemand, De Danske Gærfabrikker, Grenå, Denmark; SKÆRTOFTMØLLE, organically produced baker's yeast from Agrano, Riegel am Kaiserstuhl, Germany; RAPUNZEL, organically produced baker's yeast from Rapunzel Naturkost AG, Legau, Germany; SEMA from Lallemand, Panevezys, Lithuania; L'HIRONDELLE from Le Saffre, Marcq-en-Baroeul, France; BRUGGEMAN from Algist Bruggeman, Gent, Belgium; ZYMAROM from Algist Bruggeman, Gent, Belgium. In Paper II and V the baker's yeast named MALTESERKORS was the only yeast included in the study. All baker's yeasts were used a few days after the purchase and therefore well before their expiration dates.

2.1.3 Additional ingredients

Water is added to the dough in order to hydrate primarily the gluten proteins and starch molecules. The content of water in wheat dough is typically 55-60% depending on the water uptake of the flour (**Table 3**). The content of water showed no significant effect on dough expansion when addition of 56%, 58%, and 60% w/w flour basis, respectively was added to wheat doughs (Chevallier et al., 2012).

The content of salt in wheat bread is typically 1-2% of the flour. Salt has been found to increase the cross-linking of gluten proteins and hence strengthen the gluten network (Lynch et al., 2009; Beck et al., 2012). Furthermore, salt decreases the metabolic activity of yeast by increasing the osmotic pressure on the semi-permeable membrane of yeast cells (Lynch et al., 2009). Salt also functions as a flavour enhancer, and it is an important taste component in bread (Salovaara et al., 1982; Barylko-Pikielna et al., 1990).

The presence of fermentative sugars in the dough is essential as substrate for the yeast. The level of fermentative sugars in wheat flour is 0.5% of the flour (**Table 2**) and the level of added sugar to wheat dough is usually 1-3% of the flour. The absolute concentration of fermentative sugars in wheat dough is typically so low that the yeast has metabolised all the sugar during fermentation (Maloney and Foy, 2003).

Besides sugar and salt, a large number of additional ingredients are often used in bread making such as ascorbic acid, lipids, enzymes, emulsifiers and shortening, the purpose of these ingredients are generally to improve the bread texture, shelf-life and volume. However, the effects of these additional ingredients on dough expansion or aroma formation were not investigated in the research work of this PhD thesis and they will not be further described.

2.2 Mixing and fermentation

The overall aims of mixing are to disperse the ingredients uniformly, to hydrate particularly the gluten proteins, to add energy required for gluten network formation and to incorporate air bubbles into the dough. When the flour is hydrated under the action of mechanical energy, the gluten proteins gliadin and glutenin form a viscoelastic network as elucidated in **section 2.1.1**.

After mixing, all the oxygen that has been incorporated into the dough is rapidly used up by the yeast (Poitrenaud, 2004). At these anaerobic conditions, the yeast starts to metabolise fermentable sugars into carbon dioxide and ethanol as the two primary products (**Figure 3**) (Lagunas, 1986). *S. cerevisiae* has several transporter mechanisms for taking up mono- or disaccharides (Does and Bisson, 1989; Ozcan and Johnston, 1999). Sugar is often added to the dough to initialise the fermentation process, but also maltose, originating from starch molecules degraded by α-amylases, naturally present in the flour, can be metabolised during yeast fermentation (Bell et al., 2001). The temperature for optimal fermentative activity of *S. cerevisiae* is approximately 25°C (Attfield, 1997).

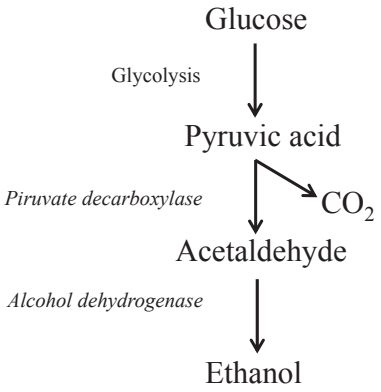

Figure 3. Yeast fermentation (Carballo, 2012).

The carbon dioxide produced initially dissolves in the free water in the dough. When it reaches the saturation point (0.0303kmol m^{-3}), carbon dioxide is transformed into the gaseous form, using the internal pressure on the impermeable gluten network (Shah et al., 1998). The gas diffuses into the air bubbles, which are incorporated and dispersed in the dough during mixing. The elastic and extensible gluten network formed during mixing retains the gas and enables the dough to rise (Maloney and Foy, 2003; Poitrenaud, 2004).

2.3 Bread making procedures used in Paper I-III and V

The mixing procedure and the fermentation conditions used in bread making can vary considerably according to the type of bread product produced (Cauvain, 1998). An overview of the bread making procedure used in the research work of this PhD thesis is shown in **Figure 4**.

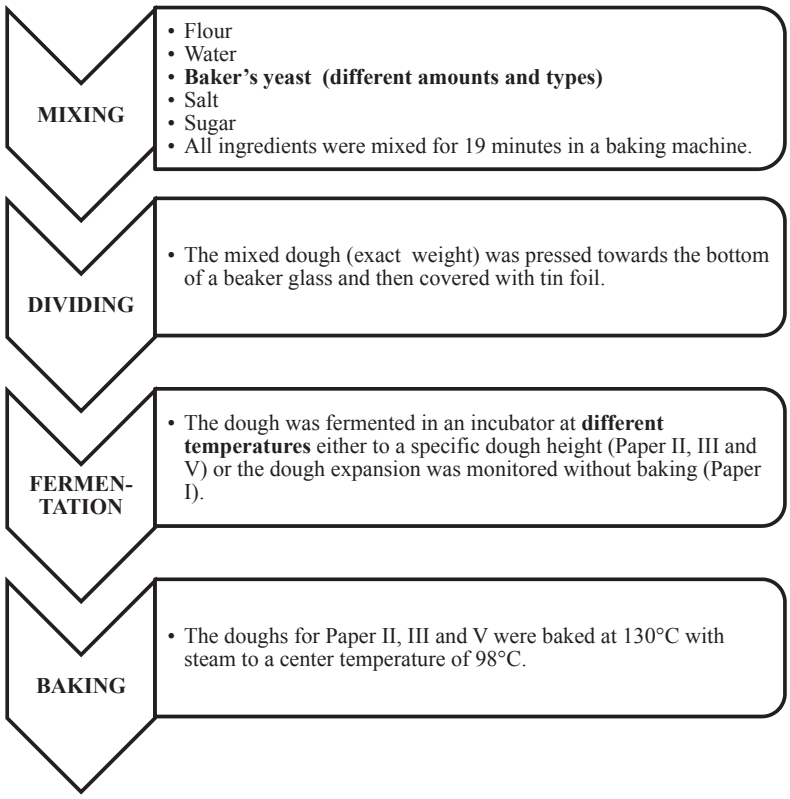

Figure 4. Overview of the bread making procedure used for Paper I-III and V. The conditions that are varied in the bread making procedures for Paper I-III are marked bold.

Initially all the ingredients were mixed, then the dough was divided and left for fermentation at the yeast concentrations and fermentation temperatures specific for Paper I-III and V as described in **section 1.2.**

Dough fermentation times varied greatly according to yeast level, type of commercial baker's yeast and fermentation temperature. The aim in Paper I was to follow the dough expansion at these different fermentation conditions (see **section 1.2**) and this was done by monitoring the dough height by a web camera as exemplified in **Figure 5**. The dough fermentations in Paper II, III and V were terminated (baked) at predefined dough heights. These predefined dough heights were the heights of fully developed doughs, and they were determined by baking experiments and subjective evaluation of the crumb structure of breads baked with different heights. The exact fermentation times to reach the predefined dough height were determined by monitoring the dough expansion in preliminary tests (as exemplified in **Figure 5**). The fermentation times for the doughs in Paper II and III are

presented in **Table 4** and **Table 5**, respectively. In Paper V the fermentation time was 1h (as the dough was fermented by 40g Malteserkors/kg flour at 15°C, **Table 4**).

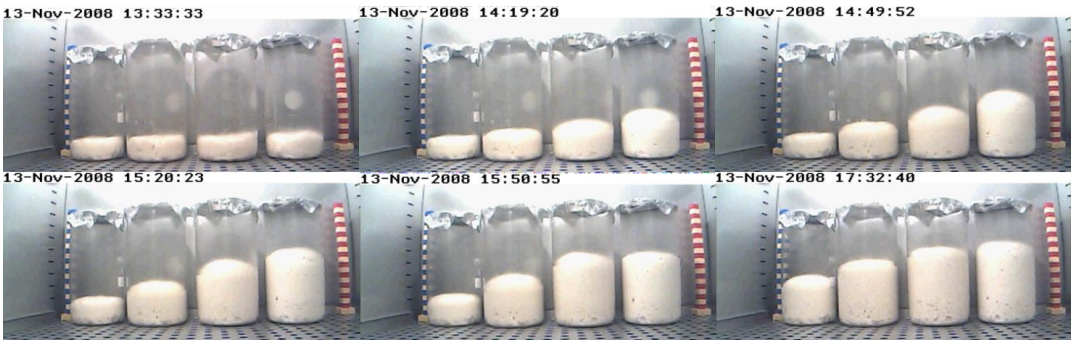

Figure 5. Monitoring dough expansion with a web camera in a temperature controlled incubator. This is an example from a pre-experiment with doughs containing four different yeast concentrations (5, 10, 20 and 40 g Malteserkors/kg flour, respectively from left to right). The dough height can be measured as each lego brick is one cm in height.

Table 4. Fermentation times for the doughs in Paper II to reach 8 cm in height according to fermentation temperature and yeast concentration (g Malteserkors/kg flour).

	Yeast concentration (g Malteserkors/kg flour)		
Fermentation temperature (°C)	20	40	60
5	21 h	3h 30 min	3h
15	3 h	1h	35 min
35	50 min	25 min	15 min

Table 5. Fermentation times in Paper III for doughs fermented with seven commercial baker´s yeasts at a concentration of 2.88• 10^{11} CFU/kg flour and 25°C to reach a total height of 10 cm.

Baker's yeast	Fermentation times (min) for the dough to reach a total height of 10 cm
Malteserkors	100 ± 7
Skærtoftmølle	71 ± 5
Rapunzel	65 ± 3
Sema	60 ± 8
Zymarom	46 ± 2
Bruggeman	40 ± 5
1´Hirondelle	40 ± 6

After fermentation the doughs in Paper II, III and V were baked at 130°C with addition of steam to a core crumb temperature of 98°C. It is generally accepted that baking to a core crumb temperature of 95°C is necessary for the structure to be adequately rigid throughout the loaf (Wiggins, 1998). The doughs in Paper I were not baked, as the aim was to follow the dough expansion (see **section 1.2**).

The procedure illustrated in **Figure 4** is related to the so-called straight dough process, where all the ingredients are added simultaneously before mixing (Cauvain, 1998). Proofing is the final dough-rising step before baking. Proofing is typically performed at a relatively high temperature (around 35°C) and relatively high humidity (80%) and this step is typically used in the straight-dough process (Wiggins, 1998). Proofing was, however, not included in the research work of this PhD thesis, as investigation of the influence of different temperatures on dough expansion and aroma formation

was not compatible with the high temperatures used in the proofing step. Furthermore, a simple bread making procedure was chosen for optimal control of the changes in the fermentation conditions.

2.4 Influence of the fermentation conditions on dough expansion (Paper I)

In Paper I the effects of commercial baker's yeasts (type and level), and fermentation temperature on dough expansion were investigated. The fermentation times for optimally developed doughs at the different fermentation conditions were estimated by mathematical modelling.

2.4.1 Kinetic rate constants and fermentation time

The majority of the expanding dough profiles in Paper I followed a first order kinetic model (**Figure 6**), which could be used to estimate the effect of the different fermentation conditions on the dough height throughout the fermentation process, as shown in

$$h(t) = b_0 + b_1 \bullet (1-e^{-k \bullet t}) \qquad \textbf{Equation 1}$$

where h(t) is the dough height (mm) at time t (min), b_0 (mm) is the estimate for the initial height, b_1 (mm) is the estimate for the increment in height and k is the kinetic rate constant (min^{-1}). As a result of this equation a high kinetic rate constant therefore corresponds to a short fermentation time. The fermentation time is the time required for optimal dough development determined by preliminary experiments (see **section 2.3**).

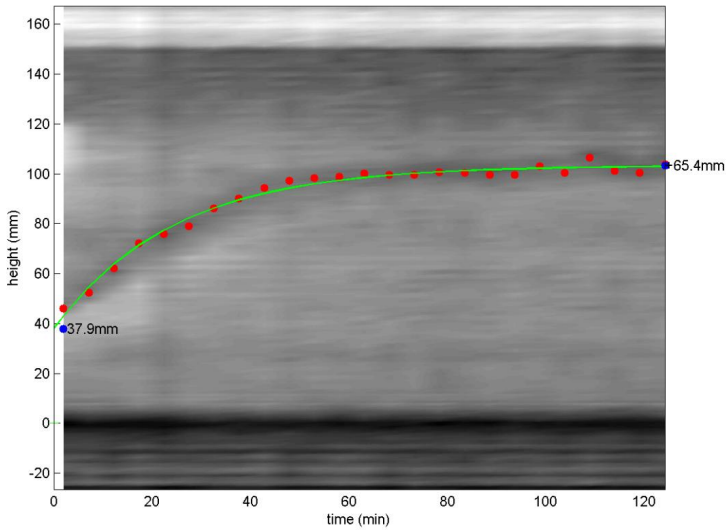

Figure 6. An example of dough expansion at discrete time points/images (●) following the first order kinetic model estimate from Equation 1. The estimated initial time point and end time point are marked with ●.The green curve is the fitted line. This dough was fermented with the baker's yeast Bruggeman at a concentration of $2.88 \bullet 10^{11}$ CFU/kg flour at 15°C.

A limited number of dough expansion profiles did not follow the first order kinetic model showing either a convex curve shape instead of a concave in the initial steep curve, or showed another unex-

pected profile, all resulting in unreliable parameter estimates in **Equation 1**; these samples will be identified distinctly in the results (**section 2.4.2** and **2.4.3**). Overall the model performance was reasonable good as the differences between the mathematical modelled and the direct determined fermentation times were small for the majority of the samples (see Figure 1B and C in Paper I). The model performance is further elaborated in Paper I.

2.4.2 Effect of fermentation temperature and yeast level on dough expansion

The four fermentation temperatures (5, 15, 25 and 35°C) were chosen to investigate the influence of a wide range of temperatures used in bakeries for dough fermentation. The typical industrial dough fermentation temperature is between 25 and 35°C (Wiggins, 1998), which is close to the optimal temperature for fermentation with *S. cerevisiae* which is approximately 25°C (Attfield, 1997). A fermentation temperature of 5°C is however also used in dough retarding (Cauvain, 1998) and this low temperature has also been shown to increase formation of esters with fruity and pleasant odours during dough fermentation compared to fermentation at higher temperatures (see **section 5.2.3**).

An overview of the effects of fermentation temperature and yeast concentration on the kinetic rate constants for the expanding doughs fermented with seven commercial baker's yeasts is shown in **Table 6.** The estimated fermentation times based on the kinetic rate constants are presented in **Figure 7**.

Doughs fermented with the baker's yeasts Malteserkors, Skærtoftmølle, Rapunzel and Sema at 25°C had significantly higher kinetic rate constants, and hence the shortest fermentation time, when the highest yeast concentration ($8.64 \cdot 10^{11}$ CFU/kg flour) was used compared to the other fermentation temperatures and yeast concentrations (**Table 6**). This tendency towards the highest kinetic rate constants for the doughs fermented at 25°C was also seen for the baker's yeasts l'Hirondelle, Bruggeman and Zymarom at the highest yeast concentration (**Table 6**).

Gujral and Singh (1999) found that wheat dough fermentation at 27°C resulted in the largest bread volume compared to fermentation at 24, 30, 33 and 36°C, which is in close agreement with the results in Paper I with 25°C as the optimal fermentation temperature. Mousia et al. (2007) investigated dough expansion rates at 30, 35, 40, 45 and 50°C and they found the highest dough expansion rate at 40°C. These results are in close agreement with Chevallier et al. (2012) which found the highest dough expansion rate at 35°C compared to 25 and 30°C. The different correlations of fermentation temperature and dough expansion rates found in the research studies are most likely explained by differences in yeast strains and process variables, however it is generally stated that the optimal fermentation temperature of *S. cerevisiae* is between 25 and 28°C (Attfield, 1997; Redon et al., 2011).

As expected, the longest fermentation times for all doughs were generally found at the lowest temperature (5°C) and the lowest yeast concentration ($2.88 \cdot 10^{11}$ CFU/kg flour) (**Table 6**). Doughs fermented with the two highest yeast concentrations ($5.76 \cdot 10^{11}$ and $8.64 \cdot 10^{11}$ CFU/kg flour) had statistically more similar kinetic rate constants compared to the doughs fermented with the lowest yeast concentration ($2.88 \cdot 10^{11}$ CFU/kg flour), which had markedly lower kinetic rate constants (**Table 6**) and hence longer fermentation times (**Figure 7**).

Table 6. Kinetic rate constants (k, min^{-1}) of expanding wheat doughs influenced by yeast concentration and fermentation temperature (Paper I). The results of the two-way ANOVA are shown for the two factors – yeast concentration (yc) and fermentation temperature (ft) – and their possible interaction (yc•ft).

	Kinetic rate constant (k, min^{-1})[a]												Two-way ANOVA[b]						
	2.88•10^{11} CFU/kg flour				5.76•10^{11} CFU/kg flour				8.64•10^{11} CFU/kg flour				yc (•10^{11}CFU/kg flour)			ft (°C)			
Yeast	5°C	15°C	25°C	35°C	5°C	15°C	25°C	35°C	5°C	15°C	25°C	35°C	2.88	5.76	8.64	5	15	25	35
Malteserkors	*0.0005g*	*0.005g*	0.01f	*0.01f*	0.005g	0.02d	0.03c	*0.03c*	0.02ef	0.02de	0.05a	*0.04b*							
Sema	0.002d	0.01cd	ne	0.01cd	0.01cd	0.03bc	0.03bc	0.03bc	0.03bc	0.03bc	0.09a	0.05b							
Skærtoftmølle	0.002g	*0.006fg*	*0.01ef*	*0.02de*	0.006fg	0.04c	0.04c	0.03d	0.004g	0.05b	0.06a	0.05b							
Bruggeman	0.02	0.04	0.03	0.05	0.03	0.04	0.07	0.06	0.03	0.05	0.1	0.07	b	ab	a	b	ab	a	ab
Zymarom	0.01	0.03	*0.02*	0.04	0.04	0.03	*0.06*	0.05	0.05	0.04	0.08	0.04	b	ab	a	a	a	a	a
Rapunzel	0.004g	0.006g	0.002g	0.03ef	0.02f	0.04de	0.04cde	0.05c	0.05cd	0.06bc	0.08a	0.07ab							
l'Hirondelle	0.03	0.04	ne	*0.03*	0.07	0.02	0.07	0.08	0.06	0.05	0.1	0.07	a	a	a	a	a	a	a

[a]Mean kinetic rate constants of duplicates; ne, not estimated. The kinetic rate constants marked with italic type are estimated from dough expansion curves which do not completely fit the first order kinetic model.

[b]Different letters in the same row indicate significant differences (significance level 95%). When the interaction between yc and ft is significant the letters are shown for each kinetic rate constant. If a significant interaction effect was not found, the Tukey HSD test is reported for the two main effects (yc and fc).

Table 7. Kinetic rate constants (k, min^{-1}) of expanding wheat doughs fermented with seven commercial baker's yeasts at different yeast concentrations (yc) and fermentation temperatures (ft) (Paper I). The results of the one-way ANOVA[a] are shown for the seven baker's yeasts.

	yc	Kinetic rate constant (k, min^{-1})[b]						
ft (°C)	(•10^{11} CFU/kg flour)	Malteserkors	Sema	Skærtoftmølle	Bruggeman	Zymarom	Rapunzel	l'Hirondelle
5	2.88	*0.0005b*	0.002b	0.002b	0.02ab	0.01ab	0.004b	0.03a
	5.76	0.005b	0.01b	0.006b	0.03ab	0.04ab	0.02ab	0.07a
	8.64	0.02cd	0.03abc	0.004d	0.03bc	0.05ab	0.05ab	0.06a
15	2.88	*0.005c*	0.01bc	*0.006c*	0.04a	0.03a	0.006c	0.03ab
	5.76	0.02c	0.03abc	0.04ab	0.04a	0.03bc	0.04ab	0.02c
	8.64	0.02	0.03	0.05	0.05	0.04	0.06	0.05
25	2.88	0.01	ne	*0.01*	0.03	*0.02*	0.02	ne
	5.76	0.03c	0.03c	0.04c	0.07a	*0.06abc*	0.04bc	0.07ab
	8.64	0.05	0.09	0.06	0.1	0.08	0.08	0.1
35	2.88	*0.01d*	0.01d	*0.02cd*	0.05a	0.04ab	0.03bc	*0.03bc*
	5.76	*0.03b*	0.03b	0.03b	0.06a	0.05ab	0.05ab	0.08a
	8.64	*0.04c*	0.05bc	0.05bc	0.07ab	0.04c	0.07a	0.07a

[a]Different letters in the same row indicate significant differences (significance level 95%).

[b]Mean kinetic rate constants of duplicates; ne= not estimated. The kinetic rate constants marked with italic type are estimated from dough expansion curves which do not completely fit the first order kinetic model.

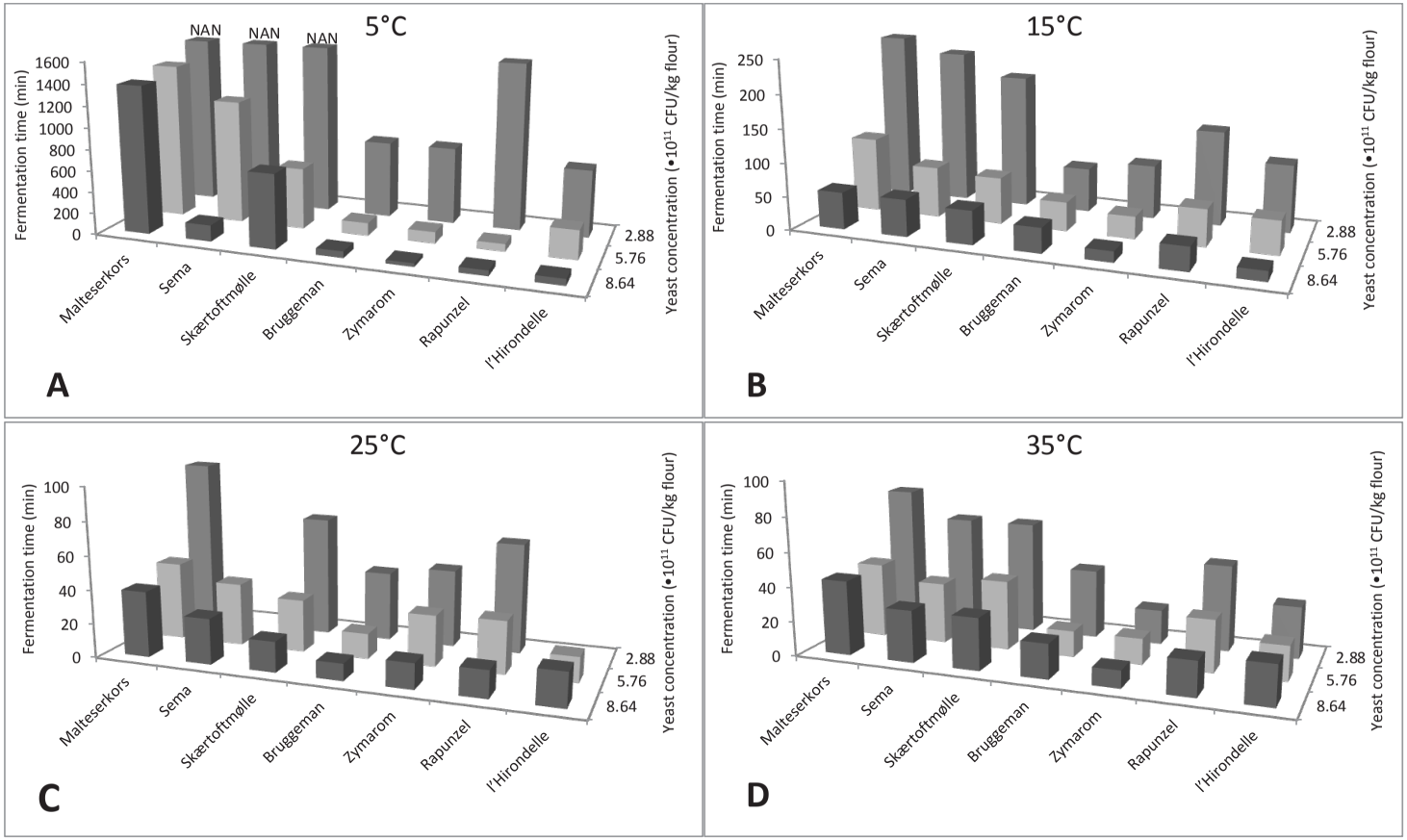

Figure 7. Estimated fermentation times for optimally fermented doughs (dough height of 90 mm for doughs fermented at 5°C and dough height of 100 mm for doughs fermented at 15, 25 and 35°C) fermented with seven commercial baker's yeasts at 5 (A), 15 (B), 25 (C) and 35°C (D) (Paper I). Note the different scale on the y-axis. If the column is marked not any number (NAN) the dough could not reach the optimal height for that temperature.

2.4.3 Effect of baker's yeast on dough expansion

The kinetic rate constants were not significantly different between the yeasts when they were fermented at 25°C at the highest yeast concentration (**Table 7**). This indicates that the activity of all baker's yeast were (statistically) equal at these fermentation conditions.

When fermentations were done at 5, 15 and 35°C higher kinetic rate constants were generally found for doughs fermented with four of the baker's yeasts (Rapunzel, l'Hirondelle, Bruggeman and Zymarom) at all yeast concentrations compared to doughs fermented with the three baker's yeasts (Malteserkors, Sema and Skærtoftmølle) (**Table 7**).

The significant differences in the kinetic rate constants were found in spite of the fact that all doughs compared had the same initial yeast CFU. These differences in fermentative activity therefore indicate that some of the commercial baker's yeasts differentiate in yeast strain. The lower kinetic rate constants estimated from the expansion of doughs fermented with Malteserkors, Skærtoftmølle and Sema are assumed to be due to a lower fermentative activity of the yeast cells in these baker's yeasts compared to the activity of the yeast cells with a higher kinetic rate constant (Bruggeman, Zymarom, Rapunzel and l'Hirondelle) (**Table 7**). If the kinetic rate constants were estimated from doughs containing equal yeast amount (g/kg flour) instead of equal number of yeast cells (CFU/kg flour) it is assumed that the kinetic rate constants would be markedly different as the number of yeast cells per g between the baker's yeasts differed significantly (see **Table 1**).

Doughs fermented with three of the seven baker's yeasts (Malteserkors, Sema and Skærtoftmølle) were not able to reach the optimal dough height at 5°C (**Table 7** and **Figure 7**). The different abilities of the seven commercial baker's yeasts to ferment at 5°C might have something to do with the lipid content of their cell membrane. At lower temperatures the fluidity of the yeast cell membrane is decreased resulting in a lower activity of the transmembrane proteins responsible for uptake of nutrients. Redon et al. (2011) found that different *S. cerevisiae* strains compensates for this by increasing the formation of triacylglycerides and medium-chain fatty acids, and yeasts having higher contents of these compounds are also better at adapting to lower fermentation temperatures.

Bell et al. (2001) also found significant differences in the fermentative activity of 39 wild and domestic baker's yeasts. The fermentative activity of *S. cerevisiae* was found to be influenced by the cultivation conditions of baker's yeast (Angelov et al., 1997). The authors found the highest fermentative activity when cultivation of *S. cerevisiae* was done at pH 6, 32°C and 20% dissolved oxygen (Angelov et al., 1997). Furthermore, the authors found that strains having a high maltase activity were also having a higher dough leaving ability (Angelov et al., 1997). Hence, the different fermentative activities observed for the seven commercial baker's yeasts in Paper I, might be caused by differences within the cultivation method of the baker's yeasts or by the mode of regulation of maltose utilisation in the yeasts.

A high fermentative activity resulting in a short fermentation time is obviously an economic benefit for the baking industry if focus is solely on bread volume. The results in Paper I elucidate that the strain of commercial baker's yeast, the fermentation temperature and the yeast concentration are three very important factors influencing the fermentation time of optimally developed doughs.

3. STALING

Staling of bread is defined as almost any changes, except microbial spoilage, that occur in bread during storage and which makes it less acceptable to a consumer (Bechtel et al., 1953). Particularly, the firming of bread during storage is unwanted in a sensory point of view (Angioloni and Collar, 2009). Starch has been found to be significantly involved in the complex staling process (Goesaert et al., 2009). The transformation of starch during storage has been found to be closely related to bread firming. The main proces is the reversible re-crystallisation of amylopectin from the completely amorpheous state of a fresh baked bread (**Figure 8**) (Zobel and Kulp, 1996). Starch retrogradation is the process in which gelatinised starch molecules re-associate to form a double helix crystalline structure (Zobel and Kulp, 1996) (**Figure 8**). It has been suggested that the increasing size and number of crystalline junction zones in the amylopectin network during storage is an important factor involved in bread firming (Fredriksson et al., 1998; Goesaert et al., 2009). Furthermore, during storage the water migrates within the crumb structure and more water is immobilised within the amylopectin crystallites, which furthermore increases crumb firmness (Goesaert et al., 2009).

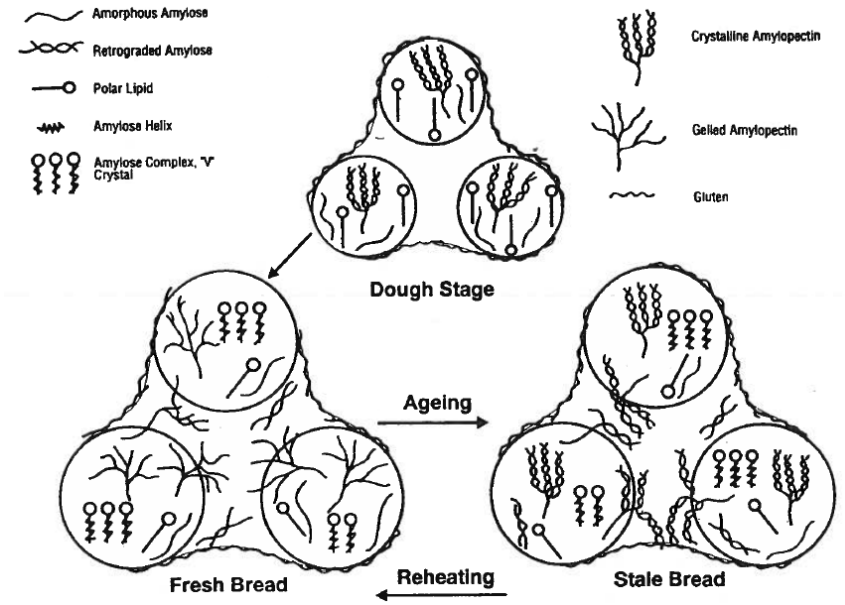

Figure 8. A model for bread crumb staling showing the molecular structures present in the dough stage, fresh bread, staled bread and reheated bread (Zobel and Kulp, 1996).

Today, addition of anti-staling enzymes is a widely used method to limit the formation and the strength of the amylopectin network and furthermore to decrease water mobilization in commercial wheat bread (Goesaert et al., 2009). Enzymes can normally be added to the dough without labeling of the bread, since enzymes are inactivated during baking at around 85°C. Amylases are widespread anti-staling enzymes used in the bread industry today. Particularly, the maltogenic α-amylases have been highlighted to prevent crumb firming without decreasing crumb elasticity during storage (Hug-

Iten et al., 2003). The maltogenic α-amylases have been found to degrade the outer amylopectin crystallisable branches and significantly lower the average chain length (Goesaert et al., 2009), whereas α-amylases, naturally present in the flour, mainly reduce the molecular size of both amylose and amylopectin, with little effect on the outer crystallisable branches of amylopectin (Hug-Iten et al., 2003).

Staling is typically measured by analysis of bread during storage by a Texture Analyser (TA) and/or by Differential Scanning Calorimetry (DSC) (Addo et al., 2001; Primo-Martin et al., 2007; Angioloni and Collar, 2009; Purhagen et al., 2012). The TA detects the firmness of the crumb by measuring the force required for compressing a bread sample and the DSC measures the energy required to melt the crystalline amylopectin in a bread sample. The crystallised amylopectin melts around 60°C, and the amount of crystalline amylopectin can be measured by integration of the peak area from the DSC curve around 60°C (Leon et al., 2006).

3.1 Influence of the fermentation time on bread staling

Staling is an important issue for the bread industry, as staling significantly decreases the shelf-life of fresh bread. Addition of enzymes to the dough has successfully proven to increase the shelf-life of bread dramatically. However, some consumers prefer bread without added enzymes and producers of organic bread typically do not add enzymes to the dough. Therefore it could be relevant to investigate if the fermentation time affects the staling rate which, to our knowledge, has not previously been investigated.

The following hypothesis was constructed: "*It is assumed that a longer fermentation time will allow the α-amylases, naturally present in the wheat flour, to degrade the starch to a higher extent than if the dough is fermented for a shorter period. A longer fermentation time will thereby decrease the staling rate".*

To investigate the hypothesis two types of bread samples were produced; short-time fermented and long-time fermented bread. Both samples were fermented to an equal dough height at 15°C. The short-time fermented bread was fermented for 1 hour with addition of 60 g baker's yeast/kg flour and the long-time fermented bread were fermented for 21 hrs with addition of 5 g baker's yeast/kg flour. The falling number was 280 sec indicating an acceptable enzyme activity in the flour (>250 Erekul and Kohn (2006)). The samples were analysed during storage (6 days) by DSC and TA. If the hypothesis was supported by the results, we furthermore wanted to investigate the aroma profile of the bread samples (short-time fermented and long-time fermented bread) during storage supplementing the DSC and TA analyses.

The results from the DSC and the TA analyses showed that no significant differences were found in the concentration of crystalline amylopectin (**Figure 9**) or in the increase of bread firming (**Table 8**) between the bread samples fermented at two different fermentation times during storage. Based on the result the hypothesis was rejected and the aroma analyses were not completed.

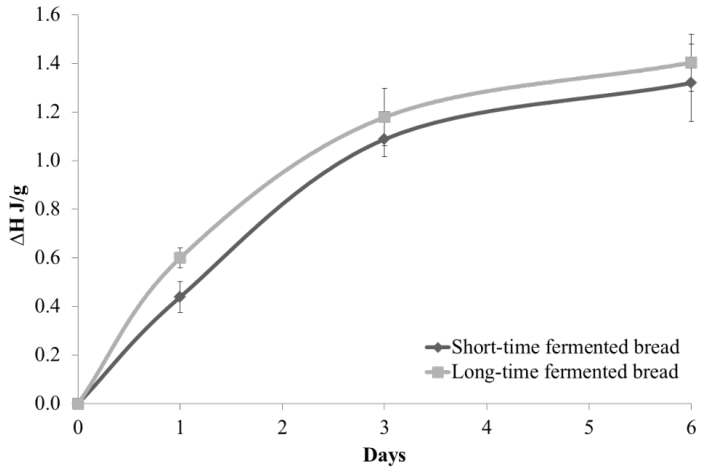

Figure 9. Transition enthalpies (ΔH, J/g) for melting of crystalline amylopectin in short- and long-time fermented bread samples stored for 6 days analysed by Differential Scanning Calorimetry (DSC). The results are based on means of 6 replicates for each sample and the CV% is 6-12. Unpublished data.

Table 8. Increase in firmness of short- and long-time fermented bread samples stored for 6 days measured by a Texture Analyser (TA). The results are based on means of 6 replicates for each sample. The firmness dimensions are from 816 to 4069 g and the CV% is from 3-12. Unpublished data.

	Days		
	1	**3**	**6**
Short-time fermented bread	1.8	2.9	3.4
Long-time fermented bread	1.6	2.4	3.4

Apparently, the increased fermentation time was not sufficient for the α-amylases to degrade the starch to an extent that it could decrease the staling rate. However, it might also be explained by the mechanism of starch degradation by α-amylases as mentioned in **section 3**. Namely, that α-amylases, naturally present in the flour, compared to maltogenic α-amylases, mainly reduce the molecular size of both amylose and amylopectin, with little effect on the outer crystallisable branches of amylopectin, and thereby little effect on the staling rate (Hug-Iten et al., 2003).

Another approach for decreasing the staling rate of bread, without addition of enzymes, have been to add sourdough to the wheat dough, which have been found to successfully decrease the staling rate (Chavan and Chavan, 2011). The technological benefits of the use of sourdough in wheat bread making has been described as a delay of starch retrogradation, higher loaf volumes and slower rate of bread firming (Corsetti et al., 1998; Corsetti et al., 2000). Addition of sourdough has furthermore been found to decrease the microbiological spoilage of the bread, since some lactic acid bacteria (LAB) contain antimicrobial and antimould activity (Elhariry et al., 2011; Chavan and Chavan, 2011).

4. ANALYSIS OF VOLATILE COMPOUNDS

The taste and odour are two very important parameters for bread quality and the term flavour is often used as an overall term including both taste and odour. Sensory studies and analysis of volatile compounds (often denoted aroma analysis) are both excellent methods to increase knowledge within bread flavour and the volatile profile of bread, respectively. Sensory analysis employs the human senses for evaluation of the bread flavour, while aroma analysis is extracting and analysing the volatile compounds by an instrumental method. In aroma analysis the detected volatile compounds, depending on their threshold values, can be correlated to the odour perception of bread. Aroma analysis, which is used in the research work of this PhD thesis, has the great advantage of being the less expensive of the two methods and furthermore the method provides a great insight into the chemical and enzymatic reactions in the dough during formation of the volatile compounds.

4.1 Sampling of volatile compounds from bread

4.1.1 Headspace sampling

Extraction of volatile compounds from a bread matrix is often performed by extraction of the headspace phase above the food matrix by static headspace sampling (Maeda et al., 2009) dynamic headspace sampling (DHS) (Seitz et al., 1998; Bianchi et al., 2008; Jensen et al., 2011) or solid phase micro extraction (SPME) (Ruiz et al., 2003; Quílez et al., 2006; Poinot et al., 2007; Poinot et al., 2010).

In static headspace sampling the volatiles are allowed to reach equilibrium, where after the volatiles in the headspace can be injected to the gas chromatograph (GC). Due to the absence of a concentration step, the sensitivity of static headspace sampling is low compared to SPME and DHS (Mestres et al., 2000). The concentration of volatiles in bread crumb is relatively small compared to more odorous food products as coffee, for which static headspace sampling has been used (Sanz et al., 2002). The reported lower sensitivity of static headspace sampling was the background for not using this sampling method in the research work of this thesis. Static headspace sampling is though a common method for sampling the headspace above other food matrices, particularly because of development of effective auto-samplers (Bicchi et al., 2004).

In DHS the sample is purged with carrier gas through a collection trap, which retains the volatile compounds while letting the carrier gas pass through. In this way, the volatiles from a large headspace volume are concentrated in the trap (Wampler, 2002). The trap is typically filled with Tenax, which is a sorbent material, capable of sorbing a wide range of organic volatiles (Wampler, 2002).

SPME, which is a combination of static headspace sampling and DHS, is an equilibrium technique, which utilises the partitioning of organic components between an aqueous or vaporous phase and the fibre in the SPME apparatus (Zeng and Noblet, 2002).

In the beginning of the PhD project, extraction of bread volatiles by SPME and DHS, respectively were compared in order to find the optimal extraction method. Both extraction methods were per-

formed by tempering the sample for 10 min at 40°C before the extractions were performed at 40°C in 40 min. The SPME fibre was a Carboxen fibre (Supelco, Bellefonte, PA) coated with polydimethylsiloxane at 85 µm thickness. The DHS was performed by extracting the volatiles in a trap containing Tenax-TA (poly-2,6-diphenyl-*p*-phenylene oxide, 200 mg) and the sample was purged with nitrogen (150 ml/min). It was found that 12 volatile compounds were extracted by SPME and the same 12 volatiles plus 38 more volatiles were extracted by DHS (unpublished results). The 50 volatiles identified by DHS were all expected bread aroma volatiles and the reproducibility of the relative peak areas was generally high.

Zeng and Noblet (2002) found that the analytical performance of SPME was largely dependent of the analyte polarity, fibre properties and sample parameters. The lower sensitivity of SPME compared to DHS in the pre-experiment for the research work of this PhD thesis was most likely due to the smaller volume of the SPME fibre (0.5 µl) compared to the amount of Tenax used in the trap when using DHS (0.2 g). Tenax, thereby, has a markedly larger surface area that can retain the volatiles, compared to the SPME fibre.

Development of specific SPME fibres increases the sensitivity of the sampling of specific volatile compounds, the purpose of this PhD project was however to analyse the entire volatile profile in bread crumb, therefore use of specific fibres was not an optimal solution.

From the pre-experiments it was concluded that DHS was clearly the most sensitive method when sampling volatiles from bread crumb, and DHS was therefore used in Paper II, III and V.

4.1.2 Sample preparation

In aroma analyses in general it is important to optimise sample preparation in order to minimise loss of volatile compounds due to evaporation. Several sample preparation methods were tested in the beginning of the PhD project. It was tested if the crumb sample should consist of grinded crumbs or larger pieces of bread, furthermore if the sample could be frozen and if the sample should be stirred during DHS. The test comparing the volatiles from frozen and fresh bread samples showed no significant differences (data not shown), and therefore freezing of the samples was done for practical reasons. The optimal sample preparation method was concluded to be: to cool down the loafs for 1 hour at room temperature, then quickly cut off the crust and the outer part of the crumb (1 cm in total). Then samples of 15 g of crumb in 4-6 pieces were packed in aluminium foil surrounded by a plastic bag and then frozen at -18°C until DHS. The frozen bread pieces were directly transferred to the DHS purge vessel without grinding and the DHS was performed without stirring in the vessel. The sample preparation procedure described here was used for the sample sets in Paper II, III and V.

4.1.3 Breakthrough volume

When using DHS it is important to consider the breakthrough volume (Bv) particularly of small polar compounds. The term Bv is defined as the volume of carrier gas that will purge a volatile compound through one (1.0) gram of adsorbent resin in a desorption tube at a specific temperature (sisweb.com, 2013). The volatile begins to elute from the sorbent material at a carrier gas volume

above the safe sampling volume (Bs = Bv*0.5) and is totally eluted from the sorbent material at a complete sample elution volume (Bf = Bv*2). The Bv depends on the nature of the compound, its volatility, the interaction between the compound and the sorbent, the amount of sorbent used, and the temperature of the trap. In practice, a Bs is typically used and Bs can for each volatile compound be calculated from a reference database (sisweb.com, 2013).

Particularly small polar alcohols (e.g. 2-methyl-1-propanol), aldehydes (e.g. 3-methylbutanal), ketones (e.g. 2,3-butanedione) and esters (e.g. ethyl acetate), which have been identified in bread crumb (Schieberle and Grosch, 1991; Frasse et al., 1992; Gassenmeier and Schieberle, 1995; Jensen et al., 2011), have small Bv's (sisweb.com, 2013). In Paper II and III the DHS was therefore performed by two different gas volumes (0.25L and 9L) applied to two different sample sets in order to extract the very volatile compounds without exceeding the Bs (0.25L) and furthermore to extract the less volatile compounds, which required a higher gas volume (9L) in order to provide reproducible relative peak areas.

From pre-experiments the influence of the sample temperature (30 and 40°C) at the two different carrier gas volumes (0.25L and 9L) was tested. The temperatures 30 and 40°C was chosen as it is close to the temperature in the human mouth, and as the release of compounds is highly temperature dependent, it is of interest to extract the volatiles at a temperature close to the temperature of the bread when it is eaten. The optimal temperature with the highest relative concentration of bread volatiles was found to be 40°C (unpublished data).

4.1.4 Water management

When performing DHS from foods, the sample matrix typically contains water. Bread crumb has a water content of approximately 35%. The presence of even 1 µl of water in the trap will result in a large water peak in the chromatogram and the retention time might be shifted (Wampler, 2002). Tenax has a very large affinity for most organic volatile compounds compared to water, however a trapping tube filled with 100–150 mg of Tenax can retain about 1 µl of water for each of 40 ml of purge gas used in the process (Wampler, 2002). Passing the dry purge gas through the trap for 5 min at a low gas flow (50 ml/min) was found to vent the water from the trap without disturbing the volatile compounds (Paper II and III).

4.2 Analysis of volatile compounds by gas chromatography mass spectrometry

Gas chromatography mass spectrometry (GC-MS) is a well-documented and sensitive technique for separating and detecting volatile compounds in foods. Before separation of the compounds in the GC, the trapped volatile compounds were thermally desorbed. A simplified illustration of the flow of volatiles as they were analysed in Paper II and III is shown in **Figure 10.**

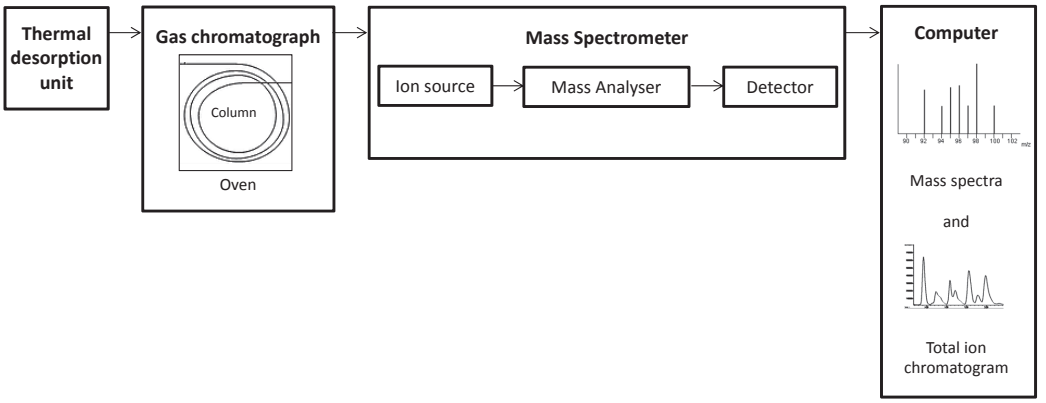

Figure 10. A simple illustration of the gas chromatography mass spectrometry instrument installed with a thermal desorption unit that desorbs the trapped volatiles from the bread sample and a computer that registers the detected mass spectra.

Gas chromatography is used for separating compounds that can be vaporised without decomposition. The GC requires supply of carrier gas, which is the mobile phase that transports the sample through the column. Best results are obtained with the smallest amount of sample that can be adequately detected therefore split injection is often used to decrease the amount of the volatiles of interest (Reineccius, 1998). Split 1:10 was used when analysing the volatiles from the sample sets in Paper II and III. The GC column contains a stationary phase that decides how the different compounds in the sample are partitioned. The capillary columns used today are very efficient, which often results in sufficient compound separation even though the phase is not optimal. Temperature programming of the oven surrounding the GC column is often used because it ensures that all compounds are eluted within reasonable time and that the separation of the peaks is fairly uniform. The column temperature programme used in Paper II, III and V had a starting temperature (10 min) at 40°C (Paper III) or 45°C (Paper II and V), hereafter heating by 6 (Paper II and V) or 8°C/min (Paper III) up to 240°C, and holding this temperature for 10 min. Temperature programmes like these are typically used for separation of volatiles in GC's (Reineccius, 1998).

In the mass spectrometer the volatiles eluting from the GC column are now introduced to an ion source where the molecules are ionised typically by electron ionization as used in Paper II, III and V. Fragmentation of the molecules occurs after the ionisation in the mass analyser (**Figure 10**). The molecular ion (having the highest m/z ratio) of a molecule represents the part of the molecule that does not fragmentise. Finally the separated, charged fragments are monitored by a detector resulting in mass spectra and a total ion chromatogram (TIC) (see **section 4.4.1**).

4.3 Quantification and evaluation of volatile compounds

4.3.1 Multiple headspace extraction

Quantification of volatile compounds in semi-solid food matrices, as bread, can be problematic, because it typically requires addition of internal standards. Multiple headspace extraction (MHE) is a quantification method that has been found to be independent of the food matrix (Kolb, 1982; Kolb

and Ettre, 1991). MHE has recently been successfully performed within food systems (Carrillo and Tena, 2006; Soria et al., 2007). The principle is that stepwise headspace extractions of volatile compounds are performed, each extraction resulting in a lower area of the volatile compound (**Figure 11**).

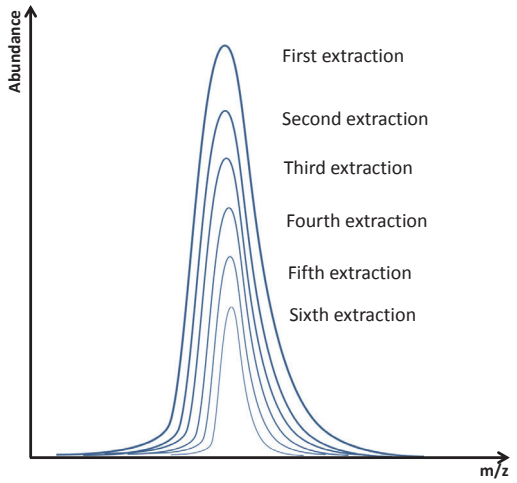

Figure 11. Illustration of stepwise headspace extractions resulting in decreased peak areas.

After an infinite number of extractions all volatile compounds are extracted from the sample. The peak area corresponding to a complete dynamic headspace extraction can be calculated assuming that the decline in peak area during MHE follows first order kinetics, i.e.:

$$\sum_{i=1}^{i=\infty} A_i = \frac{A_1}{1 - e^{-q}}$$

where A_i is the sum of all extractions from A_1 to A_∞, A_1 represents the area of a volatile compound obtained in the first extraction and q represents the slope of the regression curve of the natural logarithm to the peak area vs. number of extractions (Kolb and Ettre, 1991). q can be determined for each volatile compound, where after A_1 for each volatile compound can be used to calculate the area representing the total amount of volatile compound in each sample. Finally, areas can be transformed into absolute concentrations as described in Paper II, III and V.

MHE can besides being a quantification method also be used to check if the volatiles detected are actually volatiles present in the sample or if they might be artefacts (e.g. from breakdown of Tenax). If each extraction results in a lower area of the volatile compound, then the compound was most likely present in the sample.

MHE was originally developed for static headspace extraction however from Paper II, III and V it was found that DHS is also a well suited extraction method for quantification by MHE. MHE was found to be a relatively simple and reproducible quantification method of volatiles from bread crumb (Paper II, III and V). However, the method has been found to be improved by modifications

in the calculation procedure (Soria et al., 2007). The authors investigated the possibly great influence of an error in the peak area in the first extraction step (A_1) on the estimation of the total amount of the volatile compound. They found that the model was improved (by a decreased root mean square error) when the calculations took this factor into account. Therefore, in further studies using MHE, it could be relevant to use the calculations presented by Soria et al. (2007).

4.3.2 Odour activity value

Quantification of aroma compounds is relevant for the purpose in Paper II, III and V, since it is of interest to identify which compounds that can be sensed when the bread is eaten. In Paper II, III and V this evaluation is based on odour activity values (OAV's) defined in **Figure 12**. An aqueous odour threshold value of a volatile compound will only give an approximation of the OAV in bread crumb. It would have been more appropriate to calculate the OAV's from odour threshold in starch or cellulose. They were however, not available from the literature for the majority of the volatile compounds identified in bread crumb. To take the uncertainty of the calculated OAV's into account it was assumed in Paper II and III that volatile compounds having an OAV of 0.1 or higher might be important bread aroma compounds. It should be noted that in Paper II and V all compounds are denoted aroma compounds regardless of their OAV's, but in Paper III and this PhD thesis the (more correct) definitions shown in **Figure 12** is used.

Volatile compound	Compound extracted from headspace of bread crumb
Odour activity value (OAV)	Volatile compound concentration/odour threshold in water
Aroma compound	Compound that is likely to be sensed, when the bread is eaten (OAV>0.1)

Figure 12. Definitions of volatile compound, odour activity value and aroma compound as it is used in this PhD thesis.

In some research studies the aroma extraction dilution analysis (AEDA) is employed to analyse potent volatile compounds in bread (Schieberle and Grosch, 1991; Schieberle and Grosch, 1994). In AEDA the result for each volatile compound is presented as a flavour dilution factor (FD factor). The FD factor is the ratio of the concentration of the volatile compound in the initial liquid extract to its concentration in the most dilute extract in which the odour is still detectable by GC olfactometry (GC-O). A compound having a FD factor below 1 means that the compound was not perceptible at the sniffing port (Schieberle and Grosch, 1987). Consequently, the FD factor is a relative measure and it is proportional to the OAV calculated in the research work of this PhD thesis. The FD factors are not corrected for losses of the odourants during the isolation and concentration step; furthermore in AEDA the volatiles are completely volatilised and then evaluated by sniffing, whereas the volatility of the aroma compounds in foods depends on their solubility in the aqueous and/or lipid phase as well as on their binding to non-volatile food constituents (Grosch, 1993). Therefore calculation of the FD factors is a screening method suitable for the analysis of potent volatile compounds, however in order to find the most aroma-active compounds important when eating the food OAV's should be calculated (Grosch, 1993). AEDA has been criticised because the liquid extraction includes also semi- and non-volatile compounds in the analysis and they might not be very relevant, when the food is eaten (Mahattanatawee and Rouseff, 2011).

Quantification of volatile compounds in a food matrix is necessary when calculating OAV's and hence when evaluating which volatiles are aroma active. When the important aroma compounds are well identified in a specific food product, it is often not relevant to quantify the volatile compounds of the food product in future experiments. Quantification is time-consuming and the results of the relative peak areas are sufficient in comparison of different treatments of samples, where the OAV's are already well established.

4.4 Data analysis

4.4.1 Handling GC-MS data

A mass spectrum is normally depicted as a bar plot indicating the intensity of the various fragments produced when a molecule is subjected to ionization (**Figure 13A**). Mass spectra are obtained at a certain frequency over the entire chromatographic run and form the basis of the so-called total ion chromatogram (TIC), which is often used to present data from GC-MS analyses (**Figure 13B**). Each point on the curve represents a mass spectrum and the sum of all peaks in the spectrum determines the position of the individual point in the TIC.

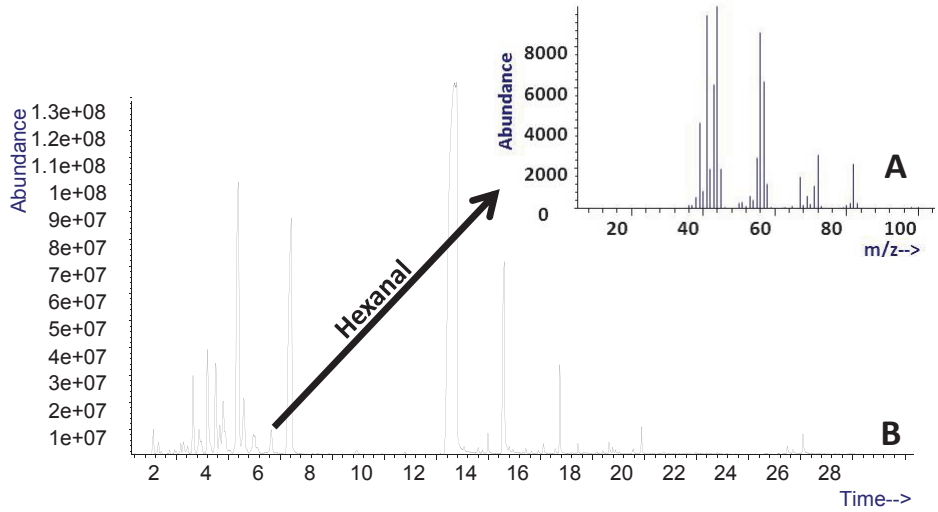

Figure 13. Example of a mass spectrum for hexanal where each line represents the m/z fragment with the abundance unique to a specific compound (A) and a total ion chromatogram for a bread crumb sample (B).

Analysis of GC-MS data can be problematic regarding shifts, baseline drift, co-eluting peaks and low-intensity peaks. The software Enhanced ChemStation (Agilent Technologies, Inc) is a common tool for relative quantification of a volatile compound by integration of either TIC or by a single ion. The mass spectra can furthermore be compared to a commercial mass spectra library (e.g. Wiley275) for identification of the volatile compounds. However, working with ChemStation is time consuming and the integration can be problematic, especially when the compound concentration is low. Furthermore, baseline drift, shifts and co-elution are not completely handled in ChemStation.

A multivariate method called parallel factor analysis 2 (PARAFAC2) has been shown to be a useful tool to solve these problems (Amigo et al., 2008; Amigo et al., 2010). PARAFAC2 decomposes the three-way GC-MS data into three matrices; one for the samples, one for the retention times and one for the mass spectra. PARAFAC2 allows the retention time profiles to be different from each other and the method can therefore handle shifted data. If a successful model is obtained, PARAFAC2 can separate different data regarding the abundance, retention time profiles and mass spectra of each volatile.

Pre-experiments during this PhD project showed that PARAFAC2 could solve the three common GC-MS data problems; co-eluting peaks (**Figure 14**), shifted data (results not shown) and low-intensity peaks (results not shown).

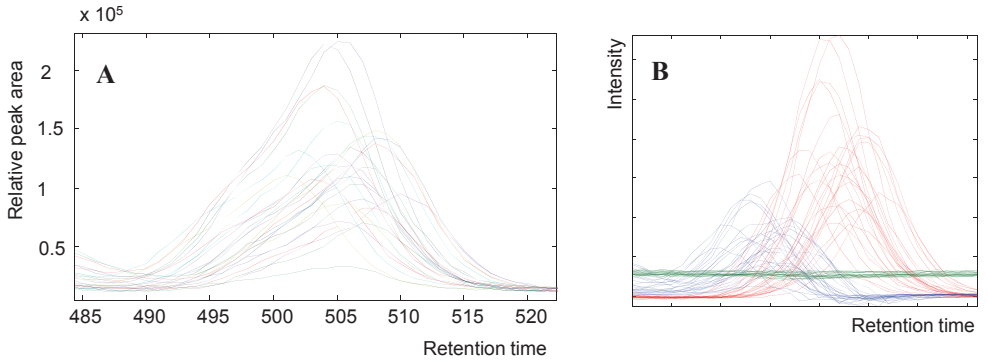

Figure 14. A: A small interval of GC-MS data from 26 bread samples showing two possible co-eluting peaks. B: Chromatographic loadings calculated by three components by PARAFAC2 showing complete separation of two peaks (Unpublished data).

Separation of co-eluting peaks by ChemStation can be handled by using the button "peak purity", showing the major extracted ion chromatograms for the co-eluting peaks. Correct integration of the peak areas of co-eluting peaks can be done by choosing different quantifier ions for each co-eluting peak. A high correlation (coefficient of determination (R^2) = 0.96-0.99) was found between the relative concentrations for the two separated peaks calculated by ChemStation and PARAFAC2, respectively (**Figure 15**).

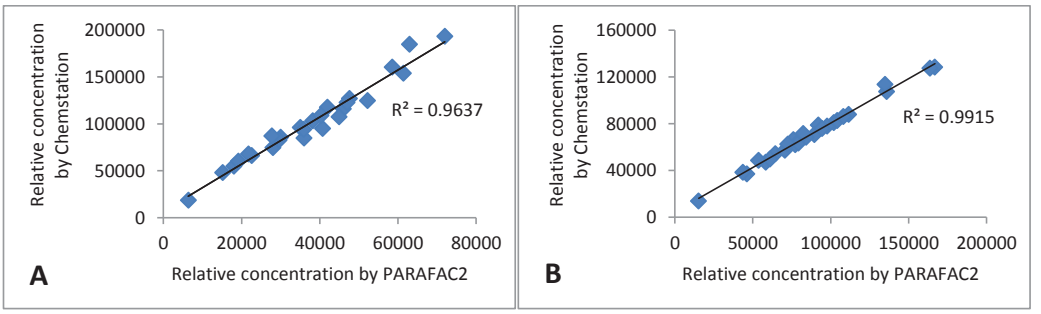

Figure 15. Correlation between the relative concentration of the two peaks A and B based on integration of the peak areas by ChemStation and by the sample score values calculated by PARAFAC2 (Unpublished data). R^2 is the coefficient of determination.

Identification of the volatiles from the mass spectra was relatively easily done in ChemStation because of the commercial mass spectra library (e.g. Wiley) adapted to the software, this is somewhat more problematic when using PARAFAC2, since it is necessary to extract the data and compare the mass spectra with a mass spectra library. Both PARAFAC2 and ChemStation are time consuming and advanced data analysis methods, however it is believed that a combination of Enhanced ChemStation and PARAFAC2 would be an advantage when handling general GC-MS data problems such as shifts, baseline drift, co-elution and low-intensity peaks.

The chromatograms representing the volatiles of bread crumb were relatively simple to analyse, because of the low content of volatiles (approximately 50-60 compounds) and only few co-eluting peaks, therefore ChemStation was chosen for handling GC-MS data in the research work of this PhD thesis.

4.4.2 Multivariate data analysis

Both univariate and multivariate data analyses were employed to investigate the influence of the fermentation conditions on bread aroma and dough expansion (Paper I-III). Univariate data analysis is a common method when testing for example if significant differences can be seen between the means in a dataset by using e.g. Student's T-test (Paper II and III) or Tukey's honestly significant difference (HSD) test (Paper I).

Multivariate data analysis involves analysis of more than one statistical variable at a time and particularly principal component analysis (PCA) formulated by Hotelling (1933) is a useful technique to describe major trends in a data set and to detect possible outliers as done in Paper II and III.

PCA decomposes the data into scores (T) and loadings (P) according to:

$$X = TP' + E,$$

where X is the raw data, T is the score matrix, P' is the transposed loadings matrix and E is the error matrix. In PCA the scores are related to the samples and the loadings are related to the variables (volatile compounds in Paper II and III). In PCA new variables (principal components) are constructed from a data matrix of samples. The first principal component captures as much as possible of the variability in all the original variables and each new principal component (applied orthogonally - i.e. being independent of - the previous principal component) accounts for as much of the remaining variability as possible. A large portion of the variability is therefore often described by a few principal components and the results in Paper II and III could be presented by PCA bi plots showed for principal component 1 and 2. A bi plot is a combination of a score plot of the samples and a loadings plot showing the correlations between the variables (e.g. volatile compounds).

Partial Least Squares (PLS) regression is another multivariate method used in Paper II to determine the relationship between variables (e.g. volatile compounds) and properties of interest (e.g. fermentation temperature or yeast concentration) (Næs et al., 2004). Calculation of PLS models were done in order to investigate the PLS regression coefficients. Volatile compounds having very low PLS

regression coefficients related to the properties of interest (fermentation temperature or yeast concentration in Paper II) do not contribute much to the variability and can therefore be removed in order to improve the PCA model.

PCA and PLS regression models were computed by the software Latentix (version 2.00, Latent5) and all GC-MS data was autoscaled prior to the analysis (**Figure 16**). Autoscaling means that all variables are divided with the standard variation and this pre-processing method is used to ensure that all volatile compounds have equal importance in the data analysis (**Figure 16**). Autoscaling is important when analysing volatile compounds, since a volatile compound with low abundance might be an important aroma compound if the odour threshold of the compound is low (see **section 4.3**).

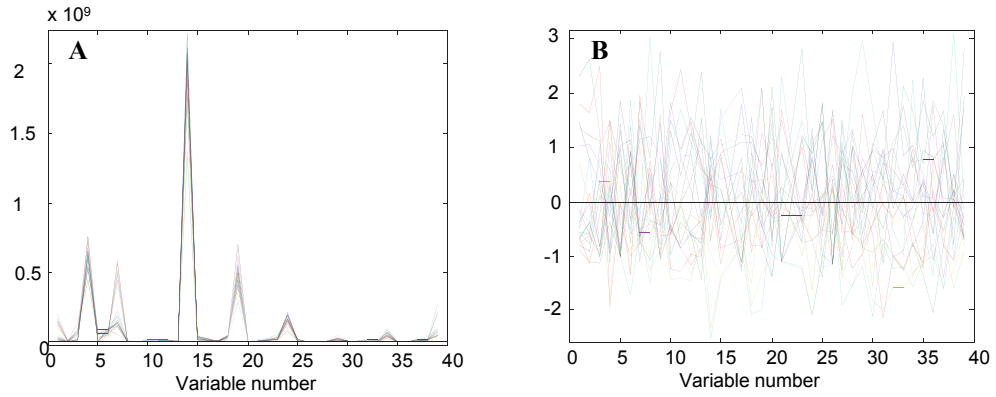

Figure 16. Raw (A) and autoscaled (B) peak areas of 39 detected volatile compounds (variables) in 21 bread samples (the dataset is from Paper III).

5. BREAD CRUMB AROMA

A wide range of volatile compounds has been identified in wheat bread crumb and a compilation of the more than 150 volatile compounds identified in wheat bread crumb from 11 research studies can be found in Table 1 in Paper IV. The 45 most aroma-active compounds found in bread crumb are presented in Table 2 in Paper IV. Many of these volatile compounds originate from different enzymatic or non-enzymatic reactions involving the fermentative activity of yeast, oxidation of flour lipids and/or Maillard reactions (in the crust). An overview of these chemical reactions is explained in Paper IV.

The volatile compounds identified from bread crumb in the research work of this PhD project are presented in this section with focus on the most aroma active compounds. Furthermore, the effects of commercial baker's yeast (level and type), fermentation temperature and fermentation time on bread aroma are discussed in this section and in Paper IV.

5.1 Aroma compounds in bread crumb (Paper II and III)

The volatile compounds identified in bread crumb from the research work of this PhD project are shown in **Table 9.** Overall the bread volatiles are dominated by alcohols, aldehydes, ketones, acids and esters. The most important bread aroma compounds found in Paper II and III is presented in **Table 9** based on their odour activity values (OAV's). The volatile compounds identified in wheat bread crumb might be sensed, when the bread is eaten, if they have OAV's > 0.1 (as elucidated in **section 4.3.2**). The most important bread aroma compounds were in Paper II and III found to be: (E)-2-nonanal (green, tallow), 3-methyl-1-butanol (balsamic, alcoholic), 3-methylbutanal (malty), hexanal (green), 2,3-butanedione (buttery, caramel), nonanal (citrus), 1-octen-3-ol (mushroom) and phenylacetaldehyde (honey-like) (**Table 9**).

A review of the important bread aroma compounds based on both OAV and FD factors from different research studies can be found in Table 2 in Paper IV. Schieberle and Grosch (1991) and Gassenmeier and Schieberle (1995) found the following compounds to have high FD factors: (E)-2-Nonenal, 3-methyl-1-butanol, hexanal, 2,3-butanedione and 2-phenylethanol, which is in close agreement with the results of Paper II and III (**Table 9**). Schieberle and Grosch (1991) identified (E,E)-2,4-decadienal, *trans*-4,5-epoxy-(E)-2-decenal and 2,4-decadienal in bread crumb and Gassenmeier and Schieberle (1995) identified 4-hydroxy-2,5-dimethyl-3(2H)-furanone and 4-vinyl-2-metoxyphenol. These five compounds were all found to have high FD factors (8 to 512). The five compounds are semi-volatile compounds with relatively high boiling points and their relevance for the aroma might be questioned. As mentioned in **section 4.3.2** AEDA, which is based on liquid extraction, also includes these semi-volatile compounds in the analysis, which might not be very relevant, when the bread is eaten (Mahattanatawee and Rouseff, 2011). The dynamic headspace method used in Paper II and III includes only the volatile compounds and this discrimination of compounds is assumed to be more comparable with the situation, where the bread is eaten.

Table 9 Volatile compounds identified in Paper II and III. The odour activity value[a] (OAV) of the compound and the odour characteristics are presented if the OAV is above 0.1. The origin of the compounds having an OAV above 0.1 is shown based on the theory in Paper IV; fermentative activity of yeast (F), lipid oxidation (L) and Maillard reactions (M). The compounds are grouped first according to chemical group then according to OAV.

Chemical group	Volatile compound	Origin	OAV	Odour
Alcohols	3-methyl-1-butanol[II and III]	F	17-25[II] and 25-35[III]	balsamic, alcoholic
	1-octen-3-ol[II]	L	9-13[II]	mushroom
	1-heptanol[II and III]	L	2-3[II] and 8-14[III]	green
	2-methyl-1-propanol[II and III]	F	0.1-0.3[II] and 0.2-0.8[III]	glue, alcohol
	1-propanol[II and III]	L	0.1-2[III]	fruity, plastic
	2-phenylethanol[II and III]	F	0.2-0.4[II] and 0-0.1[III]	flowery
	1-butanol[II and III]			
	1-penten-3-ol[II]			
	2-penten-1-ol[III]			
	3-methyl-3-buten-1-ol[II and III]			
	1-pentanol[II and III]			
	(E)-2-penten-1-ol[II and III]			
	1-hexanol[II and III]			
	3-hexenol[II]			
	2-octen-1-ol[III]			
	2-ethyl-1-hexanol[II and III]			
	1-octanol[II and III]			
	3-nonen-1-ol[II]			
	1-dodecanol[II]			
Aldehydes	(E)-2-nonenal[II, IIIb]	L	40-65[II]	green, tallowy
	3-methylbutanal[II and III]	F, L	25-54[II] and 56-236[III]	malty
	hexanal[II and III]	L	12-20[II] and 49-87[III]	green
	phenylacetaldehyde[II and III]	F	8-13[II] and 6-35[III]	honey-like
	nonanal[II and III]	L	15-20[II] and 18-34[III]	citrus
	octanal[II and III]	L	4-7 and 19-30	citrus
	heptanal[II and III]	L	5-13[II] and 14-26[III]	fatty, rancid
	decanal[II and III]	L	2-4[II] and 8-21[III]	citrus
	benzaldehyde[II and III]	F, L	0-0.2[II] and 0.1-0.2[III]	almond
	2-furancarboxaldehyde[II and III]			
Ketones	2,3-butanedione[II and III]	F, M	9-33[II] and 19-103[III]	buttery and caramel
	6-methyl-5-hepten-2-one[III]		2-5[III]	herbaceous, green
	3-hydroxy-2-butanone[II and III]	F	0.8-1.5[III]	butterscotch
	2-octanone[II]		0.1-0.2[II]	
	2-heptanone[II and III]			
	3-octen-2-one[II]			
Esters	ethyl 3-methylbutanoate[II]	F	1.5-11	fruity, apple
	ethyl hexanoate[II]	F	0.2-0.5[II]	fuity, juicy
	ethyl acetate[II and III]	F	0-0.1[III]	sweet, fuity
	ethyl octanoate[II and III]	F	0-0.1[III]	sweet, soap, fresh
	3-methylbutyl acetate[II]			
	phenylethyl acetate[II]			
Acids	acetic acid[II]		-	
	2-methylpropanoic acid[II]		-	
	butanoic acid[II]		-	
	pentanoic acid[II]		-	
	hexanoic acid[II]		-	
Other compounds	2-pentylfuran[II and III]	F, L, M	1-3[II and III]	floral, fruity
	methylbenzene[III]			
	limonene[III]			
	gamma-nonalactone[II]			
	trimethylpyrazine[II and III]			

[a]OAV is defined as the volatile compound concentration divided to the odour threshold in water.
[b]2-Nonenal was not quantified in Paper III because of a non-linear standard curve.
[II]Paper II.
[III]Paper III

5.1.1 Fermentation compounds

The most important bread aroma compounds formed from the yeast metabolism were in Paper II and III found to be; 3-methylbutanal, 3-methyl-1-butanol, 2,3-butanedione and phenylacetaldehyde, because of their high OAV's (**Table 9**). This result is in agreement with Schieberle and Grosch (1991) and Gassenmeier and Schieberle (1995) as they found 3-methyl-1-butanol, 2,3-butanedione and phenylacetaldehyde to be important bread aroma compounds based on their high FD factors (Table 2 in Paper IV). However, 3-methylbutanal has not previously been characterised as an important aroma active compound in bread by other authors.

Gassenmeier and Schieberle (1995) found the two fermentation compounds; 2-phenylethanol and ethyl octanoate to have high OAV's (2.9-11.8 and 14.6-18.8, respectively) compared to the relatively low OAV's (0-0.4) found in Paper II and III. The high contents of these two compounds in the research work of Gassenmeier and Schieberle (1995) could be due to the use of pre-ferments (flour, water and yeast) and long fermentation times (18 hrs), which increases the contents of yeast fermentation compounds. On the other hand, Gassenmeier and Schieberle (1995) reported the OAV's of another typical fermentation compound 3-methyl-1-butanol to be 9.7-18.1, which is lower than the OAV's reported in Paper II and III (17-35) (**Table 9**). Schieberle and Grosch (1991) reported the FD factor of 2-phenylethanol to be 4 in a short-time fermented bread (time not stated in their paper) and 256 in a long-time (3 hrs) fermented bread. The differences observed in the OAV's for 2-phenylethanol might also be caused by the markedly different methods for sampling the bread compounds by liquid extraction and dynamic headspace sampling, respectively as mentioned in **section 5.1**. Because of the differing results of the OAV's and FD factors of 2-phenylethanol, the role of 2-phenylethanol as an important aroma active compound in bread crumb is not clear.

Ethyl octanoate was not reported in the paper by Schieberle and Grosch (1991) indicating that the FD factor was below 2, as they only reported compounds having FD factors higher than 2. The small impact of ethyl octanoate on the overall aroma profile of bread crumb was in agreement with the results of Paper II and III, therefore ethyl octanoate is not concluded to be among the most aroma active compounds in bread crumb, though it can most likely be sensed.

The most aroma active esters in bread crumb were ethyl 3-methylbutanoate, ethyl hexanoate, ethyl acetate and ethyl octanoate (**Table 9**). Formation of esters in bread crumb is particularly interesting, since they are often characterised as having pleasant and fruity odours (**Table 9**). However, the esters are generally found to have low OAV's (**Table 9**).

It could have been interesting if the acids in bread crumb were also quantified in the research work of Paper II and III. However, this was not possible with the DHS method employed, since very low reproducibility was found for the identified acids (acetic acid, 2-methylpropanoic acid, butanoic acid, pentanoic acid and hexanoic acid, **Table 9**). It is assumed that the low reproducibility is mainly caused by disassociation of the acid groups, which strongly decreases the volatility of the acid (Sha et al., 2010). The three acids, acetic acid, butanoic acid and 3-methylbutanoic acid can most likely be sensed in bread crumb, because of FD factors above 8 (Gassenmeier and Schieberle, 1995). The odour characteristics are acetic, pungent, rancid and sweaty (Gassenmeier and Schieberle, 1995).

5.1.2 Compounds formed from the lipid oxidation

It is clear that lipid oxidation plays a very important role for the aroma profile of wheat bread crumb (**Table 9**). Especially the aldehydes, with low odour threshold values ((E)-2-nonenal, nonanal, hexanal, heptanal and octanal), account for a large part of the most important bread aroma compounds and their formation is explained in Paper IV. These aldehydes have green, tallowy, mushroom and citrus odours, and they are often denoted as off-aroma compounds (Zehentbauer and Grosch, 1998).

(E, E)-2,4-Decadienal (fatty, waxy) was found as an important aroma compound (with FD factor 512) formed from degradation of unsaturated fatty acids (Schieberle and Grosch, 1991; Gassenmeier and Schieberle, 1995). (E, E)-2,4-Decadienal was also detected by Ruiz et al. (2003), however the compound was not detected in the research work of this PhD thesis and the importance of this semi-volatile compound for the overall bread aroma was questioned in **section 5.1**.

Sensory tests or GC-O analyses could be relevant in order to clarify the sensory importance of particularly the fermentation compounds 2-phenylethanol, esters and acids as well as the aldehydes formed from oxidation of flour lipids.

5.1.3 Crust compounds

Crust aroma compounds are mainly formed from Maillard reactions during baking and they might diffuse into the crumb. An overview of the most important crust aroma compounds formed during baking can be found in Paper IV. Bread baking was, in the research work of this PhD thesis, done with steam and at 130°C to limit Maillard reactions (see **Figure 4, section 2.3**). The baked bread was very pale and the crust was relatively soft indicating that the Maillard reaction did not occur, therefore the risk of diffusion of crust compounds to the crumb was small. Only few compounds (2-furancarboxaldehyde and trimethylpyrazine) originating from Maillard reactions were found in the bread crumb samples in Paper II and III (**Table 9**).

It should be noted that some aldehydes (e.g. 3-methylbutanal, acetaldehyde and phenylacetaldehyde), some ketones (2,3-butanedione and 3-hydroxy-2-butanone) and some acids (e.g. acetic acid and 3-methylbutanoic acid) formed during yeast fermentation, can also be formed from the Maillard reaction (Hofmann et al., 2000; Prost et al., 2012). This fact elucidate that when investigating formation of volatile compounds in bread crumb it is important to separate the crust from the crumb, since some compounds can be formed from different pathways in the crumb and crust, respectively.

5.2 Influence of the fermentation conditions on bread crumb aroma

The aroma profile of wheat bread crumb has been found to be dependent on baker's yeast (level and type), fermentation temperature and fermentation time, which will be discussed in this section.

5.2.1 Yeast level (Paper II)

The effect of yeast level (20, 40 and 60 g Malteserkors/kg flour) on bread crumb aroma was investigated in Paper II. An overview of the results is shown in a PCA biplot (**Figure 17**). A two-way analysis of variance between the samples was furthermore calculated and can be found in Table 2 in Paper II. Dough fermented with a high yeast concentration (60 g /kg flour) resulted in bread with a higher concentration of the majority of the compounds formed from the fermentative activity of yeast, compared with dough fermented with lower yeast concentrations (20 and 40 g/kg flour) (**Figure 17**). 2,3-Butanedione and phenylacetaldehyde were the most aroma active of these fermentation compounds (**Table 9**).

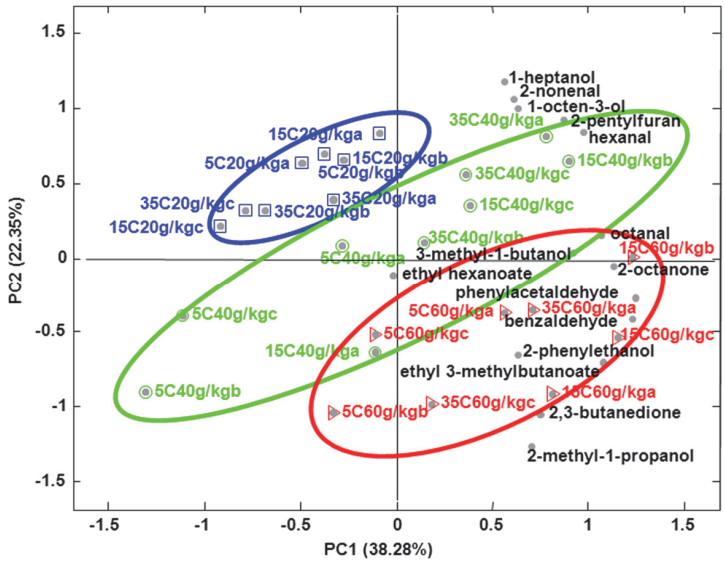

Figure 17. PCA biplot showing the effect of yeast concentration on aroma formation (Paper II). Blue squares, green circles and red triangles show samples fermented with 20, 40 and 60 g yeast/kg flour, respectively. The number before C in the sample name shows the fermentation temperature, the number before g/kg shows the yeast concentration and the letter a, b or c corresponds to the triplicate number. Only aroma compounds having an OAV of 0.1 or higher and furthermore with regression coefficients higher than ±0.1 were included in the PCA model for the PCA biplot. Regression coefficients were calculated from a PLS model with yeast concentration as the y-variable (data not shown).

It was expected that dough fermented with a high yeast concentration would result in a higher concentration of the aroma compounds formed by the yeast activity, compared to dough fermented with a lower yeast concentration. However, it is of interest that all the fermentation compounds did not increase equally in concentration, when the yeast level was increased from 20 to 60 g/kg flour. The concentration of 3-methyl-1-butanol was for example not significantly influenced by yeast level.

Gassenmeier and Schieberle (1995) investigated how two different yeast levels (15 and 46 g/kg flour in the final dough) influence formation of aroma compounds in wheat bread crumb. The authors found the lowest content of 3-methyl-1-butanol, and 2-phenylethanol in bread crumb, while the content of phenylacetaldehyde was highest in bread, when the final dough contained 46 g yeast/kg flour compared to 15 g yeast/kg flour (Gassenmeier and Schieberle, 1995). This result is not in agreement with the results from Paper II, as these three compounds were all found to increase

with increasing yeast concentration (**Figure 17**). The different results are probably explained by the markedly different fermentation conditions or sampling methods in the research work of Gassenmeier and Schieberle (1995) compared to the conditions in Paper II, as mentioned in **section 5.1** and **5.1.1**.

Richard-Molard et al. (1979) investigated the effect of yeast level from 5 to 20 g/kg flour on formation of acetic acid in bread crumb, when the fermentation was done for 5h at 22°C. They found an increase in acetic acid from 123 to 195 mg/kg when the yeast level was increased from 5 to 15 g/kg flour. A yeast level above 15 g/kg flour did not further increase the level of acetic acid in bread crumb.

Yeast added in levels from 20 to 60 g/flour did not influence formation of the compounds formed from lipid oxidation (e.g. 2-nonenal and hexanal) (**Figure 17**). Yeast contains lipids in their cell membrane, and lysis of yeast cells during baking might therefore influence the level of lipid oxidation compounds in breads fermented with different yeast levels this was however not seen from the results.

A high yeast concentration obviously decreases the fermentation time, which is an economical benefit for the bread industry. However, it is possible that non-volatile compounds from the yeast are unwanted from a sensory point of view. Research within identification and quantification of non-volatile compounds in bread could therefore be relevant in order to provide a more detailed flavour profile of bread fermented at different yeast levels.

5.2.2 Baker´s yeast (Paper III)

The effect of the type of commercial baker's yeast on bread crumb aroma was investigated in Paper III. An overview of the aroma profile of the seven commercial baker's yeasts is shown in a PCA plot (**Figure 18**). A one-way analysis of variance between the samples was furthermore calculated and can be found in Table 3 in Paper III.

Fermentation of wheat dough by seven commercial baker´s yeast resulted in a diverse aroma profile of the bread crumb depending on the type of baker's yeast (**Figure 18**). The fermentation compounds 2,3-butanedione and 1-propanol were found in a higher concentration in breads fermented with the four baker's yeasts (Bruggeman, Zymarom, Sema and l'Hirondelle) (**Figure 18**) having the shortest fermentation times (**Table 5**). Furthermore, 3-methylbutanal, 2-methyl-1-propanol and ethyl acetate were found in a higher concentrations in two of those yeasts (Bruggeman and Zymarom) (**Figure 18**).

On the other hand crumb of bread produced with the two organically produced baker's yeasts (Skærtoftmølle and Rapunzel) were found to have significantly higher concentration of the fermentation compounds phenylacetaldehyde and 2-phenylethanol, and were together with the bread fermented with Sema, furthermore characterised by having higher concentrations of the lipid oxidation compound hexanal (**Figure 18**). The higher concentration of hexanal might be due to a higher lipoxygenase activity of the yeast in Skærtoftmølle, Rapunzel and Sema compared to the other baker's yeasts. Apart from this, no significantly differences were found in the concentration of lipid oxidation compounds in the bread samples.

Bread fermented with the baker's yeast named Maltserkors had a significantly lower concentration of the majority of all the volatile compounds compared to the other six baker's yeasts (**Figure 18**). This picture would be different if the doughs were prepared with equal yeast amount (w/w) instead of equal number of yeast cells (CFU/kg flour) (see **Table 1**).

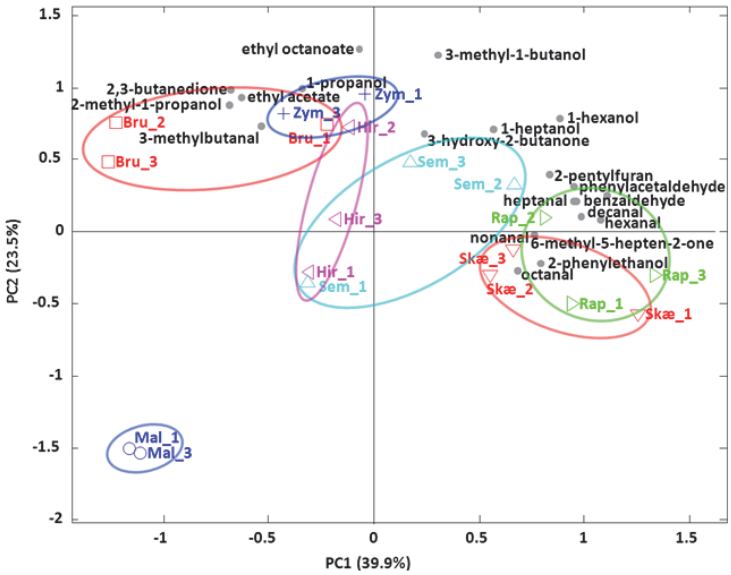

Figure 18. PCA biplot showing the aroma compounds formed in the bread samples fermented with the seven commercial baker's yeasts ($2.88 \cdot 10^{11}$ CFU/kg flour). The samples are named as following; Mal for Malteserkors, Skæ for Skærtoftmølle, Rap for Rapunzel, Hir for L´Hirondelle, Sem for Sema, Bru for Bruggeman and Zym for Zymarom. The number at the end of the sample name corresponds to the triplicate number. The seven yeast samples are manually marked with circles in order to highlight the results of the PCA. Only aroma compounds having an OAV of 0.1 or higher were included in the PCA model.

The significant differences in the aroma profile between the bread fermented with the seven baker's yeasts indicate that some of the baker's yeasts differentiate in yeast strain. The aroma profiles of the organically produced baker's yeasts Skærtoftmølle and Rapunzel are quite similar, which might indicate that they contain the same yeast strain. The differences in the aroma profiles may be due to differences in the gene-regulating mechanisms and biosynthetic pathways of aroma compound formation between these different yeast strains during dough fermentation.

Phenylacetaldehyde and 2-phenylethanol, both formed from catabolism of phenylalanine in the Ehrlich pathway (Dickinson et al., 2003), were, as mentioned above, found in higher concentrations in bread fermented with Rapunzel and Skærtoftmølle compared with bread fermented with the other five baker's yeasts (Paper III). On the other hand 1-propanol, 2-methyl-1-propanol and 3-methylbutanal formed from valin and leucin also through the Ehrlich pathway (Hazelwood et al., 2008) were found in a higher content for the two Belgian yeasts (Bruggeman and Zymarom). These apparently contradictory results might be explained by the presence of different carboxylases in the commercial baker's yeast. Different carboxylases have been found to be important for the catabolism of the branched-chain amino acids (leucin and valin) and the aromatic amino acid (phenylalanine), respectively in the Ehrlich pathway (Dickinson et al., 2003).

A high content of lactic acid bacteria (LAB) ($0.1 \bullet 10^{10}$ to $4.1 \bullet 10^{10}$ CFU of LAB/g baker's yeast) were found in the seven commercial baker's yeasts investigated in Paper III. However, it is assumed that the majority of the fermentation compounds identified in the bread samples in the research work of Paper III (**Table 9**) were produced mainly from the metabolism of yeast based on the short fermentation times (see **Table 5**).

Identification of the yeast strains in the seven baker's yeasts could be very interesting in order to increase knowledge within aroma formation during dough fermentation by baker's yeast.

5.2.3 Temperature (Paper II)

The influence of fermentation temperature (5, 15 and 35°C) on bread crumb aroma was investigated in Paper II. An overview of the results is shown in a PCA plot (**Figure 19**). Furthermore, a two-way analysis of variance between the samples was calculated and can be found in Table 2 in Paper II.

A low fermentation temperature (5°C) resulted in a higher concentration of the three esters ethyl acetate, ethyl hexanoate, and ethyl octanoate (**Figure 19**), with ethyl hexanoate (OAV's 0.2-0.5) as being the most aroma active of the esters (**Table 9**). A higher concentration of esters was also found by reducing the fermentation temperature from 25-28 to 13-15°C during wine fermentation (Molina et al., 2007; Beltran et al., 2008).

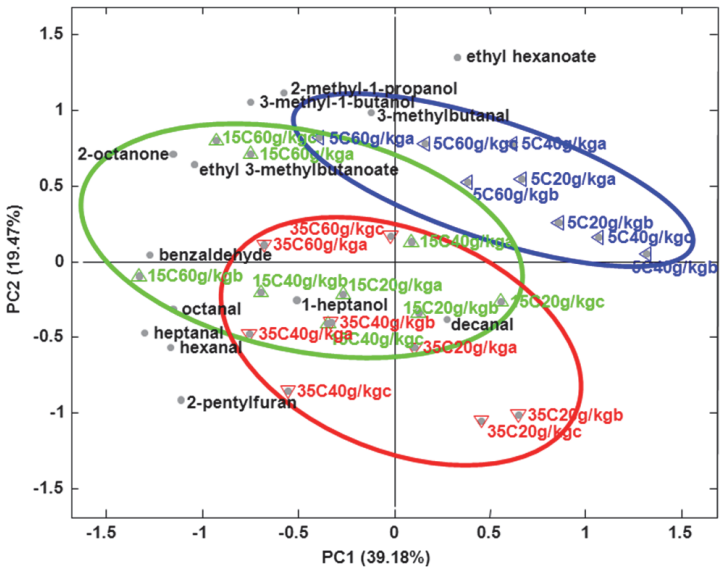

Figure 19. PCA biplot showing the effect of fermentation temperature on aroma formation (Paper II). The sample names are described in **Figure 17**. Blue, green and red triangles show samples fermented at 5, 15 and 35°C, respectively. Only aroma compounds having an OAV of 0.1 or higher and furthermore with regression coefficients higher than ±0.4 were included in the PCA model for the PCA biplot. Regression coefficients were calculated from a PLS model with fermentation temperature as the y-variable (data not shown).

The typical fermentation compound, 3-methylbutanal, was found in a significantly higher amount, when the fermentation was done at 15ºC compared to 5 and 35ºC. Formation of the other important

fermentation compounds (e.g. 3-methyl-1-butanol, 2,3-butanedione and phenylacetaldehyde) was not significantly influenced by fermentation temperature (see Table 2 in Paper II).

Gassenmeier and Schieberle (1995) found the significant highest concentrations of 3-methyl-1-butanol and 2-phenylethanol when fermentations of liquid pre-ferments were done at 35ºC, compared to 25, 30 and 40ºC. The disagreeing results regarding the effect of fermentation temperature on formation of fermentation compounds are most likely explained by different fermentation times in the two research studies. Gassenmeier and Schieberle (1995) fermented a liquid pre-ferment at different temperatures (25, 30, 35 and 40ºC) for eight hours, while the dough fermentations at different temperatures (5, 15 and 35 ºC) carried out in Paper II were terminated at a pre-defined dough height, resulting in different fermentation times (ranging from 15 min to 21 hrs (**Table 4**),

Fermentation temperatures of 15 and 35°C resulted in a higher concentration of the majority of the compounds formed from degradation of unsaturated fatty acids compared to fermentation at 5°C (**Figure 19**), with hexanal and heptanal as being the most aroma active (OAV's from 12 to 20 and 5 to 13, respectively). It is well known that a higher temperature increases the rate of lipid oxidation. However, it is of interest that (E)-2-nonenal and nonanal, evaluated as two of the most aroma active compounds in bread crumb (**Table 9**), was not found in significant higher concentrations, when the fermentation temperature was increased from 5 to 35°C (see Table 2 in Paper I).

The high influence of fermentation temperature on lipid oxidation compounds might be of great importance for the overall aroma profile of bread, and particularly the aldehydes are important, because of their relatively low odour threshold and green off-odours (Zehentbauer and Grosch, 1998).

5.2.4 Fermentation time

Yeast level, fermentation temperature, and fermentation time are three factors that are inextricably linked with each other and changes within yeast level or fermentation temperature will have a great impact on the fermentation time (Paper II). This is important to consider when investigating the effect of fermentation time on bread aroma. In Paper II it was found that the fermentation time varied greatly from 15 min to 21h when doughs were fermented at different fermentation temperatures (5, 15 and 35°C) and yeast levels (20, 40 and 60 g/kg flour). However, no direct correlation was found between fermentation time and formation of volatile compounds by this experimental design.

Maeda et al. (2009) found that a 3h dough fermentation period resulted in the highest content of alcohols, esters and aromatic compounds in bread crumb, compared to fermentation for shorter periods (15 min, 1h and 2h). On the other hand the authors found that the level of aldehydes, carboxylic acids, ketones and diketones was reduced during 3h fermentation, compared to the shorter fermentation times. The level of alkenals, sulphuric and furanic compounds was not found to be influenced by fermentation times from 15 min to 3h (Maeda et al., 2009). Schieberle and Grosch (1991) found that the concentration of the fermentation compounds 2,3-butanedione, 3-methyl-1-butanol, 2-phenylethanol and increased by 2, 8 and 64 times, respectively in long-time fermented bread (3h) compared to short-time fermented bread (time not stated). The result is in agreement with Maeda et al. (2009), however not for 2,3-butanedione, which might be due to the yeasts ability to reabsorb 2,3-butanedione (Dulieu et al., 2000).

Richard-Molard et al. (1979) investigated the effect of fermentation time from 3 to 10h on formation of acetic acid in bread crumb. The fermentations were done with 20 g yeast/kg flour (temperature not stated in the paper). They found an increasing content of acetic acid from 30 to 75 mg/kg when the fermentation time was increased from 3 to 6 hrs, where after the concentration of acetic acid stagnated. Acetic acid is described as sour and pungent (Gassenmeier and Schieberle, 1995), however it is also suggested to act as an aroma enhancer in the levels of 100-200 mg/kg (Richard-Molard et al., 1979).

It was stated in the introduction of this thesis that a longer fermentation time is generally believed to result in bread with a more pleasant aroma. The truth of this statement is not yet clarified from the research work of this thesis or from the research work of Maeda et al. (2009), Schieberle and Grosch (1991) and Richard-Molard et al. (1979), since additional sensory analysis should be done. However, from the results of Paper II it could be speculated that fermentation temperature might be more important than a long fermentation time for a pleasant bread aroma, as a low fermentation temperature (5°) resulted in bread with a higher content of esters and a lower content of compounds formed from the lipid oxidation compared to fermentation at higher temperatures (15 and 35°C).

6. CONCLUSIONS

6.1 Dough expansion

Dough expansion was found to be significantly influenced by the type and level of commercial baker's yeast, as well as by the fermentation temperature. Doughs fermented with baker's yeast I, II, III and V (Bruggeman, Zymarom, l'Hirondelle and Rapunzel) generally had higher kinetic rate constants and hence shorter fermentation times compared to doughs fermented with baker's yeast IV, VI and VII (Sema, Skærtoftmølle and Malteserkors). The highest kinetic rate constants were found for doughs fermented at 25°C with the highest yeast concentration ($8.64 \cdot 10^{11}$ CFU/kg flour) compared with doughs fermented at 5, 15 and 35°C and/or with lower yeast concentrations. This result was found with significance for baker's yeast IV, VI and VII and as a clear trend for baker´s yeast I, II, III and V.

The doughs fermented with the two highest yeast concentrations (for all baker's yeasts) had more comparable kinetic rate constants and markedly shorter fermentation times compared to dough fermented with the lowest concentration of yeast. Lower kinetic rate constants and hence longer fermentation times were generally found for doughs fermented with all baker's yeasts at 5°C and the lowest yeast concentration ($2.88 \cdot 10^{11}$ CFU/kg flour) compared with doughs fermented at higher temperatures (15, 25 and 35°C) and higher yeast concentrations ($5.76 \cdot 10^{11}$ and $8.64 \cdot 10^{11}$ CFU/kg flour). Baker's yeast IV, VI and VII were not able to reach the optimal dough height at 5°C and the lowest yeast concentration.

6.2 Bread aroma

A wide range of aroma compounds were identified in bread crumb, mainly originating from the activity of yeast and from oxidation of flour lipids. The most aroma active compounds were found to be (E)-2-nonanal (green, tallow), 3-methyl-1-butanol (balsamic, alcoholic), 3-methylbutanal (malty), hexanal (green), 2,3-butanedione (buttery, caramel), nonanal (citrus), 1-octen-3-ol (mushroom) and phenylacetaldehyde (honey-like). A relatively high level of lactic acid bacteria ($0.1 \cdot 10^{10}$ to $4.1 \cdot 10^{10}$ CFU of LAB/g baker's yeast) was found in the seven baker's yeasts however, it is assumed that the vast majority of the volatile compounds was formed from yeast activity due to the short fermentation times.

Bread aroma was found to be greatly influenced by the level and type of baker's yeast as well as the fermentation temperature. Fermentation with the highest yeast concentration (60 g/kg flour) resulted in breads with the highest concentration of compounds originating from the yeast activity compared with breads fermented at lower yeast concentrations (20 and 40 g/kg flour). Formation of compounds originating from oxidation of flour lipids was found to be independent of the yeast level. A fermentation temperature at 5°C resulted in breads with the highest concentration of esters (fruity odours), compared to breads fermented at higher temperature (15 and 35°C). Fermentation at 15 and 35°C resulted on the other hand in breads with the highest concentration of many lipid oxidation compounds often characterised as off-odours compared to breads fermented at 5°C.

Fermentation by baker's yeasts I to IV resulted in breads having a significantly higher concentration of 2,3-butanedione and 1-propanol compared to breads fermented by the other baker's yeasts. Furthermore, 3-methylbutanal, 2-methyl-1-propanol and ethyl acetate were found in significantly higher concentration in breads fermented by baker's yeasts I and II. Breads fermented by baker's yeast V and VI had a significantly higher concentration of phenylacetaldehyde and 2-phenylethanol. Breads fermented by baker's yeast VII had a significantly lower content of the majority of the aroma compounds. The fact that dough fermentation time and bread aroma was significantly influenced by the type of commercial yeast indicates that some of the seven baker´s yeasts might differentiate in yeast strain.

From the results of the research work of this PhD thesis it could be speculated that the conditions for the most optimal bread aroma might be dough fermentation by one of the two Belgian commercial baker's yeasts (Zymarom or Bruggeman) at a fermentation temperature of 5°C, as these conditions result in bread with a slightly fruity odour from the esters and a low content of lipid oxidation compounds (green and citrus odours). However, it is recommended to include sensory analysis in future research studies in order to support the results of this PhD project.

7. FUTURE PERSPECTIVES

Dough fermentation with a high concentration of yeast (60 g Maltserkors/kg flour) was found to increase formation of the majority of the fermentation compounds, without influencing the formation of lipid oxidation compounds as well as decreasing the fermentation time, compared to dough containing lower yeast concentration (20 and 40 g Malteserkors/kg flour) (Paper II). However, it is possible that non-volatile compounds from the yeast are unwanted from a sensory point of view. Very few research studies have been investigating the influence of non-volatile compounds on bread flavour (Jensen et al., 2011; Bin et al., 2012). Research within identification and quantification of non-volatile compounds as well as aliphatic acids in bread could support the findings of Paper II and hence provide a more detailed flavour profile of bread fermented at different yeast levels. Analysis of non-volatile compounds and also aliphatic acids could be done by high performance liquid chromatography (HPLC) analysis (Jensen et al., 2011; Jayaram et al., 2013) or by applying different derivatisation methods followed by GC-MS analysis (Proestos et al., 2006; Sha et al., 2010) in combination with sensory analysis.

Additionally, it could be relevant to evaluate breads fermented at different fermentation temperatures by a sensory analysis in order to elucidate if the higher concentration of esters and the lower concentration of lipid oxidation compounds found in bread fermented at 5°C compared to 15 and 35°C (Paper II) can actually be sensed in bread. Furthermore, it should be investigated, whether the breads fermented at 5°C are preferred over breads fermented at higher temperatures. It should be noted that bread fermentation at 5°C is problematic, since industrial bakeries do not typically have the facilities to store large amounts of dough at 5°C. Cooling is expensive therefore dough fermentation at 5°C might only be relevant in production of "high quality" specialty breads. Another important issue for the baking industry is the 10% lower volume of dough fermented at 5°C compared to dough fermented at 15, 25 or 35°C (Paper I).

The fermentation times for doughs fermented at different yeast levels and fermentation temperatures were achieved from model doughs (around 240 g). The fermentation times achieved by the same fermentation conditions used in the research work of this thesis would be distinctly different from the times obtained in industrial bakeries because of the markedly larger dough volumes and the additional proofing step. Therefore investigation of the differences between small scale and large scale fermentation profiles could be relevant to investigate.

Research within selection of yeast strains with improved aroma formation could be of industrial interest (Styger et al., 2011). Particularly selection of yeast strains with increased ester formation could be interesting. Studies have recently been carried out on alcohol acetyltransferases from *S. cerevisiae* and their influence on ester formation in alcoholic fermentation (Verstrepen et al., 2003; Procopio et al., 2011; Suárez-Lepe and Morata, 2012). The results from these studies might be successfully applied to dough fermentation in the search for novel ways of developing the aroma of bread. Further experiments with the use of non-Saccharomyces strains producing high concentrations of esters in the dough could also be interesting in order to develop bread with a more fruity and appealing aroma. The choice of strain could be inspired from research studies within alcoholic

fermentation, where strains of *Pichia anomala*, *Hanseniospora guillermondii* and *Hanseniospora osmophila* were found to significantly increase formation of some esters (Rojas et al., 2001; Rojas et al., 2003; Viana et al., 2008). Non-*Saccharomyces* yeasts are normally used in mixed cultures combined with *S. cerevisae* in alcoholic fermentation. Ciani et al. (2006) and Andorrá et al. (2012) found that pure cultures of some non-Saccharomyces wine yeasts (*Hanseniaspora uvarum*, *Torulaspora delbrueckii*, *Candida zemplinina* and *Kluyveromyces thermotolerans*) had reduced fermentation rate compared with pure cultures of *S. cerevisae*. Use of non-*Saccharomyces* yeast combined with *S. cerevisae* is therefore also assumed to be the best choice in future experiments within dough fermentation.

REFERENCES

AACC, 1995a. Rheological behavior of flour by farinograph: Constant flour weight procedure. Approved methods of the American Association of Cereal Chemists 9, Method 54-21.

AACC, 1995b. Wet gluten, dry gluten, water-binding capacity, and gluten index. Approved methods of the American Association of Cereal Chemists 38-12.

Addo, K., Xiong, Y. L., Blanchard, S. P., 2001. Thermal and dynamic rheological properties of wheat flour fractions. Food Research International 34, 329-335.

Amigo, J. M., Popielarz, P. J., Callejón, R. M., Morales, M. L., Troncoso, A. M., Petersen, M. A., Toldam-Andersen, T. B., 2010. Comprehensive analysis of chromatographic data by using PARAFAC2 and principal components analysis. Journal of Chromatography A 1217, 4422-4429.

Amigo, J. M., Skov, T., Coello, J., Maspoch, S., Bro, R., 2008. Solving GC-MS problems with PARAFAC2. Trends in Analytical Chemistry 27, 714-725.

Andorrá, I., Berradre, M., Mas, A., Esteve-Zarzoso, B., Guillamón, J. M., 2012. Effect of mixed culture fermentations on yeast populations and aroma profile. LWT - Food Science and Technology 49, 8-13.

Angelov, A., Hristozova, T., Roshkova, Z., 1997. Maltase activity and dough rising ability of baker's yeasts obtained at different growth conditions during the maturation stage. Zeitschrift fur Lebensmittel-Untersuchung Und-Forschung A-Food Research and Technology 205, 80-84.

Angioloni, A., Collar, C., 2009. Bread crumb quality assessment: a plural physical approach. European Food Research and Technology 229, 21-30.

Attfield, P. V., 1997. Stress tolerance: The key to effective strains of industrial baker's yeast. Nature Biotechnology 15, 1351-1357.

Barak, S., Mudgil, D., Khatkar, B. S., 2013. Relationship of gliadin and glutenin proteins with dough rheology, flour pasting and bread making performance of wheat varieties. LWT - Food Science and Technology 51, 211-217.

Barylko-Pikielna, N., Matuszewska, I., Hellemann, U., 1990. Effect of salt on time-intensity characteristics of bread. Lebensmittel-Wissenschaft und-Technologie 23, 422-426.

Bechtel, W. G., Meisner, D. F., Bradley, W. B., 1953. The effect of the crust on the staling of bread. Cereal Chemistry 30, 160-168.

Beck, M., Jekle, M., Becker, T., 2012. Sodium chloride - sensory, preserving and technological impact on yeast-leavened products. International Journal of Food Science and Technology 47, 1798-1807.

Bell, P. J. L., Higgins, V. J., Attfield, P. V., 2001. Comparison of fermentative capacities of industrial baking and wild-type yeasts of the species *Saccharomyces cerevisiae* in different sugar media. Letters in Applied Microbiology 32, 224-229.

Beltran, G., Novo, M., Guillamon, J. M., Mas, A., Rozes, N., 2008. Effect of fermentation temperature and culture media on the yeast lipid composition and wine volatile compounds. International Journal of Food Microbiology 121, 169-177.

Bianchi, F., Careri, M., Chiavaro, E., Musci, M., Vittadini, E., 2008. Gas chromatographic-mass spectrometric characterisation of the Italian protected designation of origin "Altamura" bread volatile profile. Food Chemistry 110, 787-793.

Bicchi, C., Cordero, C., Liberto, E., Rubiolo, P., Sgorbini, B., 2004. Automated headspace solid-phase dynamic extraction to analyse the volatile fraction of food matrices. Journal of Chromatography A 1024, 217-226.

Bin, Q., Jiang, D. S., Cho, I. H., Peterson, D. G., 2012. Chemical markers for bitterness in wheat bread. Flavour and Fragrance Journal 27, 454-458.

Bot, A., de Bruijne, D. W., 2003. Osmotic properties of gluten. Cereal Chemistry 80, 404-408.

Brabant, C., Fossati, D., Kleijer, G., Vincent, V., Jacques, C., 2007. Influence of the wheat variety on the bread flavour. Revue Suisse D Agriculture 39, 101-108.

Carballo, J., 2012. The role of fermentation reactions in the generation of flavour and aroma of foods. In: Fermentation. CRC Press, Boca Raton,

Carrillo, J. D., Tena, M. T., 2006. Determination of volatile compounds in antioxidant rosemary extracts by multiple headspace solid-phase microextraction and gas chromatography. Flavour and Fragrance Journal 21, 626-633.

Catterall, P., 1998. Flour milling. In: Cauvain, S P, Young, L S (Eds.), Technology of breadmaking. Blackie Academic and Proffesional, London, pp. 296-329.

Cauvain, S. P., 1998. Breadmaking processes. In: Cauvain, S P, Young, L S (Eds.), Technology of breadmaking. Blackie Academic and Professional, London, pp. 18-44.

Chang, C.-Y., Seitz, L. M., Chambers, E., 1995. Volatile flavor components of breads made from hard red winter wheat and hard white winter wheat. Cereal Chemistry 72, 237-242.

Chavan, R. S., Chavan, S. R., 2011. Sourdough technology a traditional way for wholesome foods: A review. Comprehensive Reviews in Food Science and Food Safety 10, 169-182.

Chevallier, S., Zúñiga, R., Le-Bail, A., 2012. Assessment of bread dough expansion during fermentation. Food and Bioprocess Technology 5, 609-617.

Ciani, M., Beco, L., Comitini, F., 2006. Fermentation behaviour and metabolic interactions of multistarter wine yeast fermentations. International Journal of Food Microbiology 108, 239-245.

Corsetti, A., Gobbetti, M., Balestrieri, F., Paoletti, F., Russi, L., Rossi, J., 1998. Sourdough lactic acid bacteria effects on bread firmness and staling. Journal of Food Science 63, 347-351.

Corsetti, A., Gobbetti, M., De Marco, B., Balestrieri, F., Paoletti, F., Russi, L., Rossi, J., 2000. Combined effect of sourdough lactic acid bacteria and additives on bread firmness and staling. Journal of Agricultural and Food Chemistry 48, 3044-3051.

Czerny, M., Schieberle, P., 2002. Important aroma compounds in freshly ground wholemeal and white wheat flour - Identifidation and quantitative changes during sourdough fermentation. Journal of Agricultural and Food Chemistry 50, 6835-6840.

Decock, P., Cappelle, S., 2005. Bread technology and sourdough technology. Trends in Food Science and Technology 16, 113-120.

Dickinson, J. R., Eshantha, L., Salgado, J., Hewlins, M. J. E., 2003. The catabolism of amino acids to long chain and complex alcohols in *Saccharomyces cerevisiae*. Journal of Biological Chemistry 278, 8028-8034.

Does, A. L., Bisson, L. F., 1989. Comparison of glucose-uptake kinetics in different yeasts. Journal of Bacteriology 171, 1303-1308.

Dornez, E., Gebruers, K., Joye, I. J., De Ketelaere, B., Lenartz, J., Massaux, C., Bodson, B., Delcour, J. A., Courtin, C. M., 2008. Effects of genotype, harvest year and genotype-by-harvest year interactions on arabinoxylan, endoxylanase activity and endoxylanase inhibitor levels in wheat kernels. Journal of Cereal Science 47, 180-189.

Dulieu, C., Moll, M., Boudrant, J., Poncelet, D., 2000. Improved performances and control of beer fermentation using encapsulated alpha-acetolactate decarboxylase and modeling. Biotechnology Progress 16, 958-965.

Elhariry, H. M., Mahmoud, R. M., Hassan, A. A., Aly, M. A., 2011. Development of co-culture sourdough systems for improving bread quality and delaying staling. Food Biotechnology 25, 252-272.

Erekul, O., Kohn, W., 2006. Effect of weather and soil conditions on yield components and bread-making quality of winter wheat (Triticum aestivum L.) and winter triticale (Triticosecale Wittm.) varieties in north-east Germany. Journal of Agronomy and Crop Science 192, 452-464.

Every, D., Ross, M., 1996. The role of dextrins in the stickiness of bread crumb made from pre-harvest sprouted wheat or flour containing exogenous alpha-amylase. Journal of Cereal Science 23, 247-256.

Frasse, P., Lambert, S., Levesque, C., Melcion, D., Richard-Molard, D., Chiron, H., 1992. The influence of fermentation on volatile compounds in french bread crumb. Food Science and Technology-Lebensmittel-Wissenschaft and Technologie 25, 66-70.

Fredriksson, H., Silverio, J., Andersson, R., Eliasson, A. C., Åman, P., 1998. The influence of amylose and amylopectin characteristics on gelatinization and retrogradation properties of different starches. Carbohydrate Polymers 35, 119-134.

Gassenmeier, K., Schieberle, P., 1995. Potent aromatic compounds in the crumb of wheat bread (French-type) - influence of pre-ferments and studies on the formation of key odorants during dough processing. Zeitschrift für Lebensmittel-Untersuchung und - Forschung 201, 241-248.

Goesaert, H., Brijs, K., Veraverbeke, W. S., Courtin, C. M., Gebruers, K., Delcour, J. A., 2005. Wheat flour constituents: how they impact bread quality, and how to impact their functionality. Trends in Food Science and Technology 16, 12-30.

Goesaert, H., Leman, P., Bijttebier, A., Delcour, J. A., 2009. Antifirming effects of starch degrading enzymes in bread crumb. Journal of Agricultural and Food Chemistry 57, 2346-2355.

Grosch, W., 1993. Detection of potent odorants in foods by aroma extract dilution analysis. Trends in Food Science and Technology 4, 68-73.

Gujral, H. S., Singh, N., 1999. Effect of additives on dough development, gaseous release and bread making properties. Food Research International 32, 691-697.

Hazelwood, L. A., Daran, J. M., van Maris, A. J. A., Pronk, J. T., Dickinson, J. R., 2008. The ehrlich pathway for fusel alcohol production: a century of research on *Saccharomyces cerevisiae* metabolism. Applied and Environmental Microbiology 74, 2259-2266.

Hofmann, T., Münch, P., Schieberle, P., 2000. Quantitative model studies on the formation of aroma-active aldehydes and acids be Strecker-type reactions. Journal of Agricultural and Food Chemistry 48, 434-440.

Hotelling, H., 1933. Analysis of complex of statistical variables with principal components. Journal of Educational Psychology 24, 417-441.

Hug-Iten, S., Escher, F., Conde-Petit, B., 2003. Staling of bread: Role of amylose and amylopectin and influence of starch-degrading enzymes. Cereal Chemistry 80, 654-661.

ICC, 1995. Determination of the "Falling number" according to Hagberg - as a measure of the degree of alpha-amylase activity in grain and flour. ICC-Standard No.107/1.

Jacob, H. E., 1944. Six thousand years of bread: Its holy and unholy history, Doubleday, Doran and Co, New York.

Jayaram, V. B., Cuyvers, S., Lagrain, B., Verstrepen, K. J., Delcour, J. A., Courtin, C. M., 2013. Mapping of *Saccharomyces cerevisiae* metabolites in fermenting wheat straight-dough reveals succinic acid as pH-determining factor. Food Chemistry 136, 301-308.

Jensen, S., Oestdal, H., Skibsted, L. H., Larsen, E., Thybo, A. K., 2011. Chemical changes in wheat pan bread during storage and how it affects the sensory perception of aroma, flavour, and taste. Journal of Cereal Science 53, 259-268.

Khatkar, B. S., Bell, A. E., Schofield, J. D., 1995. The dynamic rheological properties of glutens and gluten subfractions from wheats of good and poor bread-making quality. Journal of Cereal Science 22, 29-44.

Kolb, B., 1982. Multiple headspace extraction—A procedure for eliminating the influence of the sample matrix in quantitative headspace, gas chromatography. Chromatographia 15, 587-594.

Kolb, B., Ettre, L. S., 1991. Theory and practice of multiple headspace extraction. Chromatographia 32, 505-513.

Lagunas, R., 1986. Misconceptions about the energy-metabolism of *Saccharomyces cerevisiae*. Yeast 2, 221-228.

Leon, A. E., Barrera, G. N., Perez, G. T., Ribotta, P. D., Rosell, C. M., 2006. Effect of damaged starch levels on flour-thermal behaviour and bread staling. European Food Research and Technology 224, 187-192.

Li, W., Dobraszczyk, B. J., Wilde, P. J., 2004. Surface properties and locations of gluten proteins and lipids revealed using confocal scanning laser microscopy in bread dough. Journal of Cereal Science 39, 403-411.

Lynch, E. J., Dal Bello, F., Sheehan, E. M., Cashman, K. D., Arendt, E. K., 2009. Fundamental studies on the reduction of salt on dough and bread characteristics. Food Research International 42, 885-891.

Maeda, T., Kikuma, S., Araki, T., Ikeda, G., Takeya, K., Sagara, Y., 2009. The effects of mixing stage and fermentation time on the quantity of flavor compounds and sensory intensity of flavor in white bread. Food Science and Technology Research 15, 117-126.

Mahattanatawee, K., Rouseff, R., 2011. Gas Chromatography/Olfactometry (GC/O). In: Goodner, K, Rouseff, R (Eds.), Practical Analysis of Flavor and Fragrance Materials. Blackwell Publishing Ltd, pp. 69-90.

Maloney, D., Foy, J., 2003. Yeast fermentations. In: Kulp, K, Lorenz, K (Eds.), Handbook of dough fermentations. Marcel Dkker Inc., New York,

Mestres, M., Busto, O., Guasch, J., 2000. Analysis of organic sulfur compounds in wine aroma. Journal of Chromatography A 881, 569-581.

Molina, A. M., Swiegers, J. H., Varela, C., Pretorius, I. S., Agosin, E., 2007. Influence of wine fermentation temperature on the synthesis of yeast-derived volatile aroma compounds. Applied Microbiology and Biotechnology 77, 675-687.

Mousia, Z., Campbell, G. M., Pandiella, S. S., Webb, C., 2007. Effect of fat level, mixing pressure and temperature on dough expansion capacity during proving. Journal of Cereal Science 46, 139-147.

Ozcan, S., Johnston, M., 1999. Function and regulation of yeast hexose transporters. Microbiology and Molecular Biology Reviews 63, 554-569.

Pedersen, B., Eggum, B. O., 1983. The influence of milling on the nutritive value of flour from cereal grains. 2. Wheat. Qualitas Plantarum-Plant Foods for Human Nutrition 33, 51-61.

Poinot, P., Arvisenet, G., Grua-Priol, J., Fillonneau, C., Le-Bail, A., Prost, C., 2010. Influence of inulin on bread: Kinetics and physico-chemical indicators of the formation of volatile compounds during baking. Food Chemistry 119, 1474-1484.

Poinot, P., Grua-Priol, J., Arvisenet, G., Rannou, C., Semenou, M., Bail, A. L., Prost, C., 2007. Optimisation of HS-SPME to study representativeness of partially baked bread odorant extracts. Food Research International 40, 1170-1184.

Poitrenaud, B., 2004. Baker's yeast. In: Yui, Y H, Meunier-Goddik, L, Hansen, Å S, Nip, W K, Stanfield, P S, Toldrá, F (Eds.), Handbook of food and beverage fermentation technology. Marcel Dekker Inc., New York, pp. 695-719.

Popineau, Y., Deshayes, G., Lefebvre, J., Fido, R., Tatham, A. S., Shewry, P. R., 2001. Prolamin aggregation, gluten viscoelasticity, and mixing properties of transgenic wheat lines expressing 1Ax and 1Dx high molecular weight glutenin subunit transgenes. Journal of Agricultural and Food Chemistry 49, 395-401.

Primo-Martin, C., van Nieuwenhuijzen, N. H., Hamer, R. J., van Vliet, T., 2007. Crystallinity changes in wheat starch during the bread-making process: Starch crystallinity in the bread crust. Journal of Cereal Science 45, 219-226.

Procopio, S., Qian, F., Becker, T., 2011. Review Paper: Function and regulation of yeast genes involved in higher alcohol and ester metabolism during beverage fermentation. European Food Research and Technology 233, 721-729.

Proestos, C., Sereli, D., Komaitis, M., 2006. Determination of phenolic compounds in aromatic plants by RP-HPLC and GC-MS. Food Chemistry 95, 44-52.

Prost, C., Poinot, P., Rannou, C., Arvisenet, G., 2012. Bread aroma. In: Cauvain, S P (Ed.), Breadmaking: Improving quality. Woodhead Publishing ltd, Cambridge, pp. 523-561.

Purhagen, J. K., Sjoo, M. E., Eliasson, A. C., 2012. The anti-staling effect of pre-gelatinized flour and emulsifier in gluten-free bread. European Food Research and Technology 235, 265-276.

Purlis, E., 2010. Browning development in bakery products - A review. Journal of Food Engineering 99, 239-249.

Quílez, J., Ruiz, J. A., Romero, M. P., 2006. Relationships between sensory flavor evaluation and volatile and nonvolatile compounds in commercial wheat bread type baguette. Journal of Food Science 71, S423-S427.

Redon, M., Guillamon, J. M., Mas, A., Rozes, N., 2011. Effect of growth temperature on yeast lipid composition and alcoholic fermentation at low temperature. European Food Research and Technology 232, 517-527.

Reineccius, G., 1998. Gas chromatography. In: Nielsen, S S (Ed.), Food analysis. Klywer Academic/Plenum Publishers, New York, pp. 527-547.

Richard-Molard, D., Nago, M. C., Drapron, R., 1979. Influence of the bread making method on french bread flavor. Bakers Digest 53, 34-38.

Rojas, V., Gil, J. V., Piñaga, F., Manzanares, P., 2001. Studies on acetate ester production by non-Saccharomyces wine yeasts. International Journal of Food Microbiology 70, 283-289.

Rojas, V., Gil, J. V., Piñaga, F., Manzanares, P., 2003. Acetate ester formation in wine by mixed cultures in laboratory fermentations. International Journal of Food Microbiology 86, 181-188.

Ruiz, J. A., Quilez, J., Mestres, M., Guasch, J., 2003. Solid-phase microextraction method for headspace analysis of volatile compounds in bread crumb. Cereal Chemistry 80, 255-259.

Salovaara, H., Hellemann, U., Kurkela, R., 1982. Effect of salt on bread flavour. Lebensmittel-Wissenschaft und-Technologie 15, 270-274.

Sanz, C., Maeztu, L., Zapelena, M. J., Bello, J., Cid, C., 2002. Profiles of volatile compounds and sensory analysis of three blends of coffee: influence of different proportions of Arabica and Robusta and influence of roasting coffee with sugar. Journal of the Science of Food and Agriculture 82, 840-847.

Schieberle, P., Grosch, W., 1987. Evaluation of the flavour of wheat and rye bread crusts by aroma extract dilution analysis. Zeitschrift für Lebensmittel-Untersuchung und - Forschung 185, 111-113.

Schieberle, P., Grosch, W., 1991. Potent odorants of the wheat bread crumb. Zeitschrift für Lebensmittel-Untersuchung und - Forschung 192, 130-135.

Schieberle, P., Grosch, W., 1994. Potent odorants of rye bread crust - differences from the crumb and from wheat bread crust. Zeitschrift für Lebensmittel-Untersuchung und - Forschung 198, 292-296.

Seitz, L. M., Chung, O. K., Rengarajan, R., 1998. Volatiles in selected commercial breads. Cereal Chemistry 75, 847-853.

Sha, Y. F., Meng, J. R., Zhang, Y. C., Deng, C. H., Wu, D., 2010. Determination of volatile organic acids in tobacco by single-drop microextraction with in-syringe derivatization followed by GC-MS. Journal of Separation Science 33, 212-217.

Shah, P., Campbell, G. M., Mckee, S. L., Rielly, C. D., 1998. Proving of bread dough: Modelling the growth of individual bubbles. Food and Bioproducts Processing 76, 73-79.

Shewry, P. R., Tatham, A. S., 1997. Disulphide bonds in wheat gluten proteins. Journal of Cereal Science 25, 207-227.

Shewry, P. R., Tatham, A. S., Barro, F., Barcelo, P., Lazzeri, P., 1995. Biotechnology of Breadmaking - Unraveling and Manipulating the Multi-Protein Gluten Complex. Bio-Technology 13, 1185-1190.

sisweb.com, 2013. Tenax TA breakthrough volume data. http://www.sisweb.com/index/referenc/tenaxta.htm Scientific Instrument Services, Inc..

Soria, A. C., Martinez-Castro, I., Sanz, J., 2007. Estimation of recovery by multistep purge-and-trap gas chromatographic-mass spectrometric analysis of honey volatiles. Journal of Chromatography A 1157, 430-436.

Styger, G., Jacobson, D., Bauer, F. F., 2011. Identifying genes that impact on aroma profiles produced by *Saccharomyces cerevisiae* and the production of higher alcohols. Applied Microbiology and Biotechnology 91, 713-730.

Suárez-Lepe, J. A., Morata, A., 2012. New trends in yeast selection for winemaking. Trends in Food Science and Technology 23, 39-50.

Users.rcn.com, 2013. Budding Yeast: *Saccharomyces cerevisiae*. http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/Y/Yeast.html.

van der Maarel, M. J. E. C., van der Veen, B., Uitdehaag, J. C. M., Leemhuis, H., Dijkhuizen, L., 2002. Properties and applications of starch-converting enzymes of the alpha-amylase family. Journal of Biotechnology 94, 137-155.

Verstrepen, K. J., Van Laere, S. D. M., Vanderhaegen, B. M. P., Derdelinckx, G., Dufour, J. P., Pretorius, I. S., Winderickx, J., Thevelein, J. M., Delvaux, F. R., 2003. Expression levels of the yeast alcohol acetyltransferase genes ATF1, Lg-ATF1, and ATF2 control the formation of a broad range of volatile esters. Applied and Environmental Microbiology 69, 5228-5237.

Viana, F., Gil, J. V., Genovés, S., Vallés, S., Manzanares, P., 2008. Rational selection of non-Saccharomyces wine yeasts for mixed starters based on ester formation and enological traits. Food Microbiology 25, 778-785.

Wampler, T. P., 2002. Analysis of food volatiles using headspace gas chromatographic techniques. In: Flavor, Fragrance, and Odor Analysis. Marcel Dekker Inc., New York,

Weibiao, Z., Nantawan, T., 2012. Fermented bread. In: Handbook of plant-based fermented food and beverage technology. CRC Press, Boca Raton, pp. 477-492.

Wiggins, C., 1998. Proving, baking and cooling. In: Cauvain, S P, Young, L S (Eds.), Technology of breadmaking. Blackie Academic and Professional, London, pp. 120-148.

Williams, T., Pullen, G., 1998. Functional ingredients. In: Cauvain, S P, Young, L S (Eds.), Technology of Breadmaking. Blackie Academic and Professional, Gloucestershire, pp. 45-80.

Young, L. S., 2012. Applications of texture analysis to dough and bread. In: Cauvain, S P (Ed.), Breadmaking. Improving Quality. Woodhead Publishing Ltd., Cambridge, pp. 562-579.

Zehentbauer, G., Grosch, W., 1998. Crust aroma of baguettes II. Dependence of the concentrations of key odorants on yeast level and dough processing. Journal of Cereal Science 28, 93-96.

Zeng, E. Y., Noblet, J. A., 2002. Theoretical considerations on the use of solid-phase microextraction with complex environmental samples. Environmental Science and Technology 36, 3385-3392.

Zobel, H. F., Kulp, K., 1996. The staling mechanism. In: Hebeda, R E, Zobel, H F (Eds.), Baked Goods Freshness. Technology, Evaluation, and Inhibition of Staling. Marcel Dekker, Inc., New York, pp. 1-65.

PAPER I

Birch, A. N., van den Berg, F. W. J. and Hansen, Å. S.

Expansion profiles of wheat doughs fermented by seven commercial baker's yeasts

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Expansion profiles of wheat doughs fermented by seven commercial baker's yeasts

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Abbreviations: AACC, American Association of Cereal Chemists; ANOVA, Analysis of Variance; CFU, Colony Forming Units; HSD, Honest Significant Difference; ICC International Association for Cereal Science and Technology; SPO, sporulation; YPG, Yeast extract Peptone Glycerol.

Abstract

Commercial baker's yeast consist of *Saccharomyces cerevisae*, however the strain can vary in each baker's yeast, which might influence the dough fermentation time. The scope of this research was to investigate the dough expansion of wheat doughs fermented by seven commercial baker's yeasts at different yeast concentrations ($2.88 \cdot 10^{11}$, $5.76 \cdot 10^{11}$ and $8.64 \cdot 10^{11}$ colony forming units/kg flour) and fermentation temperatures (5°C, 15°C, 25°C and 35°C). Dough expansion was investigated by monitoring the dough height and it was found to be described well by a first order kinetic model. Doughs fermented with four of the seven yeasts generally had higher kinetic rate constants and hence shorter fermentation times compared to fermentation with the other three yeasts. The shortest fermentation times were found for doughs fermented at 25°C and the highest yeast concentration, a trend found for all the yeasts tested. The differences in the kinetic rate constants indicate a differentiation in yeast strain among the commercial baker's yeasts emphasising the great importance of the choice of baker's yeast for the dough fermentation time.

1. Introduction

Baker's yeast used in bread making is essential for dough fermentation. During dough fermentation baker's yeast uses simple carbohydrates to produce ethanol and carbon dioxide as the most important primary products. The carbon dioxide diffuses into the air bubbles, which are incorporated and dispersed in the dough during mixing. The elastic and extensible gluten network formed during mixing retains the gas and enables the dough to rise (Poitrenaud, 2004; Romano et al., 2007). A wide range of secondary products are furthermore produced during dough fermentation and particularly aroma active compounds such as aldehydes, acids, esters and ketones produced from the fermentative activity of yeast are very important for the overall bread aroma (Birch et al., 2013b; Frasse et al., 1992; Schieberle and Grosch, 1991).

Several analytical methods have been used to study the expansion of dough volume during dough fermentation, particularly rheofermentometer measurements (Gujral and Singh, 1999; Ktenioudaki et al., 2010; Rollini et al., 2007) and image analyses (Bellido et al., 2009; Shehzad et al., 2010). The dough surface in a cylindrical solid container has a domed shape, which is depended on dough rheology, however the volumetric expansion of dough has often been investigated by measuring dough heights (Gandikota and MacRitchie, 2005; Gujral and Singh, 1999; Ktenioudaki et al., 2010; Pérez-Nieto et al., 2010; Rollini et al., 2007; Therdthai et al., 2007) since the increase in dough height have been found to correlate well with the volume expansion of dough (Ktenioudaki et al., 2010). Dough expansion has been described previously as a first-order, non-Arrhenius kinetic model (Therdthai et al., 2007).

The dough expansion depends on several factors such as yeast concentration, type of wheat flour, additives (lactic acid, fat, sugar and sodium chloride) and process variables (relative humidity, fermentation temperature and mixing duration) (Birch et al., 2013b; Chiotellis and Campbell, 2003; Gujral and Singh, 1999; Ktenioudaki et al. 2010; Therdthai et al., 2007). Furthermore, the strain of baker's yeast might influence the dough expansion profile.

The production of commercial baker's yeast today is limited to relatively few companies and commercial baker's yeasts are typically highly selected strains of the species S*accharomyces cerevisiae*. It is likely that different strains of *S. cerevisiae* are produced in each company and they might possess different dough leavening capacities during fermentation. Knowledge about the dough expansion profiles of the baker's yeast and hence the dough fermentation times are of great industrial interest, since strains with a high fermentative activity will result in a short dough fermentation time, which is an economical benefit for the bakers when focus is solely on bread volume.

The purpose of this work was to investigate the expansion profiles of doughs fermented by seven commercial compressed baker's yeasts by development of a theoretical kinetic model. Simultaneously, the effects of temperature and yeast concentration on dough expansion were investigated.

2. Materials and methods

2.1 Flour

Wheat flour (Reform) was supplied by Lantmännen Mills A/S (Vejle, Denmark). Water content of the flour was measured the day of baking (HOH-express, Pfeuffer) and varied from 12.6 to 13.2%. The gluten content was 30.0% (wet gluten) and the gluten index was 91 (Glutomatic 2100, Perten) according to the American Association of Cereal Chemists (AACC) international approved method no. 38-12 (AACC, 1995). The falling number was 300 sec (Falling number 1500, Perten) according to the International Association for Cereal Science and Technology (ICC) standard method no. 107/1 (ICC, 1995).

2.2 Commercial baker's yeasts

The following types of compressed commercial baker's yeast were included in the study: MALTESERKORS from Lallemand, De Danske Gærfabrikker, Grenå, Denmark; SKÆRTOFTMØLLE, organically produced baker's yeast from Agrano, Riegel am Kaiserstuhl, Germany; RAPUNZEL, organically produced baker's yeast from Rapunzel Naturkost AG, Legau, Germany; SEMA from Lallemand, Panevezys, Lithuania; L'HIRONDELLE from Le Saffre, Marcq-en-Baroeul, France; BRUGGEMAN from Algist Bruggeman, Gent, Belgium; ZYMAROM from Algist Bruggeman, Gent, Belgium.
All baker's yeasts were used a few days after the purchase and therefore well before their expiration dates.

2.3 Count of yeast cells

1 g of baker's yeast was suspended in 9 ml sterile sporulation (SPO) medium (8.5 g NaCl, 1.0 g peptone, 0.3 g Na_2HPO_4, 1 L ion exchanged water, pH 5.5) to dilution 10^{-1}. The dilution was continued to 10^{-8}. 25 µL of the 10^{-6}, 10^{-7} and 10^{-8} dilutions were inoculated on sterile yeast extract peptone glycerol (YPG) agar plates (10 g glucose, 3 g yeast extract, 5 g peptone, 1 L ion exchanged water and 20 g agar, pH 5.5). The plates were incubated at 25˚C for 48 hours before counting the yeast colony forming units (CFU) (**Table 1**). The dilution series and the inoculation on YPG plates were both performed in duplicate.

2.4 Monitoring dough height

300 g of flour (adjusted to 14% water content), 185 mL water (30°C), 4 g saccharose, 4 g NaCl and an equal number of baker's yeast cells according to **Table 1** (dough containing $2.88 \cdot 10^{11}$, $5.76 \cdot 10^{11}$ and $8.64 \cdot 10^{11}$ CFU/kg flour, respectively, corresponding to 20-40, 40-80 and 60-120 g/kg flour) were mixed in a baking machine (FAB-100, Funai) for 19 minutes. A dough sample of 235 g was transferred to a 1 L beaker glass. The beaker glass was covered with aluminium foil and the dough was left for fermentation at 5°C, 15°C, 25°C and 35°C respectively in an incubator (Cooling incubator series 6000, Termaks). The dough height was monitored by a web camera (Live! Cam Voice, Creative Technology Ltd.) taking pictures of the doughs in intervals of 1-15 minutes (depending on the expected fermentation rate of each dough). The dough heights were monitored as duplicates from the same dough that was divided in two samples of 235 g dough. The dough height was measured as the highest point of the dough, as the dough height has previously been found to correlate well with the volume expansions of the dough (Ktenioudaki et al., 2010).The height for optimally developed dough was 90 mm for fermen-

tation at 5°C and 100 mm for fermentation at 15°C, 25°C and 35°C. The dough heights for optimally developed doughs were determined from pre-trials (results are not shown) where fermented doughs with different heights were baked and the crumb structures for each dough height were evaluated.

Table 1. CFU[a] of yeast cells per g compressed baker's yeast and the amount of baker's yeast in the dough corresponding to $2.88 \cdot 10^{11}$, $5.76 \cdot 10^{11}$ and $8.64 \cdot 10^{11}$ CFU/kg flour.

Yeast	CFU of yeast pr. g yeast	Amount of baker's yeast in the dough (g baker's yeast/kg flour)		
		$2.88 \cdot 10^{11}$ CFU/kg flour	$5.76 \cdot 10^{11}$ CFU/kg flour	$8.64 \cdot 10^{11}$ CFU/kg flour
Malteserkors	$(1.44 \pm 0.17) \cdot 10^{10}$	20	40	60
Sema	$(1.33 \pm 0.42) \cdot 10^{10}$	22	44	66
Skærtoftmølle	$(0.92 \pm 0.14) \cdot 10^{10}$	31	62	93
Zymarom	$(0.77 \pm 0.14) \cdot 10^{10}$	37	74	111
Bruggeman	$(0.73 \pm 0.10) \cdot 10^{10}$	39	78	117
Rapunzel	$(0.72 \pm 0.19) \cdot 10^{10}$	40	80	120
l'Hirondelle	$(0.72 \pm 0.11) \cdot 10^{10}$	40	80	120

[a]CFU = Colony Forming Units.

2.5 Data Analysis

The data obtained for the 84 different conditions (seven baker's yeasts, four temperatures and three yeast concentrations) were fitted to first-order non-Arrhenius kinetic models using non-linear regression with the Levenberg-Marquardt fitting function. For each condition the kinetic rate constant, b_0 and b_1 were estimated. Image editing, curve fitting and preparation of the box-and whisker plot were performed by the software Matlab (version 7.11/R2011b, Mathworks) using in-house routines. The Tukey Honest Significant Difference (HSD) test was used for comparison of means in the analysis of variance (ANOVA) computed by the software Jump (version 7.0, SAS Institute Inc.).

3. Results and discussion

3.1 First order kinetic model

The majority of the expanding dough profiles followed a first order kinetic model (**Figure 1A**), which could be used to estimate the change of dough height throughout the fermentation process, as shown in

$$h(t) = b_0 + b_1 \cdot (1 - e^{-k \cdot t}) \qquad \textbf{Equation 1}$$

where h(t) is the dough height (mm) at time t (min), b_0 (mm) is the estimate for the initial height, b_1 (mm) is the estimate for the increment in height and k is the kinetic rate constant (min^{-1}). A limited number of dough expansion profiles did not follow the first order kinetic model showing either a convex curve shape instead of a concave in the initial steep curve, or showed another unexpected profile, all resulting in unreliable parameter estimates in Equation 1; these samples will be identified distinctly in the results (**Section 3.2** and **3.3**). The model performance was evaluated by comparing the differences between the estimated dough fermentation times for reaching a predefined dough height for a fully developed dough (see section 2.4) by mathematically modelling and by direct determination (from pictures of the developing dough) (**Figure 1B and C**). A larger difference between the mathematically and direct determined fermentation times was generally found for the fermentation times for the samples fermented at 5°C (**Figure 1B**) compared to the samples fermented at 15°C, 25°C and 35°C. This is

mostly explained by the generally longer fermentation times of samples fermented at the low fermentation temperature (5°C) which will be further discussed in section 3.2. Generally, the mathematically modelled fermentation times were comparable with the direct determined fermentation times for samples fermented at 5°C (**Figure 1B**), as they were centred around zero in the box-and whisker plot. The mathematically modelled fermentation times for the samples fermented at 15°C, 25°C and 35°C had slightly longer fermentation times compared to the direct determined (**Figure 1C**). Overall the model performance was reasonable good as the differences between the mathematical modelled and the direct determined fermentation times were small for the majority of the samples (**Figure 1B and C**).

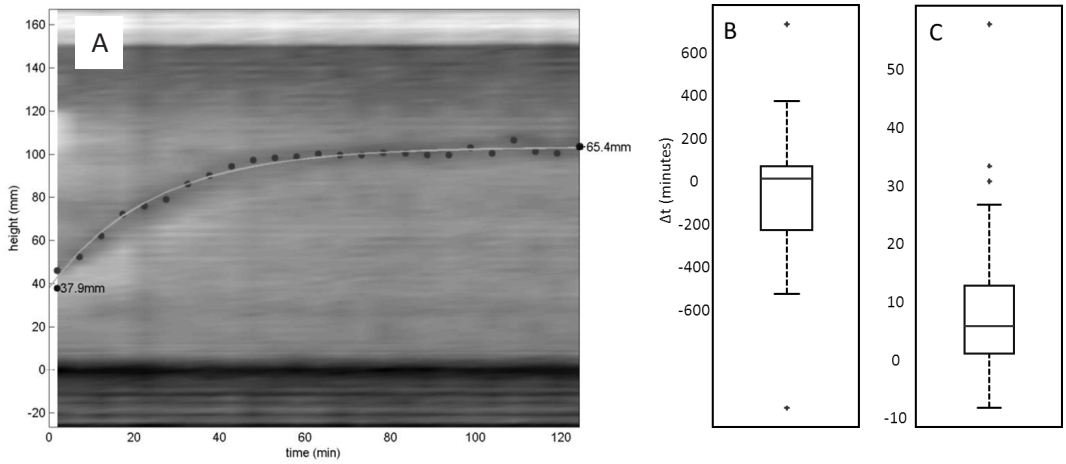

Figure 1. A: An example of dough expansion at discrete time points/images (●) following the first order kinetic model estimate from **Equation 1**. Each time-point is like a "bar" from a picture of dough expansion, these bars are placed next to each other, which result in the dough expansion profile. The white curve is the fitted line. This dough was fermented with the baker's yeast Bruggeman at a concentration of $2.88 \bullet 10^{11}$ CFU/kg flour at 15°C. B and C: Box-and-whisker plot of the differences (in minutes) between estimated dough fermentation times to reach a fully developed dough height by mathematically modelling and by direct determination - (B) 5^0C samples (N=18) and (C) 15, 25 and 35^0C samples (N=56). The black crosses mark those samples considered outliers for the median-estimate in the box-and-whisker determination.

The two dough samples fermented at 25°C with the lowest concentration ($2.88 \bullet 10^{11}$ CFU/kg flour) of the baker's yeasts l'Hirondelle and Sema were both outlying samples because of their curve shape and they were not included in the further data analysis. The mean coefficient of variation (CV%) of the kinetic rate constants for all samples was 17.1.

If the kinetic rate constants were estimated from doughs containing equal yeast amount (g/kg flour) instead of equal number of yeast cells (CFU/kg flour) it is assumed that the kinetic rate constants would be markedly different as the number of yeast cells per g between the baker's yeasts differed significantly (**Table 1**).

3.2 Effect of fermentation temperature and yeast concentration on the kinetic rate constant

The four fermentation temperatures (5°C, 15°C, 25°C and 35°C) were chosen to investigate the influence of a wide range of temperatures used in bakeries for dough fermentation. The typical industrial dough fermentation temperature is between 25 and 35°C (Wiggins, 1998), which is close to the optimal temperature for fermentation with *S. cerevisiae* which is approximately 25°C (Attfield, 1997). A fermentation temperature of 5°C is however also used in dough retarding (Cauvain, 1998) and this low temperature has also been shown to increase formation of esters (characterised as having a fruity and pleasant odour) during dough fermentation (Birch et al., 2013b).

An overview of the effects of fermentation temperature and yeast concentration on the kinetic rate constants for the expanding doughs fermented with each commercial baker's yeast is shown in **Table 2**, where a high kinetic rate constant corresponds to a short fermentation time. Doughs fermented with the baker's yeasts Malteserkors, Skærtoftmølle, Rapunzel and Sema at 25°C had significantly higher kinetic rate constants when the highest yeast concentration ($8.64 \cdot 10^{11}$ CFU/kg flour) was used compared to the other fermentation temperatures and yeast concentrations (**Table 2**). This tendency towards the highest kinetic rate constants for the doughs fermented at 25°C was also seen for the baker's yeasts l'Hirondelle, Bruggeman and Zymarom at the highest yeast concentration (**Table 2**).

Description of the dough expansion profiles as a first order kinetic model is in agreement with a previous study (Therdthai et al., 2007). These authors developed a model to investigate how relative humidity (70 to 90%) and temperature (30°C to 55°C) influence the expansion rate of rice-flour-based doughs. They found the significant highest kinetic rate constant at 46°C and 90% relative humidity. Gujral and Singh (1999) found that wheat dough fermentation at 27°C resulted in the largest bread volume compared to fermentation at 24°C, 30°C, 33°C and 36°C, which is in agreement with our results in **Table 2** with 25°C as the optimal fermentation temperature. Mousia et al. (2007) investigated dough expansion rates at 30°C, 35°C, 40°C, 45°C and 50°C and they found the highest dough expansion rate at 40°C. These results are in agreement with Chevallier et al. (2012) which found the highest dough expansion rate at 35°C compared to 25°C and 30°C. The different correlations of fermentation temperature and dough expansion rates found in the research studies are most likely explained by differences in yeast strains (type of yeast strain was not stated in the three papers) and process variables, however it is generally stated that the optimal fermentation temperature of *S. cerevisiae* is between 25°C and 28°C (Attfield, 1997; Redon et al., 2011).

The estimated fermentation times based on the kinetic rate constants are presented in **Figure 2**. Increasing the yeast concentration markedly decreased the fermentation times of all doughs (**Figure 2**). Doughs fermented with the two highest yeast concentrations ($5.76 \cdot 10^{11}$ and $8.64 \cdot 10^{11}$ CFU/kg flour) had statistically more similar kinetic rate constants compared to the doughs fermented with the lowest yeast concentration ($2.88 \cdot 10^{11}$ CFU/kg flour). The doughs fermented with the lowest yeast concentration had markedly lower kinetic rate constants (**Table 2**) and hence longer fermentation times (**Figure 2**) compared to doughs fermented with the two highest yeast concentrations. A short fermentation time

Table 2. Kinetic rate constants (k, min^{-1}) of expanding wheat doughs influenced by yeast concentration and fermentation temperature. The results of the two-way ANOVA are shown for the two factors - yeast concentration (yc) and fermentation temperature (ft) - and their possible interaction (yc•ft). The mean coefficient of variation (CV%) of the kinetic arte constant for all samples was 17.1.

	Kinetic rate constant (k, min^{-1})[a]												Two-way ANOVA[b]						
	2.88•10^{11} CFU/kg flour				5.76•10^{11} CFU/kg flour				8.64•10^{11} CFU/kg flour				yc (•10^{11}CFU/kg flour)			ft (°C)			
Yeast	5°C	15°C	25°C	35°C	5°C	15°C	25°C	35°C	5°C	15°C	25°C	35°C	2.88	5.76	8.64	5	15	25	35
Malteserkors	*0.0005g*	*0.005g*	0.01f	*0.01f*	0.005g	0.02d	0.03c	*0.03c*	0.02ef	0.02de	0.05a	*0.04b*							
Sema	0.002d	0.01cd	ne	0.01cd	0.01cd	0.03bc	0.03bc	0.03bc	0.03bc	0.03bc	0.09a	0.05b							
Skærtoftmølle	0.002g	*0.006fg*	*0.01ef*	*0.02de*	0.006fg	0.04c	0.04c	0.03d	0.004g	0.05b	0.06a	0.05b							
Bruggeman	0.02	0.04	0.03	0.05	0.03	0.04	0.07	0.06	0.03	0.05	0.1	0.07	b	ab	a	b	ab	a	ab
Zymarom	0.01	0.03	*0.02*	0.04	0.04	0.03	*0.06*	0.05	0.05	0.04	0.08	0.04	b	ab	a	a	a	a	a
Rapunzel	0.004g	0.006g	0.002g	0.03ef	0.02f	0.04de	0.04cde	0.05c	0.05cd	0.06bc	0.08a	0.07ab							
l'Hirondelle	0.03	0.04	ne	*0.03*	0.07	0.02	0.07	0.08	0.06	0.05	0.1	0.07	a	a	a	a	a	a	a

[a]Mean kinetic rate constants of duplicates; ne, not estimated. The kinetic rate constants marked with italic type are estimated from dough expansion curves which do not completely fit the first order kinetic model. [b]Different letters in the same row indicate significant differences (significance level 95%). When the interaction between yc and ft is significant the letters are shown for each kinetic rate constant. If a significant interaction effect was not found, the Tukey HSD test is reported for the two main effects (yc and fc).

Table 3. Kinetic rate constants (k, min^{-1}) of expanding wheat doughs fermented with seven commercial baker's yeasts at different yeast concentrations (yc) and fermentation temperatures (ft). The results of the one-way ANOVA[a] are shown for the seven baker's yeasts. The mean coefficient of variation (CV%) of the kinetic arte constant for all samples was 17.1.

	yc	Kinetic rate constant (k, min^{-1})[b]						
ft (°C)	(•10^{11} CFU/kg flour)	Malteserkors	Sema	Skærtoftmølle	Bruggeman	Zymarom	Rapunzel	l'Hirondelle
5	2.88	*0.0005b*	0.002b	0.002b	0.02ab	0.01ab	0.004b	0.03a
	5.76	0.005b	0.01b	0.006b	0.03ab	0.04ab	0.02ab	0.07a
	8.64	0.02cd	0.03abc	0.004d	0.03bc	0.05ab	0.05ab	0.06a
15	2.88	*0.005c*	0.01bc	*0.006c*	0.04a	0.03a	0.006c	0.03ab
	5.76	0.02c	0.03abc	0.04ab	0.04a	0.03bc	0.04ab	0.02c
	8.64	0.02	0.03	0.05	0.05	0.04	0.06	0.05
25	2.88	0.01	ne	*0.01*	0.03	*0.02*	0.02	ne
	5.76	0.03c	0.03c	0.04c	0.07a	*0.06abc*	0.04bc	0.07ab
	8.64	0.05	0.09	0.06	0.1	0.08	0.08	0.1
35	2.88	*0.01d*	0.01d	*0.02cd*	0.05a	0.04ab	0.03bc	*0.03bc*
	5.76	*0.03b*	0.03b	0.03b	0.06a	0.05ab	0.05ab	0.08a
	8.64	*0.04c*	0.05bc	0.05bc	0.07ab	0.04c	0.07a	0.07a

[a]Different letters in the same row indicate significant differences (significance level 95%). [b]Mean kinetic rate constants of duplicates; ne= not estimated. The kinetic rate constants marked with italic type are estimated from dough expansion curves which do not completely fit the first order kinetic model.

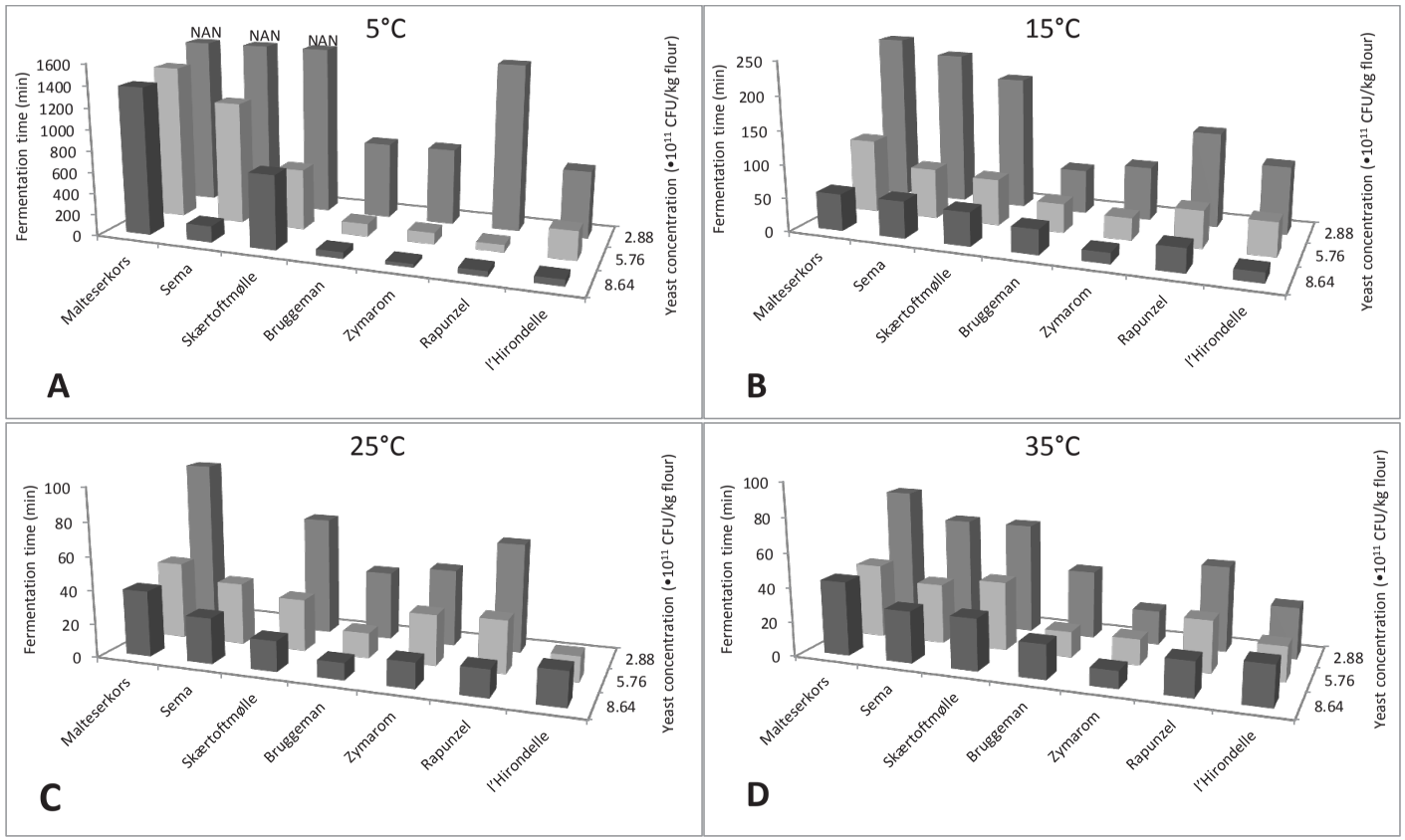

Figure 2. Estimated fermentation times for optimally fermented doughs (dough height of 90 mm for doughs fermented at 5°C and dough height of 100 mm for doughs fermented at 15, 25 and 35°C) fermented with seven commercial baker's yeasts at 5 (A), 15 (B), 25 (C) and 35°C (D). Note the different scale on the y-axis. If the column is marked not any number (NAN) the dough could not reach the optimal height for that temperature.

achieved by a high yeast concentration might be economically beneficial for the baker if focus is solely on bread volume. However, it is possible that compounds from the yeast are unwanted from a sensory point of view. The specific influence of yeast level on bread taste has to our knowledge not been investigated. Very few studies have been investigating the influence of non-volatile compounds on bread flavor, the focuses of these few studies were however not related to the level of yeast, but to the effect of storage on bread flavor and in the identification of bitterness in bread, respectively (Jensen et al., 2011; Bin et al., 2012). In future studies the effect of yeast level on non-volatile compounds in bread should be investigated by sensory analysis and by quantification of the non-volatile compounds (e.g. by HPLC). Birch et al. (2013b) found that a high yeast concentration (60 g/kg flour) resulted in bread with a high content of volatile fermentation compounds, with 2,3-butanedione (buttery and caramel odour) and phenylacetaldehyde (honey-like odour) being the most aroma active compounds, compared to breads fermented at lower yeast concentrations (20 and 40 g/kg flour).

The longest fermentation times for all doughs were generally found at the lowest temperature (5°C) and the lowest yeast concentration ($2.88 \cdot 10^{11}$ CFU/kg flour) (**Table 2**). Dough expansion at 5°C was problematic for most of the baker's yeasts, especially at the lowest yeast concentration where the standard deviations were also larger compared to the samples fermented at higher temperatures. Furthermore, doughs fermented with Malteserkors, Sema and Skærtoftmølle did not reach the optimal dough height when fermented at the lowest fermentation temperature (5°C) and yeast concentration ($2.88 \cdot 10^{11}$ CFU/kg flour) (**Figure 2A**). This phenomenon is likely associated with a lower fermentative activity of the yeast cells at this low fermentation temperature. The different abilities of the seven commercial baker's yeasts to ferment at 5°C might have something to do with the lipid content of their cell membrane. At lower temperatures the fluidity of the yeast cell membrane is decreased resulting in a lower activity of the transmembrane proteins responsible for uptake of nutrients. Redon et al. (2011) found that different *S. cerevisiae* strains compensates for this by increasing the formation of triacylglycerides and medium-chain fatty acids, and yeasts having higher contents of these compounds are also adapting better to lower fermentation temperatures.

3.3 Effect of baker's yeast on the kinetic rate constant

To investigate the effect of baker's yeast on the kinetic rate constant a one-way ANOVA was calculated (**Table 3**), it should be mentioned that the kinetic rate constants are the same as in the two-way ANOVA presented in **Table 2**. When comparing the kinetic rate constants of the doughs fermented with the seven commercial baker's yeasts, doughs fermented with Rapunzel, l'Hirondelle, Bruggeman and Zymarom generally had higher kinetic rate constants and hence shorter fermentation times compared to doughs fermented with the yeasts Malteserkors, Skærtoftmølle and Sema for nearly all fermentation temperatures and yeast concentrations (**Table 3** and **Figure 2**). No statistically significant differences were though found between the kinetic rate constants of doughs fermented with the seven baker's yeasts at 15°C with the highest yeast concentration or at 25°C with the lowest and highest yeast concentrations (**Table 3**). It is interesting to observe that although the kinetic rate constants differ significantly between doughs fermented with each baker's yeast at most temperatures and yeast concentrations, the kinetic rate constants were not significantly different between the yeasts when they were fer-

mented at 25°C at the highest yeast concentration, which corresponds to optimal and customary fermentation conditions for the majority of the baker's yeasts (**Table 3**).

The significant differences in the kinetic rate constants for doughs at most temperatures and yeast concentrations were found in spite of the fact that all doughs compared had the same initial yeast CFU (**Table 1**). The lower kinetic rate constants estimated from the expansion of doughs fermented with Malteserkors, Skærtoftmølle and Sema are assumed to be due to a lower fermentative activity of the yeast cells in these baker's yeasts compared to the activity of the yeast cells with a higher kinetic rate constant (Bruggeman, Zymarom, Rapunzel and l'Hirondelle) (**Table 3**).

A previous study also found significant differences in the fermentative activity between commercial baker's yeast by measuring the carbon dioxide production in a synthetic dough media (Bell et al., 2001). The fermentative activity of *S. cerevisiae* was found to be influenced by the cultivation conditions of baker's yeast (Angelov et al., 1997) and the highest fermentative activity was found when cultivation of *S. cerevisiae* was done at pH 6, 32°C and 20% dissolved oxygen. Furthermore, the authors found that strains having a high maltase activity also had a higher dough leaving ability. Hence, the different fermentative activities observed for the seven commercial baker's yeasts in **Table 3**, might be caused by differences within the cultivation method of the baker's yeasts or by the mode of regulation of maltose utilisation in the yeasts.

The differences in the kinetic rate constants between the seven commercial yeasts indicate that some of the yeasts differentiate in yeast strain. This result is supported by significant different aroma profiles found between breads fermented by the same seven commercial baker's yeasts (Birch et al. 2013a). As the differences in the aroma profiles may be due to differences in the gene-regulating mechanisms and biosynthetic pathways of aroma compound formation between these different yeast strains during dough fermentation.
The desirable properties of an ideal baking strain of *S. cerevisiae* are manifold and include for example high glycolytic activity, high chemical tolerance (resistance to bread additives and sodium chloride), greater ability to utilize maltose under glucose-repressing conditions and good storage ability (Walker, 1998). Furthermore, the baker's yeast producers constantly search for new strains with improved technological abilities to meet the industrial demands for strains that are better suited for sweet dough, frozen dough, etc. (Rollini et al., 2007).

Besides research within strains of baker's yeasts for specific dough formulas, additional investigations within selection of yeast strains with improved aroma formation could be of industrial interest. Particularly the selection of yeast strains with increased ester formation could be of relevance, since esters have been found to have a fruity and pleasant aroma (Lee and Noble, 2003).

4. Conclusion

Dough expansion could be described in a first order kinetic model that fitted most of the dough samples well. The optimal conditions resulting in the shortest fermentation time for all the seven baker's yeasts were fermentation of the doughs at 25°C with the highest yeast concentration ($8.64 \cdot 10^{11}$ CFU/kg flour). Dough fermentations at 5°C, 15°C and 35°C had generally shorter fermentation times when fermented by the baker's yeasts Rapunzel, l'Hirondelle, Bruggeman and Zymarom at all yeast concentrations compared to doughs fermented by Malteserkors, Sema and Skærtoftmølle. The doughs fermented with the two highest yeast concentrations had more similar fermentation times compared to the doughs fermented with the lowest yeast concentration, which had clearly longer fermentation times, a trend observed for all fermentation temperatures. The markedly lowest kinetic rate constants were found for fermentation at 5°C combined with the lowest yeast concentration ($2.88 \cdot 10^{11}$ CFU/kg flour) and doughs fermented with Malteserkors, Sema and Skærtoftmølle were not able to reach the optimal dough height at this temperature.

The results elucidate that the type of commercial baker's yeast, the fermentation temperature and the yeast concentration are three very important factors influencing the fermentation time of optimally developed doughs.

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References

AACC, 1995. Wet gluten, dry gluten, water-binding capacity, and gluten index. Approved methods of the American Association of Cereal Chemists 38-12.

Angelov, A., Hristozova, T., Roshkova, Z., 1997. Maltase activity and dough rising ability of baker's yeasts obtained at different growth conditions during the maturation stage. Zeitschrift fur Lebensmittel-Untersuchung Und-Forschung A-Food Research and Technology 205, 80-84.

Attfield, P. V., 1997. Stress tolerance: The key to effective strains of industrial baker's yeast. Nature Biotechnology 15, 1351-1357.

Bell, P. J. L., Higgins, V. J., Attfield, P. V., 2001. Comparison of fermentative capacities of industrial baking and wild-type yeasts of the species Saccharomyces cerevisiae in different sugar media. Letters in Applied Microbiology 32, 224-229.

Bellido, G. G., Scanlon, M. G., Page, J. H., 2009. Measurement of dough specific volume in chemically leavened dough systems. Journal of Cereal Science 49, 212-218.

Bin, Q., Jiang, D. S., Cho, I. H., Peterson, D. G., 2012. Chemical markers for bitterness in wheat bread. Flavour and Fragrance Journal 27, 454-458.

Birch, A. N., Petersen, M. A., Arneborg, N., Hansen, Å. S., 2013a. Influence of commercial baker's yeasts on bread aroma profiles. Food Research International 52, 160-166.

Birch, A. N., Petersen, M. A., Hansen, Å. S., 2013b. The aroma profile of wheat bread crumb influenced by yeast concentration and fermentation temperature. LWT - Food Science and Technology 50, 480-488.

Cauvain, S. P., 1998. Dough retarding and freezing. In: Cauvain, S P, Young, L S (Eds.), Technology of Breadmaking. Blackie Academic and Professional, London, pp. 149-179.

Chevallier, S., Zúñiga, R., Le-Bail, A., 2012. Assessment of bread dough expansion during fermentation. Food and Bioprocess Technology 5, 609-617.

Chiotellis, E., Campbell, G. M., 2003. Proving of bread dough II: Measurement of gas production and retention. Food and Bioproducts Processing 81, 207-216.

Frasse, P., Lambert, S., Levesque, C., Melcion, D., Richard-Molard, D., Chiron, H., 1992. The influence of fermentation on volatile compounds in french bread crumb. Food Science and Technology-Lebensmittel-Wissenschaft & Technologie 25, 66-70.

Gandikota, S., MacRitchie, F., 2005. Expansion capacity of doughs: methodology and applications. Journal of Cereal Science 42, 157-163.

Gujral, H. S., Singh, N., 1999. Effect of additives on dough development, gaseous release and bread making properties. Food Research International 32, 691-697.

ICC, 1995. Determination of the "Falling Number" according to Hagberg - as a measure of the degree of alpha-amylase activity in grain and flour. ICC-Standard No.107/1.

Jensen, S., Oestdal, H., Skibsted, L. H., Larsen, E., Thybo, A. K., 2011. Chemical changes in wheat pan bread during storage and how it affects the sensory perception of aroma, flavour, and taste. Journal of Cereal Science 53, 259-268.

Ktenioudaki, A., Butler, F., Gallagher, E., 2010. Rheological properties and baking quality of wheat varieties from various geographical regions. Journal of Cereal Science 51, 402-408.

Lee, S.-J., Noble, A. C., 2003. Characterization of odor-active compounds in californian Chardonnay wines using GC-olfactometry and GC-mass spectrometry. Journal of Agricultural and Food Chemistry 51, 8036-8044.Mousia, Z., Campbell, G. M., Pandiella, S. S., Webb, C., 2007. Effect of fat level, mixing pressure and temperature on dough expansion capacity during proving. Journal of Cereal Science 46, 139-147.

Pérez-Nieto, A., Chanona-Pérez, J. J., Farrera-Rebollo, R. R., Gutiérrez-López, G. F., Alamilla-Beltrán, L., Calderón-Domínguez, G., 2010. Image analysis of structural changes in dough during baking. LWT - Food Science and Technology 43, 535-543.

Poitrenaud, B., 2004. Baker's yeast. In: Yui, Y H, Meunier-Goddik, L, Hansen, Å S, Nip, W K, Stanfield, P S, Toldrá, F (Eds.), Handbook of Food and Beverage Fermentation Technology. Marcel Dekker Inc., New York, pp. 695-719.

Redon, M., Guillamon, J. M., Mas, A., Rozes, N., 2011. Effect of growth temperature on yeast lipid composition and alcoholic fermentation at low temperature. European Food Research and Technology 232, 517-527.

Rollini, M., Casiraghi, E., Pagani, M. A., Manzoni, M., 2007. Technological performances of commercial yeast strains (Saccharomyces cerevisiae) in different complex dough formulations. European Food Research and Technology 226, 19-24.

Romano, A., Toraldo, G., Cavella, S., Masi, P., 2007. Description of leavening of bread dough with mathematical modelling. Journal of Food Engineering 83, 142-148.

Schieberle, P., Grosch, W., 1991. Potent odorants of the wheat bread crumb. Zeitschrift für Lebensmittel-Untersuchung und - Forschung 192, 130-135.

Shehzad, A., Chiron, H., Della Valle, G., Kansou, K., Ndiaye, A., Réguerre, A. L., 2010. Porosity and stability of bread dough during proofing determined by video image analysis for different compositions and mixing conditions. Food Research International 43, 1999-2005.

Therdthai, N., Zhou, W., Jangchud, K., 2007. Modeling of the effect of relative humidity and temperature on proving rate of rice-flour-based dough. LWT - Food Science and Technology 40, 1036-1040.

Walker, G. M., 1998. Yeast technology. In: Walker, G M (Ed.), Yeast Physiology and Biotechnology. John Wiley and Sons Ltd., West Sussex, pp. 265-320.

Wiggins, C., 1998. Proving, baking and cooling. In: Cauvain, S P, Young, L S (Eds.), Technology of breadmaking. Blackie Academic and Professional, London, pp. 120-148.

PAPER II

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The aroma profile of wheat bread crumb influenced by yeast concentration and fermentation temperature

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The aroma profile of wheat bread crumb influenced by yeast concentration and fermentation temperature

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ABSTRACT

The consumers of today have an increasing interest in high quality bread with appealing aroma. The scope of this work is to investigate how aroma in wheat bread crumb is influenced by different fermentation conditions: amount of yeast (20, 40 and 60 g/kg flour) and fermentation temperature (5, 15 and 35 °C). Dough samples were fermented to equal height and baked, and the aroma compounds from the bread were extracted by dynamic headspace extraction and analyzed by gas chromatography–mass spectrometry. Quantification of the aroma compounds was performed by multiple headspace extraction. The most aroma active compounds identified were 3-methylbutanal, (E)-2-nonenal, 3-methyl-1-butanol, and 2,3-butanedione. Increasing the yeast concentration was found to increase formation of the majority of the compounds formed from the yeast metabolism, with 2,3-butanedione and phenylacetaldehyde as the most aroma active compounds. High fermentation temperature (15 and 35 °C) increased formation of many lipid oxidation compounds, with hexanal and heptanal having the highest odor activity values. Low fermentation temperature (5 °C) was found to increase formation of the three esters ethyl acetate, ethyl hexanoate, and ethyl octanoate, with ethyl hexanoate having the highest odor activity value. The odor activity values of the esters were generally low.

1. Introduction

Today, industrially produced wheat bread is often made from dough with a high concentration of yeast and the dough is fermented at high temperature in order to decrease the production time (Cauvain, 1998a). A short fermentation time results in an economical benefit and a continuous flow on the production line. However, it is generally believed that a longer fermentation time results in bread with a more pleasant aroma, although only few studies have been done in this area (Hironaka, 1986; Maeda et al., 2009). Aroma development in bread crumb has been found to be dependent on yeast concentration, mixing stage and fermentation time (Frasse et al., 1992; Gassenmeier & Schieberle, 1995; Maeda et al., 2009; Richard-Molard, Nago, & Drapron, 1979; Schieberle & Grosch, 1991). Fermentation temperature was found to have a significant effect on the crust aroma of baguettes (Zehentbauer & Grosch, 1998). The influence of fermentation temperature on the crumb aroma has only been investigated for the two fermentation products 3-methyl-1-butanol and 2-phenylethanol (Gassenmeier & Schieberle, 1995). Aroma compounds identified in fermented bread crumb are mainly derived from the metabolism of yeast and from the oxidation of flour lipids (Frasse et al., 1992; Schieberle & Grosch, 1991), whereas the aroma compounds in the crust originates from Maillard reactions occurring at high temperatures and low water activity between reducing sugars and amino acids (Purlis, 2010). Dynamic headspace extraction (DHE) followed by gas chromatography–mass spectrometry (GC–MS) has proven to be a sensitive and fast method to analyze the aroma compounds in bread or bread-like systems (Christensen, Leitao, Petersen, Jespersen, & Engelsen, 2009; Luning, Roozen, Moëst, & Posthumus, 1991). Quantification of aroma compounds by multiple headspace extraction (MHE) developed by Kolb and Pospisil (1977) is an effective quantification method, since it is independent of the food matrix. MHE has recently been successfully performed within food systems (Carrillo & Tena, 2006; Soria, Martinez-Castro, & Sanz, 2007). Numerous papers have elucidated the effects of yeast and fermentation temperature on aroma development during alcoholic fermentation in wine and beer production (Molina, Swiegers, Varela, Pretorius, & Agosin, 2007; Saerens, Verbelen, Vanbeneden, Thevelein, & Delvaux, 2008;

Abbreviations: AACC, American Association of Cereal Chemists; ANOVA, analysis of variance; DHE, dynamic headspace extraction; GC–MS, gas chromatography–mass spectrometry; ICC, International Association for Cereal Science and Technology; MHE, multiple headspace extraction; PCA, principal component analysis; PLS, partial least squares; OAV, odor activity value; OT, odor threshold.

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Tominac et al., 2008; Trelea, Titica, & Corrieu, 2004). However, knowledge within aroma development in bread fermented at different yeast concentrations and fermentation temperatures is lacking. Information on how these different fermentation conditions influence the aroma in bread crumb is of interest for the bread industry in order to improve the aroma of the bread products. The purpose of this research paper is therefore to investigate how different yeast concentrations (20, 40 and 60 g/kg flour) and dough fermentation temperatures (5, 15 and 35 °C) influence the aroma profile of wheat bread crumb. The aroma compounds were extracted by DHE followed by GC–MS analysis, and the quantification of the identified aroma compounds was performed by MHE.

2. Material and methods

2.1. Flour

Wheat flour (Reform) was supplied by Lantmännen Mills A/S (Vejle, Denmark). Moisture content of the flour was measured the day of baking (HOH-express, Pfeuffer) and varied from 11.7 to 11.9%. The gluten content was 28.9% (wet gluten) and the gluten index was 95 (Glutomatic 2100, Perten) according to the American Association of Cereal Chemists (AACC) international approved method no. 38-12 (AACC, 1995). The following Farinograph dough parameters were measured: the dough development time, 3 min; the dough stability, $1^3/_4$ min; the degree of softening, 20 BU; and the water uptake was 54.8% (Farinograph DCE 330, Brabender) according to AACC international approved method no. 54-21 (AACC, 1995). The falling number was 299 s (Falling number 1500, Perten) according to ICC standard method no. 107/1 (ICC, 1995).

2.2. Yeast

Commercial pressed baker's yeast (*Saccharomyces cerevisiae*, Malteserkors from Lallemand, De Danske Gærfabrikker, Grenå, Denmark) was used without washing the yeast cells. The dry matter was 28%. The cell count was $1.44^{*}10^{13}$ yeast cells/g yeast and $2.0^{*}10^{9}$ lactic acid bacteria/g yeast. Preliminary tests showed that the yeast cell density was the same in the dough immediately after mixing of the dough and in the end of the fermentation period. The yeast was taken from the same batch to decrease the risk of different cell count of yeast and different contaminant bacteria.

2.3. Bread making

300 g of Flour (adjusted to 14% moisture content), 190 mL water (30 °C), 4 g saccharose, 4 g NaCl and 6, 12 and 18 g yeast (corresponding to 20, 40 and 60 g yeast/kg flour), respectively were mixed in a baking machine (FAB-100 from Funai) for 19 min. Two pieces of 235 g dough was each transferred to 1 L beaker glass. The beaker glasses were sealed with aluminum foil and the doughs were left for fermentation at 5, 15 or 35 °C, respectively in an incubator. The fermentations were terminated when the doughs reached 8 cm in total height. Preliminary tests were made at each yeast level and fermentation temperature to find the right fermentation time as the dough height was monitored by a web-camera. The fermentation times are presented in Table 1. The dough was baked at 130 °C for 31 min in a convection oven with steam (Conmatic line, Hounö) to a center temperature of 98 °C. The relatively low baking temperature and steam during baking were chosen to decrease crust formation. The bread was cooled for 15 min and was then released from the beaker glass and further cooled at room temperature for 1 h on a grate. 1 cm crust was quickly removed and samples of 15 g of bread crumb were cut into 4–6 pieces and packed in tin foil surrounded by a plastic bag.

Table 1
Fermentation times for the dough to reach 8 cm in height according to fermentation temperature and yeast concentration.

Fermentation temperature (°C)	Yeast concentration (g/kg flour)		
	20	40	60
5	21 h	3 h 30 min	3 h
15	3 h	1 h	35 min
35	50 min	25 min	15 min

Approximately six samples could be taken from each bread. The samples were frozen at −18 °C until aroma analysis within few weeks. A preliminary test was made for comparison of the aroma profile of frozen and fresh bread samples. The test showed no significant differences between the treatments (data not shown), therefore freezing of the samples were done for practical reasons.

2.4. Standards

1-Penten-3-ol, 3-methyl-1-butanol, 3-methyl-3-buten-1-ol, 1-pentanol, 2-penten-1-ol, 1-hexanol, 3-hexenol, 2-ethyl-1-hexanol, 1-octanol, 3-nonen-1-ol, 2-phenylethanol, 1-dodecanol, 3-methylbutanal, heptanal, octanal, nonanal, decanal, benzaldehyde, (E)-2-nonenal, phenylacetaldehyde, 2-heptanone, 3-hydroxy-2-butanone, 2-octanone, 3-octen-2-one, ethyl acetate, ethyl octanoate, gamma-nonalactone, acetic acid, butanoic acid, hexanoic acid, 2-pentylfuran and trimethylpyrazine were purchased from Sigma–Aldrich (Gillingham, U.K.). 2-Methyl-1-propanol, 1-octen-3-ol, hexanal, 2,3-butanedione, 2-methylpropanoic acid and pentanoic acid were purchased from Fluka (Buchs, Switzerland). 1-Propanol, 1-heptanol, 2-furancarboxaldehyde, ethyl 3-methylbutanoate and ethyl hexanoate were purchased from Merck (Darmstadt, Germany). 1-Butanol was purchased from Ferak (Berlin, Germany) and phenylethyl acetate was purchased from ICN Pharmaceuticals, Inc (Costa Mesa, U.S.).

2.5. Dynamic headspace extraction (DHE)

15 g of Frozen bread crumb in pieces of 4–6 was placed in a 500 mL glass flask (7.5 cm in diameter). A trap containing Tenax-TA (200 mg) was attached to the sealed flask. The flask containing the sample was immersed in a laboratory water bath and held at 40 °C. The sample was tempered for 10 min before purging with nitrogen. One sample set (nine sample combinations in triplicates according to Table 1) was purged with nitrogen (50 mL/min) for 5 min to extract the very volatile aroma compounds. Another sample set (nine sample combinations in triplicates according to Table 1) was purged with nitrogen (150 mL/min) for 60 min to extract the less volatile compounds. Tenax-TA traps purged for 60 min were furthermore dry-purged directly with nitrogen (50 mL/min) for 10 min to remove excess water. Sealed Tenax-TA traps were kept from 1 to 3 days at 5 °C before analysis by gas chromatography–mass spectrometry.

2.6. Multiple headspace extraction (MHE)

To determine the total amount of the aroma compounds in the bread samples seven consecutive dynamic headspace extractions were performed from the same sample (the sample containing 40 g yeast/kg flour fermented at 15 °C). The same DHE procedures as described above were used, including two combinations of flow and time. All extractions were performed in triplicates. To determine the relationship between peak areas and absolute amounts of

compounds sampled on traps, standard series of pure aroma compound in heptane solution were analyzed for all above mentioned standards (Section 2.4) in concentrations of 10, 100, 500 and 1000 μL/L 2 μL of each solution was injected to Tenax-TA traps. Standard series were performed in triplicates.

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

The trapped volatiles were desorbed using an automatic thermal desorption unit (ATD 400, Perkin Elmer, Norwalk, USA). Primary desorption was carried out by heating the trap to 250 °C with a flow (60 mL/min) of carrier gas (helium) for 15.0 min. The stripped volatiles were re-trapped in a Tenax-TA cold trap (30 mg held at 5 °C), which was subsequently heated at 300 °C for 4 min (secondary desorption, outlet split 1:10). This allowed for rapid transfer of volatiles to the GC–MS (G1800A GCD System, Hewlett–Packard, Palo Alto, CA, USA) through a heated (225 °C) transfer line. Separation of volatiles was carried out on a polar DB-Wax capillary column (J&W Scientific, 30 m long × 0.25 mm internal diameter, 0.25 μm film thickness). The column flow rate was 1.0 mL/min using helium as a carrier gas. The column temperature programme was: 10 min at 45 °C, hereafter heating by 6 °C/min up to 240 °C, and holding this temperature for 10 min. The GC was equipped with an MS detector, operating in the electron ionization mode at 70 eV. Mass-to-charge ratios between 15 and 300 were scanned. Volatile compounds were identified by matching their mass spectra with those of a commercial database (Wiley275.L, HP product no. G1035A) and by comparing their retention times and mass spectra with those of authentic standards. The software program, GCD Plus ChemStation G1074B (Version A.01.00.237, Hewlett–Packard, Palo Alto, California), was used for data analysis.

2.8. Data analysis

The peak area corresponding to a complete dynamic headspace extraction was calculated assuming that the decline in peak area during MHE follows first order kinetics, i.e.:

$$\sum_{i=1}^{i=\infty} A_i = \frac{A_1}{1 - e^{-q}},$$

where A_i is the sum of all extractions from A_1 to A_∞, A_1 represents the area of an aroma compound in a new sample and q represents the slope of the regression curve of the natural logarithm to the peak area vs. number of extractions (Kolb & Pospisil, 1977). q was determined for each aroma compound, where after A_1 for each aroma compound could be used to calculate the area representing the total amount of aroma compound in each sample. Finally, areas were transformed into absolute amounts, using the standard curves obtained from analyzing heptane solutions of pure standards injected directly into the Tenax-TA traps.

The chemometric method principal component analysis (PCA) is a useful technique to describe major trends in the data by data reduction and compression. PCA decomposes the data into scores (T) and loadings (P) according to the equation $X = TP' + E$, where X is raw data, T is the score matrix, P' is the transposed loadings matrix and E is the error matrix (Wold, Esbensen, & Geladi, 1987). In PCA the scores are related to the samples and the loadings are related to the variables. Applied to a data matrix of samples and variables PCA constructs new variables known as principal components (PCs). The first PC captures as much as possible of the variability in all the original variables and each successive new variable (applied orthogonally to the previous PC and therefore being independent) accounts for as much of the remaining variability as possible. By use of PCA it is therefore often possible to describe a very large proportion of the variability in highly multivariate data with a modest number of these new variables (PCs) (Næs, Isaksson, Fearn, & Davies, 2004). Partial least squares (PLS) regression is another chemometric method that determines the relationship between variables X and properties of interest y. The PLS regression model is defined as $y = Xb + E$, where b is the vector of regression coefficients (Næs et al., 2004). Calculation of PLS models were primarily done in order to investigate the regression coefficients, since aroma compounds having regression coefficients close to zero do not contribute much to the explanation of the separation between the samples and can hence be removed in order to improve the model. PCA and PLS regression were computed by the software Latentix (version 2.00, Latent5) and all GC–MS data was autoscaled prior to the analysis. Two-way analysis of variance (two-way ANOVA) was computed by the software JMP (version 7.0, SAS Institute Inc.) with fermentation temperature and yeast concentration as model effects. Student's t-test was computed for the aroma compounds (95% significant level) to describe the effect of fermentation temperature and yeast concentration.

3. Results and discussion

3.1. Identification and quantification of aroma compounds

A total of 46 aroma compounds were identified in the bread crumb from the nine different fermented bread, of which 45 compounds were confirmed by comparing their retention times and mass spectra with authentic standards. The MHE method for quantification of the identified aroma compounds was found to perform well with generally low standard deviations. The coefficient of variation (CV%) of the slope of the regression curve of the natural logarithm to the peak area vs. number of extractions (q) was from 2.2 to 13.3% and the CV% of the slope of the standard curves of the authentic pure standards was from 1.2 to 7.3%. Quantification of 3-hydroxy-2-butanone (acetoin) by MHE was not possible, since the slope of the regression curve (q) was close to 0 (data not shown). Furthermore, quantification of five acids (acetic acid, 2-methylpropanoic acid, butanoic acid, pentanoic acid, hexanoic acid) was not possible because of poor repeatability of their peak areas. 39 of the identified aroma compounds were quantified by MHE (Table 2). The identified aroma compounds were formed mainly from the metabolism of yeast during fermentation (iso-alcohols, 3-methylbutanal, phenylacetaldehyde, 2,3-butanedione (diacetyl), 3-hydroxy-2-butanone, esters and acids) and from oxidation of flour lipids (aldehydes, ketones and 2-pentylfuran) (Table 2).

3.2. Outlying samples

One of the triplicate samples fermented at 5 °C with a yeast concentration of 20 g/kg flour (5C20g/kgc) and one of the triplicate samples fermented at 35 °C with a yeast concentration at 60 g/kg flour (35C60g/kgb) were both characterized as outlying samples based on residual variance and Hotellings T^2 (data not shown) and were hence not included in the further data analysis.

3.3. Odor activity value (OAV)

OAVs were calculated as the ratio of each aroma compound concentration found in bread crumb to the odor threshold (OT) in water. Bread is, however, a complex food matrix and therefore it should be underlined that an aqueous OT value of an aroma compound will only give an approximation of the OAV in bread crumb. It would have been more appropriate to calculate the OAVs

Table 2
Concentration (μg/kg) of aroma compounds in wheat bread crumb influenced by yeast concentration and fermentation temperature. 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[b]	Ion used for quantification[c]	Aroma compound concentration (μg/kg) in bread samples[d]										Two-way ANOVA[e]							
		20 g Yeast/kg flour			40 g Yeast/kg flour			60 g Yeast/kg flour			yc*ft	yc (g/kg flour)				ft (°C)			
		5 °C	15 °C	35 °C	5 °C	15 °C	35 °C	5 °C	15 °C	35 °C	*p*-Value	*p*-Value	20	40	60	*p*-Value	5	15	35
1-Propanol[f]	31	217	198	144	194	182	157	212	183	193	ns	ns				ns			
2-Methyl-1-propanol[f]	43	368	420	347	675	651	579	770	889	927	ns	<0.001	c	b	a	ns			
1-Butanol[f]	56	27.0	28.3	27.3	22.9	27.9	29.2	27.5	26.8	26.0	ns	ns				ns			
1-Penten-3-ol[g]	57	8.99	9.00	8.55	7.46	8.36	8.16	7.54	6.99	5.83	ns	0.004	a	a	b	ns			
3-Methyl-1-butanol[g]	55	5660	4940	4250	4840	5660	5400	6140	5720	4710	ns	ns				ns			
3-Methyl-3-buten-1-ol[g]	41	1.75	2.61	1.85	2.27	2.30	2.26	2.72	2.76	2.44	ns	0.003	b	b	a	0.03	b	a	b
1-Pentanol[g]	42	23.0	22.8	21.7	19.6	22.7	22.8	23.3	24.0	21.1	ns	ns				ns			
2-Penten-1-ol[g]	57	3.77	3.59	3.85	3.23	3.54	3.60	3.15	3.15	2.74	ns	0.01	a	a	b	ns			
1-Hexanol[g]	56	53.4	52.9	43.9	43.5	47.4	47.3	43.4	47.1	42.5	ns	ns				ns			
3-Hexenol[g]	41	3.08	2.81	2.61	2.82	2.57	2.60	2.02	2.04	2.04	ns	<0.001	a	a	b	ns			
1-Octen-3-ol[g]	57	12.5	10.8	10.6	10.6	11.8	12.1	10.0	11.1	9.16	ns	ns				ns			
1-Heptanol[g]	70	9.32	8.32	8.56	7.47	8.27	8.82	7.99	8.13	7.20	ns	ns				ns			
2-Ethyl-1-hexanol[g]	57	5.13	5.25	5.03	4.52	5.00	8.89	5.84	4.86	6.93	ns	ns				ns			
1-Octanol[g]	56	1.99	1.86	2.05	1.83	2.03	2.17	1.77	1.87	1.81	ns	ns				ns			
3-Nonen-1-ol[g]	55	10.4	9.15	8.78	10.5	10.8	12.2	10.2	10.2	9.37	ns	ns				ns			
2-Phenylethanol[g]	91	362ab	190d	336ab	246cd	342ab	291bc	367a	395a	371a	<0.001	–				–			
1-Dodecanol[g]	55	7.43	5.48	6.44	4.43	5.85	5.55	7.71	5.67	5.87	ns	ns				ns			
3-Methylbutanal[f]	44	9.19	10.75	5.00	8.11	8.80	5.03	7.48	10.8	7.45	ns	ns				<0.001	b	a	c
Hexanal[g]	44	63.7b	87.7a	67.1b	53.2b	89.2a	90.3a	60.7b	86.3a	58.0b	0.034	–				–			
Heptanal[g]	44	18.4c	30.4b	34.1ab	15.3c	34.7ab	39.0ab	33.4ab	40.3a	33.8ab	0.013	–				–			
Octanal[g]	41	3.05	3.52	3.41	3.36	4.29	4.60	3.68	4.55	3.94	ns	0.026	b	a	a	0.036	b	a	a
Nonanal[g]	57	14.59	16.85	14.65	17.85	20.38	19.36	16.07	18.79	17.66	ns	ns				ns			
2-Furancarboxaldehyde[g]	95	3.30b	3.90ab	4.28a	2.50c	4.04ab	4.51a	3.48b	3.87ab	3.47b	0.013	–				–			
Decanal[g]	57	4.29	3.76	5.10	7.16	5.69	4.99	4.03	4.56	4.05	ns	ns				ns			
Benzaldehyde[g]	106	15.7e	22.6e	42.4d	20.8e	56.6bc	57.5bc	46.4cd	79.8a	62.6b	0.007	–				–			
(E)-2-nonenal[g]	55	5.16a	3.67bcd	3.89bcd	3.21d	4.18abc	4.36ab	3.25cd	4.26ab	2.97d	0.003	–				–			
Phenylacetaldehyde[g]	91	33.86	31.87	34.16	27.25	46.74	42.27	48.23	50.54	48.25	ns	<0.001	b	b	a	ns			
2,3-Butanedione[h]	43	75.5de	99.5de	60.1e	125cd	151bc	116cd	123cd	193ab	214a	0.033	–				–			
2-Heptanone[g]	43	2.45	3.68	3.31	2.52	3.62	3.67	2.82	3.93	3.13	ns	ns				<0.001	b	a	a
3-Hydroxy-2-butanone	45	nq	nq	nq	nq	nq	nq	nq	nq	nq	–	–				–			
2-Octanone[g]	43	5.79	7.14	4.77	5.41	6.12	6.42	6.80	8.41	6.45	ns	0.015	b	b	a	0.014	b	a	b
3-Octen-2-one[g]	43	1.49bc	1.90abc	2.25ab	1.39c	1.80bc	2.65a	1.91abc	2.30ab	1.48bc	0.043	–				–			
Ethyl acetate[f]	43	205	195	56.8	240	184	88.5	229	235	147	ns	ns				<0.001	a	a	b
Ethyl 3-methylbutanoate[g]	88	0.30d	0.00e	0.49d	0.34d	0.79c	1.05b	1.23b	2.20a	0.58cd	<0.001	–				–			
3-Methylbutyl acetate[h]	43	nq	nq	nq	nq	nq	nq	nq	nq	nq	–	–				–			
Ethyl hexanoate[g]	88	0.55	0.28	0.18	0.39	0.29	0.25	0.48	0.30	0.22	ns	ns				<0.001	a	b	c
Ethyl octanoate[g]	88	0.88b	0.64be	0.33c	0.84b	0.57bc	0.40c	1.83a	0.74b	0.62bc	0.005	–				–			
Phenylethyl acetate[g]	104	0.97	0.83	1.66	1.89	3.17	2.61	4.55	4.35	4.37	ns	<0.001	b	b	a	ns			
Gamma-nonalactone[g]	85	3.16	2.82	3.13	2.57	2.74	4.64	2.83	2.64	2.95	ns	ns				ns			
2-Pentylfuran[g]	81	10.6cd	16.4ab	13.9bc	6.93d	15.9ab	18.5a	8.86cd	16.1ab	10.0cd	0.016	–				–			
Trimethylpyrazine[g]	57	2.12	1.35	2.20	2.07	1.98	2.51	1.90	2.18	1.73	ns	ns				ns			

[a] If a significant interaction effect was found, the two-way ANOVA is not performed on the main effects; yeast concentration and fermentation temperature separately, hence the interaction effect is then the most important effect.
[b] Identification of all aroma compounds was obtained by comparing authentic standards with their mass spectra, except for 3-methylbutyl acetate, which is identified only by mass spectra.
[c] Mass fragment used for quantification.
[d] Mean concentrations of triplicates; nq, not quantitated.
[e] Different letters in the same row indicate significant differences (significant level 95%). When the interaction between yc and ft is significant the letters are shown for each concentration.
[f] Quantified from the samples purged with nitrogen (50 mL/min) for 5 min.
[g] Quantified from the samples purged with nitrogen (150 mL/min) for 60 min.
[h] No standard available.

from OT in starch or cellulose. They were however, not available from the literature for the majority of the compounds. To take the uncertainty of the calculated OAVs into account it was assumed that aroma compounds having an OAV of 0.1 or higher might be important in bread crumb aroma. This was the case for 19 compounds (Table 3) which are emphasized in the further data analysis and discussion.

In the discussion of which aroma compounds are sensorily most important in bread crumb it is relevant to emphasize that chewing, and several saliva constituents have been found to influence the release of aroma compounds from food matrices in the human mouth (Buettner, 2002; Van Ruth & Roozen, 2000). How these in mouth conditions influence the aroma release from bread crumb were not investigated in the present study.

3.4. Effect of yeast concentration on aroma formation in bread crumb

Of the 19 aroma compounds having OAVs of 0.1 or higher, the formation of four compounds (2-methyl-1-propanol, octanal, phenylacetaldehyde, and 2-octanone) was significantly positively influenced by yeast concentration (Table 2). For an overview of the effect of yeast concentration in the complex dataset a PCA was calculated (Fig. 1). A clear positive effect of yeast concentration on the production of the majority of aroma compounds formed from the fermentative activity of yeast (2-methyl-1-propanol, 2-phenylethanol, phenylacetaldehyde, 2,3-butanedione and ethyl 3-methylbutanoate) was found (Fig. 1). 3-Methyl-1-butanol and 3-methylbutanal were found to have high OAVs in the bread samples

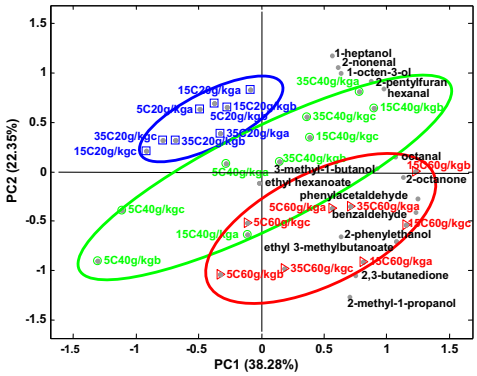

Fig. 1. PCA biplot showing the effect of yeast concentration on aroma formation. Blue squares, green circles and red triangles show samples fermented with 20, 40 and 60 g yeast/kg flour, respectively. The number before C in the sample name shows the fermentation temperature, the number before g/kg shows the yeast concentration and the letter a, b or c corresponds to the triplicate number. Only aroma compounds having an OAV of 0.1 or higher and furthermore with regression coefficients higher than ±0.1 were included in the PCA model for the PCA biplot. Regression coefficients were calculated from a PLS model with yeast concentration as the *y*-variable (data not shown). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Odor activity values[a] (OAVs) of the aroma compounds in bread samples, their odor thresholds (OT) and odors. Only aroma compounds having an OAV of 0.1 or higher[b] are presented. Odor threshold (OT) values in water and odor descriptions of the identified aroma compounds are collected from scientific papers.

Aroma compound	OAVs of aroma compounds in bread samples[c]									OT in water (μg/kg)	Odor
	20 g Yeast/kg flour			40 g Yeast/kg flour			60 g Yeast/kg flour				
	5 °C	15 °C	35 °C	5 °C	15 °C	35 °C	5 °C	15 °C	35 °C		
2-Methyl-1-propanol	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.3	0.3	3200[d]	Glue, alcohol[e]
3-Methyl-1-butanol	22.7	19.8	17.0	19.4	22.6	21.6	24.6	22.9	18.8	250[f]	Balsamic, alcohol[e]
1-Octen-3-ol	12.5	10.8	10.6	10.6	11.8	12.1	10.0	11.1	9.2	1[g]	Mushroom[h]
1-Heptanol	3.1	2.8	2.9	2.5	2.8	2.9	2.7	2.7	2.4	3[i]	Green[h]
2-Phenylethanol	0.3	0.2	0.3	0.2	0.3	0.3	0.3	0.4	0.3	1100[f]	Flowery[j]
3-Methylbutanal	46.0	53.8	25.0	40.6	44.0	25.2	37.4	54.2	37.2	0.2[f]	Malty[j]
Hexanal	14.1	19.5	14.9	11.8	19.8	20.1	13.5	19.2	12.9	4.5[f]	Green[k]
Heptanal	6.1	10.1	11.4	5.1	11.6	13.0	11.1	13.4	11.3	3[g]	Fatty, rancid[h]
Octanal	4.4	5.0	4.9	4.8	6.1	6.6	5.3	6.5	5.6	0.7[g]	Citrus[h]
Nonanal	14.6	16.9	14.7	17.9	20.4	19.4	16.1	18.8	17.7	1[g]	Citrus[h]
Decanal	2.1	1.9	2.6	3.6	2.8	2.5	2.0	2.3	2.0	2[g]	Citrus[h]
Benzaldehyde	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.2	0.1	350[g]	Almond[h]
(E)-2-nonenal	64.6	45.9	48.6	40.1	52.2	54.5	40.7	53.3	37.1	0.08[g]	Beans, cucumber[h]
Phenylacetaldehyde	8.5	8.0	8.5	6.8	11.7	10.6	12.1	12.6	12.1	4[g]	Honey-like[j]
2,3-Butanedione	11.6	15.3	9.2	19.3	23.3	17.8	19.0	29.7	32.9	6.5[d]	Buttery, caramel[k]
2-Octanone	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	50[g]	–
Ethyl 3-methylbutanoate	1.5	0.0	2.5	1.7	3.9	5.2	6.1	11.0	2.9	0.2[l]	Fruity, apple[e]
Ethyl hexanoate	0.5	0.3	0.2	0.4	0.3	0.2	0.5	0.3	0.2	1[m]	Fruity-juicy[e]
2-Pentylfuran	1.8	2.7	2.3	1.2	2.7	3.1	1.6	2.7	1.7	6[f]	Floral, fruit[h]

[a] The OAV is the ratio of the concentration of aroma compound and the OT of the compound.
[b] An OAV of 0.1 or higher and not an OAV above 1 is chosen to limit the risk of disregarding aroma compounds in bread crumb that can be sensorily detected, since the OAVs are based on OT in water and not in bread crumb.
[c] Mean OAVs of triplicates.
[d] From Mulders (1973).
[e] From Lee and Noble (2003).
[f] From Buttery, Teranishi, Ling, and Turnbaugh (1990).
[g] From Buttery et al. (1988).
[h] From Yang et al. (2008).
[i] From Fazzalari (1978).
[j] From Frauendorfer and Schieberle (2006).
[k] From Le Guen et al. (2001).
[l] From Schieberle and Hofmann (1997).
[m] From Takeoka et al. (1989).

(Table 3). 3-Methyl-1-butanol has been characterized as having a balsamic or alcoholic odor (Lee & Noble, 2003) and 3-methylbutanal as having a malty odor (Frauendorfer & Schieberle, 2006) and both compounds can undoubtedly be sensed in the samples. Neither 3-methyl-1-butanol nor 3-methylbutanal were, however, significantly influenced by yeast concentration. 3-Methyl-1-butanol and 2-phenylethanol have previously been described as the two most sensorily important aroma compounds formed in yeast fermented bread crumb (Frasse et al., 1992; Gassenmeier & Schieberle, 1995; Schieberle & Grosch, 1991). 2-Phenylethanol is mentioned as one of the aroma compounds formed in highest concentration in bread crumb (Frasse et al., 1992) and this was confirmed from our results with concentrations around 300 μg/kg (Table 2). However, 2-phenylethanol might not be one of the sensorily most important aroma compounds having OAVs between 0.2 and 0.4. There is a non-significant tendency to increasing formation of 2-phenylethanol with increasing yeast concentration (Table 2 and Fig. 1). 2,3-Butanedione was found to be an important aroma compound with OAVs from 9 to 33 (Table 3) and the concentration of the compound increased with higher yeast level (Table 2 and Fig. 1). The high level of 2,3-butanedione is expected to be of sensory importance and the odor is described as buttery and caramel (Le Guen, Prost, & Demaimay, 2001). Formation of both 2,3-butanedione and 3-hydroxy-2-butanone has previously been reported as being directly linked to the fermentative yeast activity in dough (Frasse et al., 1992; Schieberle & Grosch, 1991). Quantification of 3-hydroxy-2-butanone was as mentioned in Section 3.1 not possible, but when computing an analysis of variance on the raw peak areas the amount of 3-hydroxy-2-butanone was significantly increased with increasing yeast concentration (data not shown) as was also the case for 2,3-butanedione. The similar effect of yeast level on formation of both 2,3-butanedione and 3-hydroxy-2-butanone might be explained from the two compounds being formed from the same pathways of respectively oxidative decarboxylation and decarboxylation of 2-acetolactate. 2,3-Butanedione can furthermore be enzymatically reduced to 3-hydroxy-2-butanone or 2,3-butanediol by the yeast (Rothe & Stockel, 1978; Wainwright, 1973). Phenylacetaldehyde was produced in a significantly higher concentration in bread fermented with a yeast concentration of 60 g/kg flour compared to the lower yeast concentrations (20 and 40 g/kg flour) (Table 2). Phenylacetaldehyde, characterized as having a honey-like odor (Buttery, Turnbaugh, & Ling, 1988), might be a sensorily important compound due to the relatively high OAVs (Table 3). 2-Methyl-1-propanol was produced in a significantly higher concentration with increasing yeast concentration (Table 2), however, the OAVs of 2-methyl-1-propanol were calculated as being close to 1 (Table 3), so the sensorily importance of 2-methyl-1-propanol is therefore expected to be small.

Gassenmeier and Schieberle also investigated how two different yeast levels influence formation of aroma compounds in wheat bread crumb (Gassenmeier & Schieberle, 1995). However, the fermentation conditions (particularly addition of pre-ferments and several fermenting periods) in their study were different from our study. This may explain why they found that the concentration of both 3-methyl-1-butanol and 2-phenylethanol decreased in bread with high yeast concentration, while phenylacetaldehyde increased with a high yeast concentration (Gassenmeier & Schieberle, 1995) which is not in agreement with the results from this work (Table 2).

The second most important path in aroma formation in bread crumb is the lipid oxidation. Formation of the lipid oxidation products 2-octanone and octanal was significantly increased with increasing yeast concentration (Fig. 1 and Table 2). The level of lipid oxidation products was found to be independent of yeast concentration for the majority of the lipid oxidation products; 1-octen-3-ol, 1-heptanol, hexanal, (E)-2-nonenal and decanal (Table 2). 1-Octen-3-ol, hexanal, heptanal, nonanal and (E)-2-nonenal were the five lipid oxidation products having the highest OAVs in bread crumb (Table 3). The OAVs for 2-octanone and benzaldehyde were very close to 0.1 (Table 3), the sensory importance of 2-octanone and benzaldehyde is therefore probably small.

3.5. Effect of fermentation temperature on aroma formation in bread crumb

The three fermentation temperatures (5, 15 and 35 °C) were chosen to investigate the extremes within the temperatures of dough fermentation. 35 °C is the typical industrial dough fermentation temperature, since this is close to the optimal temperature for fermentation with *Saccharomyces cerevisiae*, which is between 25 and 30 °C. However, also a low fermentation temperature of 5 °C is widely used in dough retarding (Cauvain, 1998b) and as low fermentation temperature has been shown to increase formation of esters during beer and wine fermentation (Beltran, Novo, Guillamon, Mas, & Rozes, 2008; Molina et al., 2007), this relatively low fermentation temperature was also chosen.

Of the 19 aroma compounds having OAVs of 0.1 or higher the formation of four compounds (3-methylbutanal, octanal, 2-octanone, and ethyl hexanoate) was significantly influenced by fermentation temperature (Table 2). The fermentation compound 3-methylbutanal was formed in a significantly higher amount at 15 °C compared to 5 and 35 °C (Table 2). The significant effect of the fermentation temperature on formation of the ester ethyl hexanoate will be discussed in the following section. The remaining fermentation compounds were not significantly influenced by fermentation temperature. This is not in agreement with Gassenmeier and Schieberle, they investigated how formation of the two fermentation compounds 3-methyl-1-butanol and 2-phenylethanol was influenced by different fermentation temperatures (25, 30, 35 and 40 °C) in a liquid pre-ferment after incubation for 8 h, and they found the highest concentration of both fermentation products at 35 °C (Gassenmeier & Schieberle, 1995).

The concentration of the lipid oxidation products 1-heptanol, hexanal, heptanal, octanal, decanal and 2-pentylfuran increased when increasing the fermentation temperature from 5 °C to 15 or 35 °C (Fig. 2), though the trend was not significant for 1-heptanol and decanal (Table 2). Hexanal and heptanal, characterized as having a respectively green (Le Guen et al., 2001) and fatty or rancid (Yang, Lee, Jeong, Kim, & Kays, 2008) odor, have high OAVs (Table 3), and might therefore be sensorily important compounds in bread crumb. 2-Octanone was formed in a significantly higher concentration when the fermentation was done at 15 °C compared to 5 and 35 °C (Table 2), however this compound might be of minor sensorily importance due to OAVs close to 0.1 (Table 3). It is well known that the rate of lipid oxidation increases with increasing temperature, this explains the trend of higher concentrations of the majority of the lipid oxidation products when fermenting at 15 and 35 °C compared to 5 °C. Zehentbauer and Grosch also found a higher formation of crust lipid oxidation products ((E)-2-nonenal and 1-octen-3-one) when the fermentation temperature was increased from 4 to 26 °C (Zehentbauer & Grosch, 1998). The authors suggested that by lowering the fermentation temperature and hereby decreasing the level of lipid oxidation products might result in a sensorily improved bread crust because the compounds are often characterized as off-flavors.

For eight of the compounds (2-phenylethanol, hexanal, heptanal, (E)-2-nonenal, benzaldehyde, 2,3-butanedione, ethyl 3-methylbutanoate, and 2-pentylfuran) a significant interaction effect was found between yeast concentration and fermentation

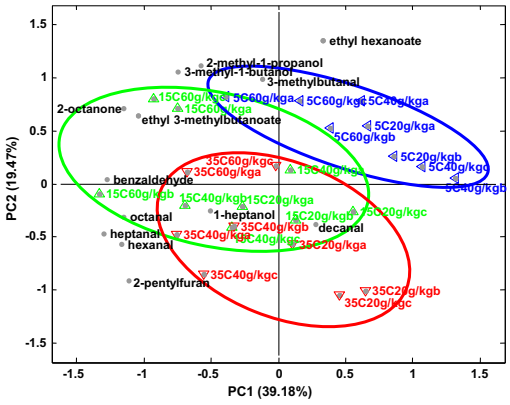

Fig. 2. PCA biplot showing the effect of fermentation temperature on aroma formation. The sample names are described in Fig. 1. Blue, green and red triangles show samples fermented at 5, 15 and 35 °C, respectively. Only aroma compounds having an OAV of 0.1 or higher and furthermore with regression coefficients higher than ±0.4 were included in the PCA model for the PCA biplot. Regression coefficients were calculated from a PLS model with fermentation temperature as the y-variable (data not shown). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

temperature (Table 2). Formation of the fermentation products (2-phenylethanol, 2,3-butanedione and ethyl 3-methylbutanoate) does not share the same trends within the combined effects of yeast concentration and fermentation temperature (Table 2). The formation of the three lipid oxidation products hexanal, (E)-2-nonenal and 2-pentylfuran seems to be increased for the samples fermented with 40 and 60 g yeast per kg flour and at a fermentation temperatures of 15 and 35 °C (Table 2, Figs. 1 and 2).

3.6. *Effect of yeast concentration and fermentation temperature on ester formation in bread crumb*

The esters identified in the bread crumb have OAVs below 0.1 except for ethyl 3-methylbutanoate and ethyl hexanoate (Table 3). In spite of the low OAVs, it is of interest to explore how the ester formation was influenced by the fermentation conditions, since esters are often characterized as having pleasant, fruity and sweet odors. Furthermore, limited work has been done on the effect of fermentation temperature on ester formation during dough fermentation, compared to the detailed research within ester formation in alcoholic fermentation in general. Formation of the identified esters; ethyl acetate, ethyl 3-methylbutanoate, ethyl hexanoate, ethyl octanoate and phenylethyl acetate increased with increasing yeast concentration (Fig. 3A), however, this tendency was though not significant for ethyl acetate and ethyl hexanoate (Table 2). Ethyl acetate was the most abundant ester identified in bread crumb (Table 2). Ethyl 3-methylbutanoate has not previously been detected in bread crumb, however, this compound has been identified in alcoholic yeast fermentation (King et al., 2008). Formation of ethyl acetate, ethyl hexanoate and ethyl octanoate were significantly higher at the low fermentation temperature (5 °C) compared to fermentation at 15 and 35 °C (Fig. 3B). No clear effect of the fermentation temperature on the formations of the two esters ethyl 3-methylbutanoate and phenylethyl acetate was found (Fig. 3B). Ethyl acetate, ethyl hexanoate and ethyl octanoate are well known esters formed during fermentation of bread (Frasse, Lambert, Richardmolard, & Chiron, 1993; Maeda et al., 2009) and increasing formation of those esters with lower fermentation temperature has also been found during wine fermentation (Beltran et al., 2008; Molina et al., 2007). Gamma-nonalactone was not significantly influenced by either yeast concentration nor fermentation temperature (Table 2), which might be explained by the fact that gamma-nonalactone is not produced from the yeast fermentation but rather from the oxidation of oleic and linoleic acid (Tressl, Haffner, Lange, & Nordsiek, 1996).

It is assumed that the concentration of acids (particularly acetic acid, hexanoic acid and octanoic acid) in the bread crumb would increase with decreased fermentation temperature, which has also been found during wine fermentation (Beltran et al., 2008; Molina et al., 2007). This would also explain the higher concentration of ethyl acetate, ethyl hexanoate and ethyl octanoate in the crumb with decreasing temperature fermentation temperature (Fig. 3B). It is of interest that Richard-Molard et al. suggested that acetic acid might act as an aroma enhancer in wheat bread crumb (Richard-Molard et al., 1979). Sensory tests or GC–Olfactometry analyses

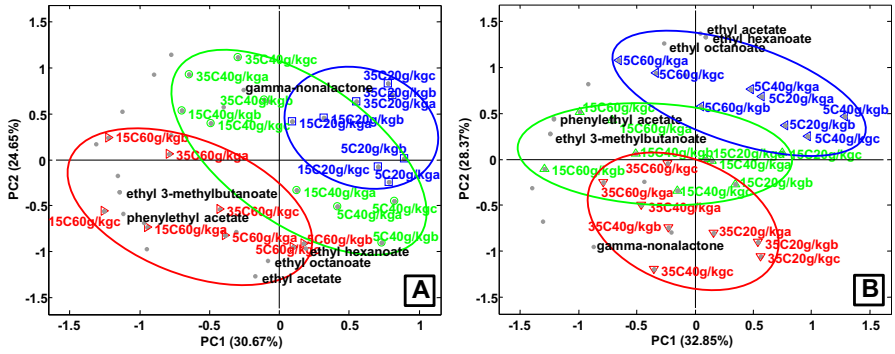

Fig. 3. PCA biplot showing the effects of yeast concentration (A) and fermentation temperature (B) on ester formation in bread crumb. The sample names are described in Fig. 1. Blue squares, green circles and red triangles in Fig. 3A show samples fermented with 20, 40 and 60 g yeast/kg flour, respectively. Blue, green and red triangles in Fig. 3B show samples fermented at 5, 15 and 35 °C, respectively. The two PCA models were calculated as described in Figs. 1 and 2 with additionally four esters; ethyl acetate, ethyl octanoate, phenylethyl acetate and gamma-nonalactone included as variables. The esters are shown with labels and the remaining aroma compounds are shown as gray dots without labels. All identified esters are included in the plot regardless of OAVs or regression coefficients. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

could be relevant in order to clarify the sensorily importance of the esters and acids, and also if a possible synergy effect could be found between the identified aroma compounds.

4. Conclusion

The result of this study showed that increasing the yeast concentration caused an increased formation of the majority of aroma compounds (2-methyl-1-propanol, 2-phenylethanol, phenylacetaldehyde, 2,3-butanedione, ethyl acetate, ethyl 3-methylbutanoate, ethyl hexanoate, ethyl octanoate and phenylethyl acetate) formed from the fermentative activity of yeast. Phenylacetaldehyde and 2,3-butanedione as being the sensory most important of these compounds. Formation of the majority of the lipid oxidation compounds was independent of yeast concentration. Increasing the fermentation temperature from 5 °C to 15 or 35 °C was found to increase the concentration of the lipid oxidation products 1-heptanol, hexanal, heptanal, octanal, decanal and 2-pentylfuran. Hexanal and heptanal were the most aroma active of these compounds, and they are often characterized as off-flavors. Decreasing the fermentation temperature to 5 °C was found to increase formation of the three esters ethyl acetate, ethyl hexanoate and ethyl octanoate in bread, which are often characterized as having a fruity and pleasant aroma. From the results it is therefore suggested to ferment the dough at low temperature (5 °C) with high concentration of yeast (60 g/kg flour) in order to develop bread with a relatively short production time combined with a high concentration of esters and a low concentration of hexanal and heptanal. It could be of interest to further investigate how the recognized differences in the aroma profile of bread crumb can be evaluated by a sensory panel, particularly since the OAVs of esters were found to be low.

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References

AACC. (1995). *Approved methods of the American Association of Cereal Chemists* (9th ed.). Minnesota.

Beltran, G., Novo, M., Guillamon, J. M., Mas, A., & Rozes, N. (2008). Effect of fermentation temperature and culture media on the yeast lipid composition and wine volatile compounds. *International Journal of Food Microbiology, 121*(2), 169–177.

Buettner, A. (2002). Influence of human saliva on odorant concentrations. 2. Aldehydes, alcohols, 3-alkyl-2-methoxypyrazines, methoxyphenols, and 3-hydroxy-4,5-dimethyl-2(5H)-furanone. *Journal of Agricultural and Food Chemistry, 50*, 7105–7110.

Buttery, R. G., Teranishi, R., Ling, L. C., & Turnbaugh, J. G. (1990). Quantitative and sensory studies on tomato paste volatiles. *Journal of Agricultural and Food Chemistry, 38*(1), 336–340.

Buttery, R. G., Turnbaugh, J. G., & Ling, L. C. (1988). Contribution of volatiles to rice aroma. *Journal of Agricultural and Food Chemistry, 36*(5), 1006–1009.

Carrillo, J. D., & Tena, M. T. (2006). Determination of volatile compounds in antioxidant rosemary extracts by multiple headspace solid-phase microextraction and gas chromatography. *Flavour and Fragrance Journal, 21*(4), 626–633.

Cauvain, S. P. (1998a). Breadmaking processes. In S. P. Cauvain, & L. S. Young (Eds.), *Technology of bread making* (1st ed.) (pp. 18–44). London: Blackie Academic and Professional.

Cauvain, S. P. (1998b). Dough retarding and freezing. In S. P. Cauvain, & L. S. Young (Eds.), *Technology of bread making* (1st ed.) (pp. 149–179). London: Blackie Acedemic and Professional.

Christensen, N. J., Leitao, S. M. D., Petersen, M. A., Jespersen, B. M., & Engelsen, S. B. (2009). A quantitative structure–property relationship study of the release of some esters and alcohols from barley and oat beta-glucan matrices. *Journal of Agricultural and Food Chemistry, 57*(11), 4924–4930.

Fazzalari, F. A. (1978). *Compilation of odor and taste threshold data*. Philadelphia: The Society.

Frasse, P., Lambert, S., Levesque, C., Melcion, D., Richard-Molard, D., & Chiron, H. (1992). The influence of fermentation on volatile compounds in French bread crumb. *Food Science and Technology – Lebensmittel-Wissenschaft & Technologie, 25*(1), 66–70.

Frasse, P., Lambert, S., Richardmolard, D., & Chiron, H. (1993). The influence of fermentation on volatile compounds in French bread dough. *Food Science and Technology – Lebensmittel-Wissenschaft & Technologie, 26*(2), 126–132.

Frauendorfer, F., & Schieberle, P. (2006). Identification of the aroma compounds in cocoa powder based on molecular sensory correlations. *Journal of Agricultural and Food Chemistry, 54*(15), 5521–5529.

Gassenmeier, K., & Schieberle, P. (1995). Potent aromatic compounds in the crumb of wheat bread (French-type) – influence of pre-ferments and studies on the formation of key odorants during dough processing. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 201*(3), 241–248.

Hironaka, Y. (1986). Relationship between sensory flavor evaluation and gas-chromatographic profiles of French bread. *Cereal Chemistry, 63*(4), 369–372.

ICC. (1995). *Determination of the "falling number" according to Hagberg – As a measure of the degree of alpha-amylase activity in grain and flour*. ICC-standard no. 107/1. Wien, Austria: International Association for Cereal Science and Technology.

King, E. S., Swiegers, J. H., Travis, B., Francis, I. L., Bastian, S. E. P., & Pretorius, I. S. (2008). Coinoculated fermentations using *Saccharomyces* yeasts affect the volatile composition and sensory properties of *Vitis vinifera* L. cv. sauvignon blanc wines. *Journal of Agricultural and Food Chemistry, 56*(22), 10829–10837.

Kolb, B., & Pospisil, P. (1977). A gas chromatographic assay for quantitative analysis of volatiles in solid materials by discontinuous gas extraction. *Chromatographia, 10*(12), 705–711.

Le Guen, S., Prost, C., & Demaimay, M. (2001). Evaluation of the representativeness of the odor of cooked mussel extracts and the relationship between sensory descriptors and potent odorants. *Journal of Agricultural and Food Chemistry, 49*(3), 1321–1327.

Lee, S.-J., & Noble, A. C. (2003). Characterization of odor-active compounds in californian chardonnay wines using GC–olfactometry and GC–mass spectrometry. *Journal of Agricultural and Food Chemistry, 51*(27), 8036–8044.

Luning, P. A., Roozen, J. P., Moëst, R. A. F. J., & Posthumus, M. A. (1991). Volatile composition of white bread using enzyme active soya flour as improver. *Food Chemistry, 41*(1), 81–91.

Maeda, T., Kikuma, S., Araki, T., Ikeda, G., Takeya, K., & Sagara, Y. (2009). The effects of mixing stage and fermentation time on the quantity of flavor compounds and sensory intensity of flavor in white bread. *Food Science and Technology Research, 15*(2), 117–126.

Molina, A. M., Swiegers, J. H., Varela, C., Pretorius, I. S., & Agosin, E. (2007). Influence of wine fermentation temperature on the synthesis of yeast-derived volatile aroma compounds. *Applied Microbiology and Biotechnology, 77*(3), 675–687.

Mulders, E. J. (1973). The odor of white bread. IV. Quantitative determination of constituents in the vapour and their odour values. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 151*, 310–317.

Næs, T., Isaksson, T., Fearn, T., & Davies, T. (2004). *A userfriendly guide to multivariate calibration and classification*. Chichester: NIR Publications.

Purlis, E. (2010). Browning development in bakery products – a review. *Journal of Food Engineering, 99*(3), 239–249.

Richard-Molard, D., Nago, M. C., & Drapron, R. (1979). Influence of the bread making method on French bread flavor. *Baker's Digest, 53*, 34–38.

Rothe, M., & Stockel, J. (1978). Diacetyl within flavor of foods. 1. Formation and degradation by enzymes from bakers-yeast. *Nahrung – Food, 22*(8), 711–719.

Saerens, S. M. G., Verbelen, P. J., Vanbeneden, N., Thevelein, J. M., & Delvaux, F. R. (2008). Monitoring the influence of high-gravity brewing and fermentation temperature on flavour formation by analysis of gene expression levels in brewing yeast. *Applied Microbiology and Biotechnology, 80*(6), 1039–1051.

Schieberle, P., & Grosch, W. (1991). Potent odorants of the wheat bread crumb. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 192*, 130–135.

Schieberle, P., & Hofmann, T. (1997). Evaluation of the character impact odorants in fresh strawberry juice by quantitative measurements and sensory studies on model mixtures. *Journal of Agricultural and Food Chemistry, 45*(1), 227–232.

Soria, A. C., Martinez-Castro, I., & Sanz, J. (2007). Estimation of recovery by multi-step purge-and-trap gas chromatographic–mass spectrometric analysis of honey volatiles. *Journal of Chromatography A, 1157*(1–2), 430–436.

Takeoka, G. R., Buttery, R. G., Flath, R. A., Teranishi, R., Wheeler, E. L., Wieczorek, R. L., et al. (1989). Volatile constituents of pineapple. In R. Teranishi, R. G. Buttery, & F. Shahidi (Eds.), *Flavor chemistry trends and developments* (388th ed.) (pp. 223–237). Washington, DC: American Chemical Society.

Tominac, V. P., Gamnic, K. K., Komes, D., Gracin, L., Banovic, M., & Maric, V. (2008). Influence of media composition and temperature on volatile aroma production by various wine yeast strains. *Czech Journal of Food Sciences, 26*(5), 376–382.

Trelea, I. C., Titica, M., & Corrieu, G. (2004). Dynamic optimisation of the aroma production in brewing fermentation. *Journal of Process Control, 14*(1), 1–16.

Tressl, R., Haffner, T., Lange, H., & Nordsiek, A. (1996). Formation of gamma- and delta-lactones by different biochemical pathways. In A. J. Taylor, & D. S. Mottram (Eds.), *Flavour science – Recent developments* (pp. 141). Cambridge: The Royal Society of Chemistry.

Van Ruth, S. M., & Roozen, J. P. (2000). Influence of mastication and saliva on aroma release in a model mouth system. *Food Chemistry, 71*, 339–345.

Wainwright, T. (1973). Diacetyl a review. Part I – analytical and biochemical considerations: part II – brewing experience. *Journal of the Institute of Brewing, 79*, 451–470.

Wold, S., Esbensen, K., & Geladi, P. (1987). Principal component analysis. *Chemometrics and Intelligent Laboratory Systems, 2*(1–3), 37–52.

Yang, D. S., Lee, K.-S., Jeong, O.-Y., Kim, K.-J., & Kays, S.-J. (2008). Characterization of volatile aroma compounds in cooked black rice. *Journal of Agricultural and Food Chemistry, 56*(1), 235–240.

Zehentbauer, G., & Grosch, W. (1998). Crust aroma of baguettes II. Dependence of the concentrations of key odorants on yeast level and dough processing. *Journal of Cereal Science, 28*(1), 93–96.

PAPER III

Birch, A. N., Petersen, M. A. Arneborg, N. and Hansen, Å. S.

Influence of commercial baker's yeasts on bread aroma profiles

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Influence of commercial baker's yeasts on bread aroma profiles

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ABSTRACT

Baker's yeast is used for bread fermentation throughout the world and it is very important for the bread quality. The scope of this work was to investigate how the aroma of wheat bread crumb is influenced by yeast fermentation by comparing the aroma formation of bread fermented by seven commercial baker's yeasts. Dough samples were fermented with equal number of yeast cells to equal height and then baked. The volatile components were extracted by dynamic headspace sampling and analyzed by gas chromatography mass spectrometry. The dough fermentation time varied significantly from 40 to 100 min. The fermentation compounds 2,3-butanedione and 1-propanol were found in significantly higher concentration in bread fermented with the four baker's yeasts having the shortest fermentation times. Furthermore, 3-methylbutanal, 2-methyl-1-propanol and ethyl acetate were found in significantly higher concentration in two of the yeasts. On the other hand phenylacetaldehyde and 2-phenylethanol were found in significantly higher concentration in bread fermented with two other yeasts. It can be concluded that use of the seven commercial baker's yeasts for bread fermentation resulted in significantly different bread aroma profiles.

1. Introduction

Baker's yeast, used for bread fermentation throughout the world, is very important for the bread quality and different commercial baker's yeasts are each highly selected strains of the species *Saccharomyces cerevisiae*. The fermentative activity of baker's yeast is essential not only for the rising action of the dough by production of CO_2, but also in production of the wide range of aroma compounds identified in bread (Birch, Petersen, & Hansen, 2013; Frasse et al., 1992; Schieberle & Grosch, 1991). They found that most of the aroma compounds in the crumb of fermented bread are derived from the metabolism of yeast and the dominating compounds are alcohols, aldehydes as well as 2,3-butanedione (diacetyl), 3-hydroxy-2-butanone (acetoin) and esters. These aldehydes or their corresponding alcohols are formed inside the yeast cell from degradation of the flour amino acids via the Ehrlich pathway (Hazelwood, Daran, van Maris, Pronk, & Dickinson, 2008). The esters are produced in the yeast cell by an enzymatic reaction between acetyltransferases, acetyl coenzyme A and various alcohols (Lilly, Lambrechts, & Pretorius, 2000). The di-ketones 2,3-butanedione and 3-hydroxy-2-butanone are formed from acetohydroxy acids leaked from the yeast cell through non-enzymatic chemical reactions outside the yeast cell (Wainwright, 1973). Furthermore, products from oxidation of flour lipids, such as alcohols, aldehydes and ketones, contribute highly to the aroma profile of bread crumb (Birch et al., 2013; Frasse et al., 1992; Schieberle & Grosch, 1991). Recently, aroma of bread has attained more focus as a quality criterion for bread (Birch et al., 2013; Jensen, Oestdal, Skibsted, Larsen, & Thybo, 2011; Poinot et al., 2008).

Formation of aroma compounds in bread crumb is highly influenced by the fermentation temperature, fermentation time and yeast level (Birch et al., 2013; Frasse et al., 1992; Gassenmeier & Schieberle, 1995; Maeda et al., 2009; Richard-Molard, Nago, & Drapron, 1979; Schieberle & Grosch, 1991). Furthermore, it is known from studies of alcoholic beverage fermentations that the choice of yeast strain is very important for the aroma formation and by this the quality of the final product (Procopio, Qian, & Becker, 2011; Suárez-Lepe & Morata, 2012). It has been suggested that differences in the genes of *S. cerevisiae* strains play a central role in explaining the diverse aroma profile produced by the different strains during alcoholic fermentation (Styger, Jacobson, & Bauer, 2011).

The European production of commercial baker's yeast is today limited to relatively few companies. Potentially different strains of *S. cerevisiae* are produced in each company, which might result in diverse aroma formation during dough fermentation. Knowledge within this field is of great commercial interest, since the choice of yeast strain is assumed to be very important for the aroma formation in bread. Furthermore, it is of interest to investigate if lactic acid bacteria can be found in commercial baker's yeast, since they might also contribute to the aroma of the bread (Hansen & Hansen, 1994).

The purpose of this research is therefore to investigate the influence of baker's yeasts on the formation of bread aroma by comparing the aroma profile of bread fermented with seven commercial compressed

Abbreviations: ANOVA, Analysis of Variance; CFU, Colony Forming Units; DHE, Dynamic Headspace Extraction; GC–MS, Gas Chromatography Mass Spectrometry; MHE, Multiple Headspace Extraction; PCA, Principal Component Analysis; LAB, Lactic Acid Bacteria; OAV, Odor Activity Value; OT, Odor Threshold.

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Table 1
Colony forming units (CFU) of yeast and lactic acid bacteria (LAB) per g baker's yeast and the amount of baker's yeast in the dough corresponding to $2.88 * 10^{14}$ CFU/kg flour.

Baker's yeast	CFU of yeast per g baker's yeast	CFU of LAB per g baker's yeast	Amount of baker's yeast in the dough (g baker's yeast/kg flour)	Amount of LAB in the dough (CFU of LAB/kg flour)
Malteserkors	$1.44 * 10^{13} \pm 0.17 * 10^{13}$	$0.2 * 10^{10} \pm 0.02 * 10^{10}$	20	$0.4 * 10^{11} \pm 0.04 * 10^{11}$
Sema	$1.33 * 10^{13} \pm 0.42 * 10^{13}$	$4.1 * 10^{10} \pm 0.62 * 10^{10}$	22	$9.0 * 10^{11} \pm 1.4 * 10^{11}$
Skærtoftmølle	$0.92 * 10^{13} \pm 0.14 * 10^{13}$	$2.7 * 10^{10} \pm 0.31 * 10^{10}$	31	$8.4 * 10^{11} \pm 0.9 * 10^{11}$
Zymarom	$0.77 * 10^{13} \pm 0.14 * 10^{13}$	–	37	–
Bruggeman	$0.73 * 10^{13} \pm 0.10 * 10^{13}$	$0.5 * 10^{10} \pm 0.01 * 10^{10}$	39	$2.0 * 10^{11} \pm 0.04 * 10^{11}$
Rapunzel	$0.72 * 10^{13} \pm 0.19 * 10^{13}$	$2.9 * 10^{10} \pm 0.45 * 10^{10}$	40	$11.6 * 10^{11} \pm 1.8 * 10^{11}$
l'Hirondelle	$0.72 * 10^{13} \pm 0.11 * 10^{13}$	$0.1 * 10^{10} \pm 0.03 * 10^{10}$	40	$0.4 * 10^{11} \pm 0.1 * 10^{11}$

baker's yeasts. This is done by using dynamic headspace extraction (DHE) followed by gas chromatography mass spectrometry (GC–MS) analysis. Quantification of the volatiles in the bread samples was performed by multiple headspace extraction (MHE).

2. Materials and methods

2.1. Flour

Wheat flour (Reform) was supplied by Lantmännen Mills A/S (Vejle, Denmark). Moisture content of the flour was measured the day of baking (HOH-express, Pfeuffer) and varied from 12.6 to 13.2%. The gluten content was 30.0% (wet gluten) and the gluten index was 91 analyzed according to Birch et al. (2013). The falling number was 300 s analyzed according to Birch et al. (2013).

2.2. Commercial baker's yeasts

The following types of baker's yeast were included in the study: MALTESERKORS from Lallemand, De Danske Gærfabrikker, Grenå, Denmark; SKÆRTOFTMØLLE, organically produced baker's yeast from Agrano, Riegel am Kaiserstuhl, Germany; RAPUNZEL, organically produced baker's yeast from Rapunzel Naturkost AG, Legau, Germany; SEMA from Lallemand, Panevezys, Lithuania; L'HIRONDELLE from Le Saffre, Marcq-en-Baroeul, France; BRUGGEMAN+ from Algist Bruggeman, Gent, Belgium; and ZYMAROM from Algist Bruggeman, Gent, Belgium.

All baker's yeasts were used a few days after the purchase and therefore well before their expiration dates.

2.3. Count of yeast

1 g of baker's yeast was suspended in 9 mL sterile SPO medium (8.5 g NaCl, 1.0 g peptone, 0.3 g Na_2HPO_4, 1 L ion exchanged water, pH 5.5) to dilution 10^{-1}. The dilution was continued to 10^{-8}. 25 μL of the 10^{-6}, 10^{-7} and 10^{-8} dilutions were inoculated on sterile YPG agar plates (10 g glucose, 3 g yeast extract, 5 g peptone, 1 L ion exchanged water and 20 g agar, pH 5.5). The plates were incubated at 25 °C for 48 h before counting the yeast colony forming units (CFU) (Table 1). The dilution series and the inoculation on YPG plates were both performed in duplicate.

2.4. Count of lactic acid bacteria (LAB)

Dilution series were performed as described for count of yeast. 25 μL of the 10^{-2}, 10^{-3} and 10^{-4} dilutions were inoculated on MRS media (10.00 g tryptone, 5.00 g meat extract, 3.00 g yeast extract, 15 mL fresh yeast extract (50 g fresh yeast dissolved in 200 mL ion exchanged water, autoclaved and cooled for 12 h), 7.00 g glucose, 7.00 g fructose, 7.00 g maltose, 2.00 g Na-gluconate, 5.00 g Na-acetate·H_2O, 2.60 g $K_2HPO_4 \cdot 3H_2O$, 0.10 g $MgSO_4 \cdot 7H_2O$, 0.05 g $MnSO_4 \cdot 4H_2O$, 0.50 g cystein-HCL·H_2O, 1.00 mL Tween 80, 18.25 g agar, 1000 mL ion exchanged water. pH 5.5, sterilized for 15 min at 118 °C). The plates were incubated anaerobically at 30 °C for 4–5 days before counting the CFU of LAB (Table 1). The dilution series and the inoculation on MRS plates were both performed in duplicate.

2.5. Bread making

300 g of flour (adjusted to 14% moisture content), 185 mL water (30 °C), 4 g saccharose, 4 g NaCl and compressed baker's yeast according to Table 1 (each dough containing $2.88 * 10^{14}$ CFU/kg flour, corresponding to approximately 20 g baker's yeast/kg flour) were mixed in a baking machine (FAB-100, Funai, Japan) for 19 min. Two pieces of 235 g dough were each transferred to a 1 L beaker glass. The beaker glasses were sealed with aluminum foil and the dough was left to ferment at 25 °C in an incubator (Termaks, series 6000, cooling incubator).

The fermentations were terminated when the dough reached 10 cm in total height. Preliminary tests were made for each commercial baker's yeast to determine the exact fermentation time to reach a dough height of 10 cm, as the dough height was monitored by a web-camera during fermentation. The fermentation times are presented in Table 2. Baking and sample preparation were done according to Birch et al. (2013).

2.6. Standards

For quantification and confirmation of the identity of the volatile compounds the following standards were used: 3-methyl-1-butanol, 3-methyl-3-buten-1-ol, 1-pentanol, 1-hexanol, 2-ethyl-1-hexanol, 1-octanol, 2-phenylethanol, 3-methylbutanal, heptanal, octanal, nonanal, decanal, benzaldehyde, phenylacetaldehyde, 2-heptanone, 3-hydroxy-2-butanone, ethyl acetate, ethyl octanoate, methyl-benzene, limonene, 2-pentylfuran and trimethylpyrazine (purchased from Sigma-Aldrich, Gillingham, U.K.); 2-methyl-1-propanol, hexanal and 2,3-butanedione (purchased from Fluka, Buchs, Switzerland); 1-propanol, 1-heptanol and 2-furancarboxaldehyde (purchased from Merck, Darmstadt, Germany); 1-butanol (purchased from Ferak, Berlin, Germany) and 6-methyl-5-hepten-2-one (purchased from Acros Organics, Geel, Belgium).

2.7. Dynamic headspace extraction (DHE)

Extraction of volatiles from the bread crumb samples was done according to Birch et al. (2013).

Table 2
Fermentation times for dough fermented with seven baker's yeasts to reach a total height of 10 cm. The fermentation times are calculated from monitoring the dough heights during fermentation by a video camera.

Baker's yeast	Fermentation times (min) for the dough to reach a total height of 10 cm
Malteserkors	100 ± 7
Skærtoftmølle	71 ± 5
Rapunzel	65 ± 3
Sema	60 ± 8
Zymarom	46 ± 2
Bruggeman	40 ± 5
l'Hirondelle	40 ± 6

2.8. Multiple headspace extraction (MHE)

To determine the total amount of volatiles in the bread samples, seven consecutive dynamic headspace extractions were performed from the same sample (the bread fermented with Malteserkors). The same DHE procedures as described in Birch et al. (2013) were used.

2.9. Gas chromatography mass spectrometry (GC–MS)

The trapped volatiles were desorbed using an automatic thermal desorption unit (ATD 400, Perkin Elmer, Norwalk, CT) according to Birch et al. (2013), the carrier gas in this work was however hydrogen (instead of helium). The volatiles were rapidly transferred to the GC–MS (G3440A GC System and G3170A VL MSD with triple axis detector, Agilent Technologies, Palo Alto, California, USA) through a heated (225 °C) transfer line. Separation of volatiles was carried out on a ZB-Wax capillary column (Zebron, 30 m long × 0.25 mm internal diameter, 0.25 μm film thickness, Phenomenex, Torrance, California, USA). The column flow rate was 1.0 mL/min using hydrogen as a carrier gas. The column temperature program was: 10 min at 40 °C, hereafter heating by 8 °C/min up to 240 °C, and holding this temperature for 10 min. The GC was equipped with an MS detector as described in Birch et al. (2013). Volatile compounds were identified and peak areas for each volatile compound integrated according to Birch et al. (2013).

2.10. Odor activity value (OAV)

OAVs were calculated as the ratio of each volatile compound concentration found in bread crumb to the odor threshold (OT) in water. Bread is, however, a complex food matrix and therefore it should be underlined that an aqueous OT value of a volatile compound will only give an approximation of the OAV in bread crumb. It would have been more appropriate to calculate the OAVs from OT in starch or cellulose. They were however, not available from the literature for the majority of the compounds. To take the uncertainty of the calculated OAVs into account it was assumed that volatiles having an OAV of 0.1 or higher might be important in bread crumb aroma. Volatiles with an OAV of 0.1 or higher are therefore named aroma compounds and they are the compounds which are emphasized in the data analysis and discussion.

2.11. Data analysis

Quantification of the volatile compounds identified in the bread samples by MHE was done according to Birch et al. (2013).

Principal Component Analysis (PCA) formulated by Hotelling (1933) is a useful technique to describe major trends in a data set and to detect possible outliers. PCA decomposes the data into scores and loadings, where the scores are related to the samples and the loadings are related to the variables (in this paper volatile compounds). In PCA new variables (principal components) are constructed from a data matrix of samples. The first principal component captures as much as possible of the variability in all the original variables and each new principal component (applied orthogonal on the previous principal component) accounts for as much of the remaining variability as possible. A large portion of the variability is therefore often described by a few principal components. The results can be presented by a PCA bi plot, as done in this paper, which is a combination of a score plot of the samples and a loadings plot showing the correlations between the variables volatile compounds. Principal Component Analysis (PCA) was computed by Matlab and all GC–MS data was autoscaled prior to the analysis.

One-way analysis of variance (one-way ANOVA) was computed by the software JMP (version 7.0, SAS Institute Inc.). Student's *t*-test was computed for the aroma compounds (95% significant level).

3. Results and discussion

3.1. Yeast and LAB count

The CFU of both yeast and LAB in the baker's yeasts are shown in Table 1. The CFU of yeast were high and relatively equally for the seven baker's yeasts. The highest CFU of yeast was found in the baker's yeast named Malteserkors ($1.44 * 10^{13}$ CFU/g baker's yeast) and the lowest in Rapunzel and l'Hirondelle (both $0.72 * 10^{13}$ CFU/g baker's yeast). The numbers of LAB per g baker's yeasts were surprisingly high, varying from $0.1 * 10^{10}$ CFU/g baker's yeast in l'Hirondelle to $4.1 * 10^{10}$ CFU/g baker's yeast in Sema (Table 1). Because equal amounts of yeast cells was added to the dough, the actual amount of LAB in the dough was highest in dough fermented with Rapunzel ($11.6 * 10^{11}$ CFU/kg flour) and lowest in dough fermented with Malteserkors and l'Hirondelle (both containing $0.4 * 10^{11}$ CFU/kg flour) (Table 1). The high contents of LAB are of interest since they might also contribute to the aroma of the bread crumb (Hansen & Schieberle, 2005).

3.2. Fermentation time

The fermentation times for the dough to reach a total height of 10 cm differed significantly between the different baker's yeasts (Table 2) from 40 min in the dough fermented with l'Hirondelle and Bruggeman to 100 min in the dough fermented with Malteserkors. This is in spite of the fact that all doughs had the same initial yeast concentration. This indicates that the baker's yeast named Malteserkors has lower fermentative activity than e.g. l'Hirondelle and Bruggeman. A high fermentative activity resulting in a short fermentation time is obviously an economic benefit for the bakers if focus is solely on bread volume. Bell, Higgins, and Attfield (2001) also found significant differences in the fermentative activity of 39 wild and domestic baker's yeasts. The fermentative activity of *S. cerevisiae* have been found to be influenced by pH, temperature and dissolved oxygen during an 8 h fed-batch cultivation, with the highest fermentative activity at pH 6, 32 °C and 20% dissolved oxygen in the phase of maturation of the baker's yeast (Angelov, Hristozova, & Roshkova, 1997). Furthermore, the authors found that strains having a high maltase activity were also having a higher dough leaving ability. Hence, the different fermentation times observed for doughs fermented with the seven commercial baker's yeasts (Table 1) might be caused by differences within the cultivation method of the baker's yeasts or by the mode of regulation of maltose utilization in the yeasts.

3.3. Identification and quantification of aroma compounds

A total of 32 volatile compounds were identified in the crumb of bread fermented with the seven baker's yeasts (Table 3). Among them, 30 were quantified by MHE. This method was found to perform well with generally low standard deviations as also found recently (Birch et al., 2013). The coefficient of variation (CV%) of the slope of the regression curve of the natural logarithm to the peak area vs. number of extractions (q) was from 2.5 to 19.4%.

The identified iso-alcohols, 3-methylbutanal and phenylacetaldehyde (Table 3) are typically fermentation compounds likely formed via the Erhlich pathway in the yeast cell (Hazelwood et al., 2008). The identified 2,3-butanedione and 3-hydroxy-2-butanone (Table 3) were most likely formed by oxidative decarboxylation and decarboxylation, respectively of acetohydroxy acids outside the yeast cell. The yeast cell has been found to be responsible for the synthesis and excretion of these acetohydroxy acids (Wainwright, 1973). The identified esters; ethyl acetate and ethyl octanoate (Table 3) were most probable formed from enzymatic reactions inside the yeast cell (Lilly et al., 2000). The remaining aldehydes, ketones and 2-pentylfuran identified in the samples (Table 3) were most likely formed from oxidation of flour lipids (Frasse et al., 1992; Schieberle & Grosch, 1991).

Table 3
Concentration (μg/kg) of volatile compounds in wheat bread crumb fermented with seven baker's yeasts. Different letters in the same row indicate significant differences (significant level 95%). The compounds marked bold are the compounds with significant differences.

Volatile compound[a]	Ion used for quantification (m/z)	Volatile compound concentration (μg/kg) in bread samples[b]						
		Malteserkors	Skærtoftmølle	Rapunzel	l'Hirondelle	Sema	Bruggeman	Zymarom
1-Propanol[c]	**57**	**531c**	**440c**	**980c**	**8186b**	**12740a**	**7739b**	**11608ab**
2-Methyl-1-propanol[c]	**42**	**797c**	**849c**	**554c**	**964c**	**746c**	**3010a**	**2460b**
1-Butanol[c]	**56**	**22bc**	**18c**	**18c**	**19c**	**23bc**	**51a**	**34b**
2-Penten-1-ol[d]	57	nq	nq	nq	nq	nq	nq	nq
3-Methyl-1-butanol[e]	**70**	**6313b**	**7770a**	**8304a**	**8706a**	**8131a**	**8768a**	**8076a**
3-Methyl-3-buten-1-ol[e]	**41**	**8ab**	**5b**	**5b**	**7ab**	**10a**	**11a**	**8ab**
1-Pentanol[e]	**42**	**78b**	**110ab**	**129a**	**107ab**	**121a**	**92ab**	**92ab**
1-Hexanol[e]	56	360	515	589	523	575	436	450
2-Octen-1-ol[d]	57	nq	nq	nq	nq	nq	nq	nq
1-Heptanol[e]	43	24	35	33	38	38	27	41
2-Ethyl-1-hexanol[e]	**57**	**9d**	**22bc**	**41a**	**24bc**	**23bc**	**15cd**	**27b**
1-Octanol[e]	55	2	3	3	3	2	2	3
2-Phenylethanol[e]	**91**	**39cd**	**86b**	**178a**	**61c**	**34d**	**59c**	**32d**
3-Methylbutanal[c]	**44**	**14c**	**25bc**	**14c**	**12c**	**11c**	**47a**	**33ab**
Hexanal[e]	**56**	**230b**	**393a**	**352a**	**241b**	**329a**	**222b**	**251b**
Heptanal[e]	70	50	77	73	41	69	41	44
Octanal[e]	41	16	17	21	15	17	13	16
Nonanal[e]	57	18	28	34	27	30	20	26
2-Furancarboxaldehyde[e]	**95**	**2c**	**3a**	**3a**	**3abc**	**3ab**	**2c**	**3bc**
Decanal[e]	**57**	**15c**	**32ab**	**41a**	**28bc**	**28abc**	**21bc**	**29ab**
Benzaldehyde[e]	**77**	**23c**	**56a**	**46ab**	**30c**	**35bc**	**30c**	**34bc**
Phenylacetaldehyde[e]	**91**	**24d**	**130a**	**141a**	**89b**	**77bc**	**57c**	**83bc**
2,3-Butanedione[c]	**43**	**123c**	**143c**	**121c**	**473ab**	**422b**	**669a**	**513ab**
2-Heptanone[e]	43	4	4	3	2	3	2	3
3-Hydroxy-2-butanone[e]	**43**	**638d**	**794c**	**875bc**	**1216a**	**947b**	**816bc**	**936b**
6-Methyl-5-hepten-2-one[e]	**43**	**99c**	**242a**	**184b**	**129c**	**108c**	**124c**	**135bc**
Ethyl acetate[c]	**43**	**38c**	**69c**	**60c**	**99c**	**52c**	**398a**	**284b**
Ethyl octanoate[e]	**88**	**1c**	**4bc**	**6ab**	**7ab**	**7ab**	**10a**	**8ab**
Methylbenzene[e]	**92**	**208ab**	**191bc**	**183c**	**214a**	**183c**	**146d**	**150d**
Limonene[e]	**68**	**4c**	**6d**	**7a**	**3cd**	**5bc**	**3d**	**4cd**
2-Pentylfuran[e]	81	9	15	13	8	13	8	9
Trimethylpyrazine[e]	**42**	**9b**	**0d**	**0d**	**16a**	**3c**	**9b**	**10b**

[a] Identification of all volatile compounds was obtained by comparing authentic standards with their mass spectra.
[b] Mean concentrations of triplicates; nq, not quantitated.
[c] Quantified from the samples purged with nitrogen (50 mL/min) for 5 min.
[d] No standard available.
[e] Quantified from the samples purged with nitrogen (150 mL/min) for 60 min.

The typical fermentation volatiles identified (Table 3) were therefore formed mainly from the metabolism of yeast. However, many of the identified fermentation compounds have also been identified from sourdough (Hansen & Hansen, 1994, 1996) thus questioning if the compounds presented in Table 3 were formed from the metabolism of LAB or yeast. Hansen and Hansen (1994) concluded that a higher number of fermentation compounds were produced during sourdough fermentation when yeast was added to the sourdough. Furthermore, the authors found a higher concentration of fermentation compounds when yeast was added to the sourdough. It is therefore assumed that the majority of the fermentation compounds identified in the bread samples in this research work (Table 3) were produced mainly from the metabolism of yeast.

3.4. Outlying samples

One of the triplicate samples fermented with Malteserkors (Mal_2) and one of the triplicate samples fermented with Zymarom (Zym_2) were characterized as outlying samples based on residual variance and Hotellings T^2 (data not shown) and were hence not included in the further data analysis.

3.5. Crumb aroma of bread fermented with baker's yeasts

A total of 20 aroma compounds was found having an OAV of 0.1 or higher (Table 4). Of those, the concentration of 14 compounds was significantly different between the bread samples (Table 3). A PCA was calculated based on the 20 aroma compounds having an OAV of 0.1 or higher to provide an overview of the differences in aroma compound concentration in the seven samples (Fig. 1).

The sensorily most important fermentation aroma compounds were predicted to be 3-methylbutanal, 3-methyl-1-butanol, phenylacetaldehyde and 2,3-butanedione, based on their OAVs (Table 4). The same compounds were stated as the most aroma active in wheat bread crumb in a recent study (Birch et al., 2013).

The fermentation compounds 2,3-butanedione and 1-propanol were found in significantly higher concentration in bread fermented with Bruggeman, Zymarom, Sema and l'Hirondelle (Table 3 and Fig. 1). 2,3-Butanedione was found to be an important aroma compound with OAVs from 19 to 103 (Table 4). The high OAVs of 2,3-butanedione is expected to be of sensory importance and the odor is described as buttery and caramel (Le Guen et al., 2001). 3-Methylbutanal, 2-methyl-1-propanol, ethyl acetate and ethyl octanoate were found in significantly higher concentration in bread fermented with Bruggeman and Zymarom (Table 3 and Fig. 1). The higher concentrations of esters in the bread fermented with Bruggeman and Zymarom is of interest, since esters are often characterized as having a fruity and pleasant aroma (Lee & Noble, 2003). However, the OAVs of the esters were generally found to be low (Table 4). 3-Methylbutanal is characterized as having a malty odor (Frauendorfer & Schieberle, 2006) and because of the high OAVs this aroma compound can highly likely be sensed in the samples (Table 4). The concentration of the important fermentation compound 3-methyl-1-butanol was only differentiated by having significantly lower concentration in bread fermented with Malteserkors (Table 3 and Fig. 1). The OAVs of 3-methyl-1-butanol were generally high for all samples (Table 4) and the balsamic or alcoholic odor

Table 4
Odor activity values[a] (OAVs) of aroma compounds in bread samples, odor thresholds (OT) and odor quality. Only compounds having an OAV of 0.1 or higher[b] are presented. Odor threshold (OT) values in water and odor descriptions of the identified aroma compounds are collected from scientific papers.

Aroma compound	OAVs of aroma compounds in bread samples[c]							OT (μg/kg)	Odor
	Malteserkors	Skærtoftmølle	Rapunzel	l'Hirondelle	Sema	Bruggeman	Zymarom		
1-Propanol	0.1	0.1	0.1	1.2	1.9	1.2	1.8	6600[d]	Fruity, plastic[e]
2-Methyl-1-propanol	0.2	0.3	0.2	0.3	0.2	0.9	0.8	3200[f]	Glue, alcohol[g]
3-Methyl-1-butanol	25	31	33	35	33	35	32	250[h]	Balsamic, alcohol[g]
1-Hexanol	0.1	0.2	0.2	0.2	0.2	0.2	0.2	2500[i]	Green grass[g]
1-Heptanol	8.0	12	11	13	13	9.0	14	3[j]	Green[k]
2-Phenylethanol	0.0	0.1	0.2	0.1	0.0	0.1	0.0	1100[h]	Flowery[l]
3-Methylbutanal	71	126	70	61	56	236	167	0.2[h]	Malty[l]
Hexanal	51	87	78	54	73	49	56	4.5[h]	Green[e]
Heptanal	17	26	24	14	23	14	15	3[i]	Fatty, rancid[k]
Octanal	22	25	30	21	25	19	23	0.7[i]	Citrus[k]
Nonanal	18	28	34	27	30	20	26	1[i]	Citrus[k]
Decanal	7.7	16	21	14	14	11	15	2[i]	Citrus[k]
Benzaldehyde	0.1	0.2	0.1	0.1	0.1	0.1	0.1	350[i]	Almond[k]
Phenylacetaldehyde	6.0	33	35	22	19	14	21	4[i]	Honey-like[l]
2,3-Butanedione	19	22	19	73	65	103	79	6.5[f]	Buttery, caramel[e]
3-Hydroxy-2-butanone	0.8	1.0	1.1	1.5	1.2	1.0	1.2	800[i]	Butterscotch[g]
6-Methyl-5-hepten-2-one	2.0	4.8	3.7	2.6	2.2	2.5	2.7	50[h]	Herbaceous, green[m]
Ethyl acetate	0.0	0.0	0.0	0.0	0.0	0.1	0.0	6200[f]	Sweet, fruity[g]
Ethyl octanoate	0.0	0.0	0.1	0.1	0.1	0.1	0.1	92[n]	Sweet, soap, fresh[g]
2-Pentylfuran	1.5	2.6	2.2	1.3	2.2	1.3	1.5	6[h]	Floral, fruity[k]

[a] The OAV is the ratio of the concentration of aroma compound and the OT of the compound.
[b] An OAV of 0.1 or higher and not an OAV above 1 is chosen to limit the risk of disregarding aroma compounds in bread crumb that can be sensorily detected, since the OAVs are based on OT in water and not in bread crumb.
[c] Mean OAVs of triplicates.
[d] From Rychlik, Schieberle, and Grosch (1998).
[e] From Le Guen, Prost, and Demaimay (2001).
[f] From Mulders (1973).
[g] From Lee and Noble (2003).
[h] From Buttery, Teranishi, Ling, and Turnbaugh (1990).
[i] From Buttery, Turnbaugh, and Ling (1988).
[j] From Fazzalari (1978).
[k] From Yang, Lee, Jeong, Kim, and Kays (2008).
[l] From Frauendorfer and Schieberle (2006).
[m] From Carbonell-Barrachina, Agustí, and Ruiz (2006).
[n] From Takeoka, Buttery, and Flath (1992).

(Lee & Noble, 2003) is assumed to be easily sensed in the samples. Generally, the bread fermented with Malteserkors was characterized by having significantly lower concentrations of nearly all aroma compounds compared to the other breads (Table 3 and Fig. 1).

The fermentation compounds phenylacetaldehyde and 2-phenylethanol showed a different tendency than the other fermentation compounds by being found in the highest concentration in bread fermented with Skærtoftmølle and Rapunzel (Table 3 and Fig. 1). The odors of phenylacetaldehyde and 2-phenylethanol are described as honey-like and flowery, respectively (Frauendorfer & Schieberle, 2006). Phenylacetaldehyde might be a sensorily important aroma compound because of the relatively high OAVs (Table 4). 2-Phenylethanol is mentioned as one of the aroma compounds formed in highest concentration in bread crumb (Frasse et al., 1992) and this was confirmed from our results with concentrations from 32 to 178 μg/kg bread crumb. However, 2-phenylethanol might not be one of the sensorily most important aroma compounds due to the low OAVs (between 0.01 and 0.2).

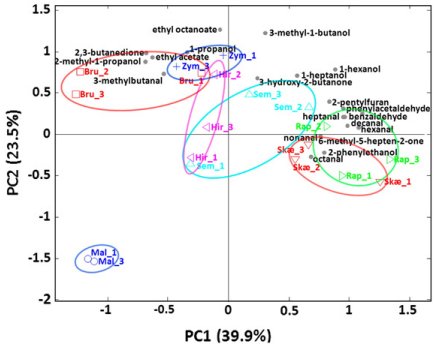

Fig. 1. PCA biplot showing the aroma compounds formed in the bread samples fermented with the seven commercial baker's yeasts. The samples are named as following; Mal for Malteserkors, Skæ for Skærtoftmølle, Rap for Rapunzel, Hir for L'Hirondelle, Sem for Sema, Bru for Bruggeman and Zym for Zymarom. The number at the end of the sample name corresponds to the triplicate number. The seven yeast samples are manually marked with circles in order to highlight the results of the PCA. Only aroma compounds having an OAV of 0.1 or higher were included in the PCA model.

The most aroma active lipid oxidation compounds were found to be hexanal, heptanal, octanal, nonanal and decanal, based on their OAVs (Table 4). For the majority of the aroma compounds formed from the lipid oxidation, no significant differences were found between the bread samples (Table 3). However, hexanal was found in a significantly higher concentration in the bread fermented with Skærtoftmølle, Rapunzel and Sema (Table 3). Hexanal, characterized as having a green odor (Le Guen et al., 2001), has high OAVs (Table 4), and might therefore be a sensorily important compound in bread crumb. Lipid oxidation compounds are often characterized as being off-flavors, therefore the high level of hexanal in bread fermented with Skærtoftmølle, Rapunzel and Sema respectively, might result in a lower acceptance of these breads if a sensory evaluation were performed with all seven breads.

The differences in the aroma profile between the breads fermented with the seven commercial baker's yeasts may be due to differences in the gene-regulating mechanisms and biosynthetic pathways of aroma compound formation for each yeast strain that take place during the dough fermentation. The genes important for the aroma formation

formed via the Ehrlich pathway have recently been investigated (Styger et al., 2011). Phenylacetaldehyde and 2-phenylethanol, both formed from catabolism of phenylalanine in the Ehrlich pathway (Dickinson, Eshantha, Salgado, & Hewlins, 2003), were as mentioned found in a higher concentration in bread fermented with Rapunzel and Skærtoftmølle compared with breads fermented with the other five baker's yeast (Table 3). This result is interesting, since the compounds (1-propanol, 2-methyl-1-propanol and 3-methylbutanal) formed from valin and leucin through the Ehrlich pathway (Hazelwood et al., 2008) were found in a higher content for the two Belgian yeasts (Bruggeman and Zymarom, Table 3). These results might be explained by different carboxylases in the commercial baker's yeast, since different carboxylases have been found to be important for the catabolism of the branched-chain amino acids (leucin and valin) and the aromatic amino acid (phenylalanine), respectively in the Ehrlich pathway (Dickinson et al., 2003). The aroma profiles of the organically produced baker's yeasts Skærtoftmølle and Rapunzel are quite similar, which might indicate that they contain the same yeast strain.

The difference in the aroma profile might also be caused by different concentrations and strains of LAB in the baker's yeasts. As mentioned in Section 3.3 the majority of the volatile compounds are assumed to be formed from the yeast metabolism and not from LAB. However, the doughs fermented with Rapunzel, Sema and Skærtoftmølle were containing the highest amounts of LAB (Table 1) and were as previously described having a generally higher content of compounds formed from the lipid oxidation, which was significant for hexanal (Table 3 and Fig. 1). The increased formation of lipid oxidation compounds could be due to an increased lipase activity in Skærtoftmølle, Rapunzel and Sema compared to the other baker's yeasts. However, Czerny and Schieberle (2002) found that lactic acid bacteria decreased the amounts of unsaturated aldehydes such as (E)-2-nonenal and (Z)-4-heptenal during sourdough fermentation. The LAB metabolism are often linked with formation of acids (Hansen & Hansen, 1996), unfortunately quantification of acids were not possible by the DHE GC–MS method used in this paper, because of very poor repeatability of the peak areas as also found in Birch et al. (2013). Quantification of the acids in the bread crumb combined with identification of the yeast and LAB strains in the different baker's yeasts could be of interest in order to explain the significant differences in the production of aroma compounds in the bread samples.

Additional research within selection of yeast strains with improved aroma formation could be of industrial interest (Styger et al., 2011). Particularly selection of yeast strains with increased ester formation could be of relevance, since esters have fruity and pleasant aroma (Lee & Noble, 2003). Studies have recently been carried out on *S. cerevisiae* alcohol acetyltransferases and their influence on ester formation in alcoholic fermentation (Procopio et al., 2011; Suárez-Lepe & Morata, 2012; Verstrepen et al., 2003). Knowledge from these studies might be successfully applied to dough fermentation in the search for novel ways of developing the aroma of bread.

4. Conclusions

Fermentation of wheat dough with seven commercial baker's yeast resulted in a diverse aroma profile of the bread crumb. Furthermore, a significant difference was found in the fermentation time for the dough to reach 10 cm in total dough height, ranging from 40 min in dough fermented with the baker's yeasts named l'Hirondelle and Bruggeman to 100 min in dough fermented with Malteserkors. The powerful odor compounds were 3-methylbutanal, 2,3-butanedione, 3-methyl-1-butanol and phenylacetaldehyde. The fermentation compounds 2,3-butanedione and 1-propanol were found in significantly higher concentration in breads fermented with Bruggeman, Zymarom, Sema and l'Hirondelle, the baker's yeasts having the shortest fermentation time. 3-Methylbutanal, 2-methyl-1-propanol and ethyl acetate were found in significantly higher concentration in the breads fermented with Bruggeman and Zymarom. Crumb of bread produced with the two organically produced baker's yeasts Skærtoftmølle and Rapunzel were found to have significantly higher concentration of the fermentation compounds phenylacetaldehyde and 2-phenylethanol, and were together with the bread fermented with Sema furthermore characterized by having significantly higher concentration of the lipid oxidation compound hexanal. Apart from this, no significantly differences were found in the concentration of lipid oxidation compounds in the bread samples.

The significantly differences in the aroma profile and fermentation time between the breads fermented with the seven baker's yeasts indicates that some of the baker's yeasts differentiate in yeast strain. The choice of baker's yeast is therefore very important in respect of both the aroma of the bread and the fermentation time of the dough. It could be speculated from the results that the two Belgian yeasts (Bruggeman and Zymarom) might have the most balanced aroma profile with a low content of lipid oxidation compounds combined with the shortest fermentation time.

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References

Angelov, A., Hristozova, T., & Roshkova, Z. (1997). Maltase activity and dough rising ability of baker's yeasts obtained at different growth conditions during the maturation stage. *Zeitschrift fur Lebensmittel-Untersuchung Und-Forschung A — Food Research and Technology*, *205*, 80–84.

Bell, P. J. L., Higgins, V. J., & Attfield, P. V. (2001). Comparison of fermentative capacities of industrial baking and wild-type yeasts of the species *Saccharomyces cerevisiae* in different sugar media. *Letters in Applied Microbiology*, *32*, 224–229.

Birch, A. N., Petersen, M. A., & Hansen, Å. S. (2013). The aroma profile of wheat bread crumb influenced by yeast concentration and fermentation temperature. *LWT — Food Science and Technology*, *50*, 480–488.

Buttery, R. G., Teranishi, R., Ling, L. C., & Turnbaugh, J. G. (1990). Quantitative and sensory studies on tomato paste volatiles. *Journal of Agricultural and Food Chemistry*, *38*, 336–340.

Buttery, R. G., Turnbaugh, J. G., & Ling, L. C. (1988). Contribution of volatiles to rice aroma. *Journal of Agricultural and Food Chemistry*, *36*, 1006–1009.

Carbonell-Barrachina, A., Agustí, A., & Ruiz, J. (2006). Analysis of flavor volatile compounds by dynamic headspace in traditional and hybrid cultivars of Spanish tomatoes. *European Food Research and Technology*, *222*, 536–542.

Czerny, M., & Schieberle, P. (2002). Important aroma compounds in freshly ground whole meal and white wheat flour — Identification and quantitative changes during sourdough fermentation. *Journal of Agricultural and Food Chemistry*, *50*, 6835–6840.

Dickinson, J. R., Eshantha, L., Salgado, J., & Hewlins, M. J. E. (2003). The catabolism of amino acids to long chain and complex alcohols in *Saccharomyces cerevisiae*. *The Journal of Biological Chemistry*, *278*, 8028–8034.

Fazzalari, F. A. (1978). *Compilation of odor and taste threshold data*. Philadelphia: The Society.

Frasse, P., Lambert, S., Levesque, C., Melcion, D., Richard-Molard, D., & Chiron, H. (1992). The influence of fermentation on volatile compounds in French bread crumb. *Food Science and Technology — Lebensmittel-Wissenschaft & Technologie*, *25*, 66–70.

Frauendorfer, F., & Schieberle, P. (2006). Identification of the aroma compounds in cocoa powder based on molecular sensory correlations. *Journal of Agricultural and Food Chemistry*, *54*, 5521–5529.

Gassenmeier, K., & Schieberle, P. (1995). Potent aromatic compounds in the crumb of wheat bread (French-type) — Influence of pre-ferments and studies on the formation of key odorants during dough processing. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, *201*, 241–248.

Hansen, B., & Hansen, Å. (1994). Volatile compounds in wheat sourdoughs produced by lactic acid bacteria and sourdough yeasts. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, *198*, 202–209.

Hansen, Å., & Hansen, B. (1996). Flavour of sourdough wheat bread crumb. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, *202*, 244–249.

Hansen, A., & Schieberle, P. (2005). Generation of aroma compounds during sourdough fermentation: Applied and fundamental aspects. *Trends in Food Science & Technology*, *16*, 85–94.

Hazelwood, L. A., Daran, J. M., van Maris, A. J. A., Pronk, J. T., & Dickinson, J. R. (2008). The Ehrlich pathway for fusel alcohol production: A century of research on *Saccharomyces cerevisiae* metabolism. *Applied and Environmental Microbiology*, *74*, 2259–2266.

Hotelling, H. (1933). Analysis of complex of statistical variables with principal components. *Journal of Educational Psychology*, *24*, 417–441.

Jensen, S., Oestdal, H., Skibsted, L. H., Larsen, E., & Thybo, A. K. (2011). Chemical changes in wheat pan bread during storage and how it affects the sensory perception of aroma, flavour, and taste. *Journal of Cereal Science*, *53*, 259–268.

Le Guen, S., Prost, C., & Demaimay, M. (2001). Evaluation of the representativeness of the odor of cooked mussel extracts and the relationship between sensory descriptors and potent odorants. *Journal of Agricultural and Food Chemistry*, *49*, 1321–1327.

Lee, S. -J., & Noble, A. C. (2003). Characterization of odor-active compounds in Californian Chardonnay wines using GC–olfactometry and GC–mass spectrometry. *Journal of Agricultural and Food Chemistry*, *51*, 8036–8044.

Lilly, M., Lambrechts, M. G., & Pretorius, I. S. (2000). Effect of increased yeast alcohol acetyltransferase activity on flavor profiles of wine and distillates. *Applied and Environmental Microbiology*, *66*, 744–753.

Maeda, T., Kikuma, S., Araki, T., Ikeda, G., Takeya, K., & Sagara, Y. (2009). The effects of mixing stage and fermentation time on the quantity of flavor compounds and sensory intensity of flavor in white bread. *Food Science and Technology Research*, *15*, 117–126.

Mulders, E. J. (1973). The odor of white bread. IV. Quantitative determination of constituents in the vapour and their odour values. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, *151*, 310–317.

Poinot, P., Arvisenet, G., Grua-Priol, J., Colas, D., Fillonneau, C., Le Bail, A., et al. (2008). Influence of formulation and process on the aromatic profile and physical characteristics of bread. *Journal of Cereal Science*, *48*, 686–697.

Procopio, S., Qian, F., & Becker, T. (2011). Review paper: Function and regulation of yeast genes involved in higher alcohol and ester metabolism during beverage fermentation. *European Food Research and Technology*, *233*, 721–729.

Richard-Molard, D., Nago, M. C., & Drapron, R. (1979). Influence of the bread making method on French bread flavor. *Bakers Digest*, *53*, 34–38.

Rychlik, M., Schieberle, P., & Grosch, W. (1998). *Compilation of odor thresholds, odor qualities and retention indices of key food odorants*. München: Deutsche Forschungsanstalt für Lebensmittelchemie, Institut für Lebensmittelchemie der Technischen Universität.

Schieberle, P., & Grosch, W. (1991). Potent odorants of the wheat bread crumb. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, *192*, 130–135.

Styger, G., Jacobson, D., & Bauer, F. F. (2011). Identifying genes that impact on aroma profiles produced by *Saccharomyces cerevisiae* and the production of higher alcohols. *Applied Microbiology and Biotechnology*, *91*, 713–730.

Suárez-Lepe, J. A., & Morata, A. (2012). New trends in yeast selection for winemaking. *Trends in Food Science & Technology*, *23*, 39–50.

Takeoka, G. R., Buttery, R. G., & Flath, R. A. (1992). Volatile constituents of Asian pear (*Pyrus serotina*). *Journal of Agricultural and Food Chemistry*, *40*, 1925–1929.

Verstrepen, K. J., Van Laere, S. D. M., Vanderhaegen, B. M. P., Derdelinckx, G., Dufour, J. P., Pretorius, I. S., et al. (2003). Expression levels of the yeast alcohol acetyltransferase genes ATF1, Lg-ATF1, and ATF2 control the formation of a broad range of volatile esters. *Applied and Environmental Microbiology*, *69*, 5228–5237.

Wainwright, T. (1973). Diacetyl: A review. Part I — Analytical and biochemical considerations: Part II — Brewing experience. *Journal of the Institute of Brewing*, *79*, 451–470.

Yang, D. S., Lee, K. -S., Jeong, O. -Y., Kim, K. -J., & Kays, S. -J. (2008). Characterization of volatile aroma compounds in cooked black rice. *Journal of Agricultural and Food Chemistry*, *56*, 235–240.

Corrigendum

Corrigendum to “Influence of commercial baker's yeasts on bread aroma profiles”

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In the paper " Influence of commercial baker's yeasts on bread aroma profiles " by Anja N. Birch*, Mikael A. Petersen, Nils Arneborg and Åse S. Hansen. (Food Research Int., 52 160-166, doi: http://dx.doi.org/10.1016/j.foodres.2013.03.011 [2013]),

The authors have been aware of a mistake in the calculation of yeast and lactic acid bacteria cells. The results in Table 1 should:

- for all CFU of yeast per g baker’s yeast be $*10^{10}$ instead of 10^{13}.
- for all CFU of LAB per g baker’s yeast be $*10^{7}$ instead of 10^{10}.
- for amount of LAB in the dough be $*10^{8}$ instead of 10^{11}.
- The yeast concentration added to the dough should be changed to $2.88 * 10^{11}$ CFU/kg flour instead of $2.88 * 10^{14}$ CFU/kg flour (this number should also be changed in 2.5 Bread making).

These corrections do not change the discussion or the conclusions.

The authors apologize for this oversight.

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PAPER IV

Birch, A. N., Petersen, M. A. and Hansen, Å. S.

Aroma of wheat bread crumb
Review

Submitted to *Cereal Chemistry*. The outline was pre-approved by *Cereal Chemistry*.

REVIEW

Aroma of Wheat Bread Crumb

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Keywords: dough fermentation; baker's yeast; bread crumb; aroma compounds

ABBREVIATIONS

AAT, alcohol acetyltransferase; ATF1, acyltransferase 1; CoA, Coenzym A; EHT1, ethanol hexanoyl transferase; GCO, Gas Chromatography Olfactometry; AEDA, Aroma Extract Dilution Analysis; OAV, Odour Activity Value; FD, Flavour Dilution.

ABSTRACT

Bread aroma is an important parameter for bread quality and this review aims to provide an overview of aroma compounds identified in bread crumb and how these compounds are formed. More than 150 volatile compounds were identified in bread crumb and they mainly originate from the fermentative activity of yeast, from oxidation of flour lipids and from Maillard reactions (diffused from the crust). Of those volatile compounds, 45 compounds can be characterised as aroma compounds, as they most likely can be sensed when the bread is eaten due to high odour activity values and flavour dilution factors. The influence of ingredients and mixing conditions on bread aroma has only been scarcely investigated. The fermentation conditions; yeast level and strain as well as fermentation temperature and time were found to significantly influence the aroma of bread crumb. Yeast level and strain mainly influence formation of compounds directly related to the fermentative activity of yeast, while fermentation temperature and time also influence formation of compounds from oxidation of flour lipids.

INTRODUCTION

Flour, water, salt and yeast are the four basic ingredients which by mixing, fermentation and baking can undergo the unique transformation to aromatic bread. Bread making has been known for over 6000 years (Jacob, 1997) and bread is still a very important part of the diet for people in most parts of the world. The quality of bread is often described by the texture, the volume and the aroma. For decades research has mainly been focussing on improving bread texture and increasing volume (Young, 2012). Recently, focus from both the consumers and the bread industry has, however, been directed more towards aroma as a quality criterion.

The influence of the flour type on bread aroma has been scarcely investigated. Chang et al. (1995) found that ethyl acetate, ethanol, 2-ethyl-3-methylpyrazine, and ethyl octanoate were higher in relative quantities in hard red winter wheat breads than in hard white winter wheat breads, and 2-butoxy-ethanol and 2-furfural were more abundant in hard white winter wheat breads. However, formation of bread aroma is mainly a result of multiple enzymatic and non-enzymatic reactions occurring during fermentation and baking. The aroma of wheat bread crumb is mainly formed from the fermentative activity of yeast and from oxidation of flour lipids (Schieberle and Grosch, 1991; Frasse et al., 1992; Birch et al., 2013b), while the aroma of bread crust is mostly formed by Maillard reactions during baking (Maillard, 1912; Nursten, 1981; Purlis, 2010). Changes in the fermentation conditions will therefore primarily influence the aroma formation in the crumb (Birch et al., 2013b).

Understanding how the dough fermentation conditions influence the aroma formation in bread will support the bakers in order to control and improve the aroma profile of the final bread product. The purpose of this review is to present the most important volatile aroma compounds identified in wheat bread crumb based on 12 research studies made on yeast and naturally fermented wheat doughs from 1979 to 2013. Furthermore, an overview is presented of the complex reactions involved in formation of the volatile compounds in bread crumb. Crust aroma compounds formed by Maillard reactions during baking might diffuse into the crumb, as they are found in bread crumb, therefore a brief overview of those reactions are presented.

AROMA IN WHEAT BREAD CRUMB

Volatile Compounds Identified In Wheat Bread Crumb

A wide range of volatile compounds were identified in wheat bread crumb (**Table 1**). Overall, the volatiles in bread crumb are found from a wide variety of chemical groups such as aldehydes, alcohols, ketones, esters, acids, benzenes, terpenes, lactones and furans.

The different bread volatile profiles among the papers cited in **Table 1** can be explained by several factors, such as variations within; ingredients, fermentation conditions, baking conditions, sample preparation method, the method for isolation of volatile compounds, and/or sensitivity of the gas chromatography mass spectrometry instrument.

Table 1 Volatile compounds identified in wheat bread crumb from 12 research studies. The compounds are in each chemical group arranged according to the number of references that have identified the compound in wheat bread crumb, with the compound being identified most often in the top.

Chemical group	Volatile compound	References
Aldehydes	Hexanal	*b, c, e, f, g, h, i, j, k, l*
	Nonanal	*c, e, f, g, h, i, j, k, l*
	Benzaldehyde	*c, e, f, g, h, i, j, k, l*
	Heptanal	*c, e, g, h, i, j, k, l*
	(E)-2-Nonenal	*c, d, e, f, g, h, k*
	3-Methylbutanal	*c, e, g, h, j, k, l*
	(E)-2-Octenal	*b, c, e, f, g, h*
	Phenylacetaldehyde	*e, d, h, j, k*
	(E)-2-Heptenal	*c, e, f, g, h*
	Octanal	*f, g, j, k, l*
	Pentanal	*c, f, g, h*
	2-Methylbutanal	*g, h, i, l*
	Decanal	*g, j, k, l*
	(E, E)-2,4-Decadienal	*c, b, f*
	Methional	*b, d, e*
	Propanal	*g, l*
	2-Methylpropanal	*e, l*
	2-Propenal	*g, l*
	Butanal	*g, l*
	(Z)-2-Butenal	*h*
	Acetaldehyde	*h*
	2-Methyl-2-propenal	*g*
	(E)-2-hexenal	*e*
	(Z)-4-Heptenal	*b*
	(Z)-2-Nonenal	*b*
	(E, E)-2,4-Nonadienal	*b*
	2,4-Decadienal	*b*
	Vanillin	*d*
	4,5-Epoxy-(E)-2-decenal	*d*
	Trans-4,5-Epoxy-(E)-2-decenal	*b*
	5-Ethyl-1-cyclopentene-carboxaldehyde	*e*
Alcohols	3-Methyl-1-butanol	*b, c, d, e, f, g, h, i, j, k*
	1-Pentanol	*c, e, f, g, h, i, j, k, l*
	1-Hexanol	*c, e, f, g, h, i, j, k, l*
	2-Phenylethanol	*c, d, f, g, h, i, j, k*
	1-Propanol	*e, g, h, i, j, k, l*
	2-Methyl-1-propanol	*e, f, h, i, j, k, l*
	1-Octen-3-ol	*c, e, f, g, i, k*
	Ethanol	*e, f, g, h, i, l*
	1-Heptanol	*e, f, h, j, k*
	1-Butanol	*e, g, j, k*
	(E)-2-Penten-1-ol	*g, h, j, k*
	2-Ethyl-1-hexanol	*e, g, j, l*
	1-Octanol	*f, j, k*
	3-Nonen-1-ol	*f, g, k*
	2-Methyl-1-butanol	*c, h, l*
	1-Penten-3-ol	*e, k*
	3-Methyl-3-buten-1-ol	*j, k*
	2-Octen-1-ol	*j, l*
	Phenylmethanol	*f*

Chemical group	Volatile compound	References
	2-Hexenol	*g*
	3-Hexenol	*k*
	1-Dodecanol	*k*
	3-Pentanol	*g*
	1-Nonanol	*f*
	3-Decen-1-ol	*f*
	2-Ethyl-1-ethanol	*f*
	2-Butoxyethanol	*f*
	3-Ethoxy-1-propanol	*f*
Ketones	2,3-Butanedione	*b, d, e, f, g, h, i, j, k, l*
	3-Hydroxy-2-butanone	*c, e, f, h, i, j, k, l*
	2-Heptanone	*c, g, h, j, k, l*
	2-Octanone	*f, g, h, k*
	2-Butanone	*g, h, i, l*
	2,3-Pentanedione	*e, g, h, l*
	6-Methyl-5-hepten-2-one	*f, g, j, l*
	1-Octen-3-one	*b, d, f*
	Acetone	*g, h, l*
	3-Octen-2-one	*g, k*
	2-cyclopenten-1,4-dione	*g, l*
	3-Methyl-2-butanone	*h*
	1-Hydroxy-2-propanone	*i*
	2,3-Octanedione	*e*
	(Z)-1,5-Octadien-3-one	*b*
Esters	Ethyl octanoate	*d, e, f, h, i, j, k*
	Ethyl acetate	*e, g, h, i, j, k, l*
	Ethyl 2-hydroxypropanoate	*e, f, i*
	Ethyl hexanoate	*h, i, k*
	Ethyl propanoate	*h, i*
	Butyl acetate	*c, f*
	3-Methylbutyl acetate	*h, k*
	2-Phenylethyl acetate	*f, k*
	Ethyl nonanoate	*d*
	2-Methylpropyl acetate	*f*
	Ethyl 3-methylbutanoate	*j*
	Methyl 2-methylpropanoate	*c*
	Ethyl 2-methylpropanoate	*b*
	Ethyl decanoate	*h*
	Methyl 2-cyanopropanoate	*e*
	Methyl cyanoacetate	*e*
Acids	Acetic acid	*a, d, e, f, h, i, k*
	3-Methylbutanoic acid	*a, d, f, h, i*
	2-Methylpropanoic acid	*a, f, h, i, k*
	Butanoic acid	*a, d, f, k*
	Hexanoic acid	*a, f, i, k*
	Propanoic acid	*a, f, h*
	2-Methylbutanoic acid	*d, h*
	Pentanoic acid	*f, k*
	Heptanoic acid	*f*
	Octanoic acid	*f*
Benzenes	Methylbenzene (toluene)	*e, f, g, h, j*
	Styrene	*e, f, h*
	Ethylbenzene	*f, g, h*
	1-Ethyl-2-methylbenzene	*f, g, h*

Chemical group	Volatile compound	References
	1, 2-Dimethylbenzene	*f, h*
	1,2,4-Trimethylbenzene	*f, h*
	1,3-Dichlorobenzene	*f, h*
	Xylene	*g, h*
	Butylbenzene	*g*
	Benzene	*h*
	1, 4-Dimethylbenzene	*f*
	1,4-Ethylmethylbenzene	*f*
	1,3,5-Trimethylbenzene	*h*
	1,4-Dichlorobenzene	*e*
Terpenes	Limonene	*f, g, h, i, j*
	γ-Nonalactone	*f, k*
	Linalool	*f*
	α-Terpineol	*f*
	α-Phellandrene	*g*
	γ -Butyrolactone	*c*
	γ -Valerolactone	*c*
	γ -Caprolactone	*c*
	4-Hydroxy-2,5-dimethyl-3(2H)-Furanone (furaneol)	*d*
Furans	2-Furancarboxaldehyde (furfural)	*c, e, f, g, h, i, j, k, l*
	2-Pentylfuran	*e, f, g, h, i, j, k, l*
	2-Furanmethanol (furfuryl alcohol)	*e, f, g, i, l*
	2-Methylfuran	*g, i, l*
	Furan	*g, h*
	2-Ethylfuran	*g, h*
	2-Butyl furan	*e*
	3-Furancarboxaldehyde	*g*
	2,3,5-Trimethylfuran	*g*
	Tetrahydrofuran	*g*
	1-(2-Furanyl)-ethanone	*e*
	5-Furfuryl-5-methyl-2(5H)-furanone	*g*
	Furfurylformate	*g*
	Furfurylmethyl ketone	*g*
	5-methyl-2-furfural	*g*
	2,2-Bifuran	*g*
	2-Acetylfuran	*l*
	5-Methyl-2-furaldehyde	*l*
Sulphides	Dimethyltrisulphide	*e, f, h*
	Dimethyldisulphide	*e, g, h*
	Dimethylsulphide	*h*
	Thiazole	*g*
Pyrazines	Trimethylpyrazine	*j, k*
	2-Methylpyrazine	*e, g*
	2-Ethylpyrazine	*g, l*
	2-Ethyl-3-methylpyrazine	*g*
	2-Methoxy-3-isopropylpyrazine	*d*
Pyridines	3-Methylpyradine	*h*
Pyrrolines	2-Acetyl-1-pyrroline	*c, d*
Phenols	4-Vinyl-2-methoxyphenol	c, *f*
	Phenol	*f*
Alkanes	Octane	*h, i*
	Decane	*f, i*
	Heptane	*h*

Chemical group	Volatile compound	References
Others	Azulene	*f*
	2-Octene	*g*
	1,3-Octadiene	*h*

[a] Richard-Molard et al. (1979). [b]Schieberle and Grosch (1991). [c]Frasse et al. (1992). [d]Gassenmeier and Schieberle (1995). [e]Seitz et al. (1998). [f]Ruiz et al. (2003). [g]Bianchi et al. (2008). [h]Maeda et al. (2009). [i]Jensen et al. (2011). [j]Birch et al. (2013a). [k]Birch et al. (2013b). [l]Chiavaro et al. (2008).

Aroma Active Compounds in Bread Crumb

Odour activity value and flavor dilution factor. Aroma compounds can be defined as the volatile compounds that are likely to be sensed when the food product is eaten. Calculation of odour activity values (OAV's) are often carried out in order to evaluate the most aroma active compounds in a food product. OAV is defined as the ratio of the concentration of the volatile compound in the food product to the compound odour threshold. A compound might therefore be sensed when the concentration of the volatile compound exceeds the odour threshold of the compound (OAV>1). When calculating OAV's of bread volatiles, odour threshold values in water are used. Bread is, however, a complex food matrix and therefore it should be underlined that an aqueous odour threshold value of an aroma compound will only give an approximation of the OAV in bread crumb. It would have been more appropriate to calculate the OAV's from odour thresholds in starch.

Aroma extraction dilution analysis (AEDA) is based on gas chromatography olfactometry (GCO) of a liquid extract of the food. Results are presented as flavour dilution (FD) factors, which is defined as the ratio of the concentration of the volatile compound in the initial extract to its concentration in the most dilute extract in which the odour is still detectable by a judge sniffing the volatiles leaving the GC coloumn (Schieberle and Grosch, 1987). Consequently, the FD factor is a relative measure and it is proportional to the OAV. However, the FD factors are not corrected for losses of the odourants during the isolation and concentration step; furthermore in AEDA the volatiles are completely volatilised and then evaluated by sniffing, whereas the volatility of the aroma compounds in foods depends on their solubility in the aqueous and/or lipid phase as well as on their binding to non-volatile food constituents (Grosch, 1993). Therefore calculation of the FD factors is a screening method suitable for the analysis of potent volatile compounds, however in order to find the most aroma-active compounds important when eating the food, Grosch (1993) suggests that OAV's should be calculated. It should be noted that the OAV's and the FD factors provides information about the aroma activity of the volatile compounds, however information of whether the compounds contribute positively or negatively to the overall aroma profile can only be evaluated from the odour descriptions and/or from further sensory analysis.

The volatile compounds in wheat bread crumb which are the most important for the sensory quality will be those with a higher OAV's and/or FD factors. An overview of compounds having OAV's higher than 0.1 and FD factors higher than 8 is presented in **Table 2**.

When comparing the results of the OAV's and FD factors in **Table 2**, the most important bread aroma compounds were found to be: (E)-2-nonanal (green, tallow), 3-methyl-1-butanol (balsamic, alcoholic), hexanal (green), 2,3-butanedione (buttery, caramel), and phenylacetaldehyde (honey-like). Furthermore, 3-methylbutanal (malty), nonanal (citrus), 1-octen-3-ol (mushroom), heptanal (fatty, rancid), and octanal (citrus) were concluded to be important bread crumb aroma compounds with OAV's at 4 or higher.

Moreover, 2-phenylethanol (flowery), ethyl octanoate (sweet, soap), (E, E)-2,4-decadienal (fatty, waxy), *trans*-4,5-epoxy-(E)-2-decenal (metallic), acetic acid (acid, pungent), (Z)-2-nonenal (green), methional (potato-like), 2,4-decadienal (fatty, green), 1-octen-3-one (mushroom-like), 4-hydroxy-2,5- dimethyl-3(2H)-furanone (caramel-like), and 4-vinyl-2-methoxyphenol (spicy) were found to be important aroma compounds having FD factors at 64 or higher (Schieberle and Grosch, 1991; Gassenmeier and Schieberle, 1995) (**Table 2**). The semi-volatile compounds (E, E)-2,4-decadienal, *trans*-4,5-epoxy-(E)-2-decenal, 2,4-decadienal, 4-hydroxy-2,5-dimethyl-3(2H)-furanone and 4-vinyl-2-methoxyphenol were identified in the research studies using AEDA and not by the research studies using headspace methods. AEDA is based on liquid extraction and hence semi- and non-volatile compounds are also included in the analysis (Mahattanatawee and Rouseff, 2011). The headspace methods include only the volatile compounds.

Table 2. Important aroma compounds with odour activity values[a] (OAV's) above 0.1 and flavor dilution factors[a] (FD factors) above 8 and their odour threshold (OT) values in water and their odour descriptions. The origin of the volatile compound is shown: (F) from the fermentative activity of yeast, (L) from lipid degradation, and (M) from Maillard reaction. The compounds are arranged first according to their chemical group, then according to their OAV and then according to their FD factor.

Chemical group	Volatile compound	Origin	OAV	FD factor	OT in water (µg/kg)	Odour[c,d,e,f]
Aldehydes	(E)-2-Nonenal	L	40-65[f]	256 and 128[d], 512 and 512[c]	0.08	Green, tallowy
	3-Methylbutanal	F, M	25-54[f] and 56-236[e]		0.2	Malty
	Nonanal	L	15-20[f] and 18-34[e]		1	Citrus
	Hexanal	L	12-20[f] and 49-87[e]	16[c]	4.5	Green
	Phenylacetaldehyde	F, M	8-13[f] and 6-35[e]	<8 and 16[d]	4	Honey-like
	Heptanal	L	5-13[f] and 14-26[e]		3	Fatty, rancid
	Octanal	L	4-7[f] and 19-30[e]		0.7	Citrus
	Decanal	L	2-4[f] and 8-21[e]		2	Citrus
	Benzaldehyde	F, L	0-0.2[f] and 0.1-0.2[e]		350	Almond
	(E, E)-2,4-Decadienal	L		512 and 512[c]	0.07	Fatty, waxy
	Trans-4,5-Epoxy-(E)-2-decenal	L		512 and 512[c]	0.02	Metallic
	(Z)-2-Nonenal	L		128 and 128[c]	0.02	Green
	Methional	L		16 and 64[d], 64 and 128[c]	0.04	Potato-like
	2,4-Decadienal	L		64 and 64[c]	0.1	Fatty, green
	(E, E)-2,4-Nonadienal	L		32 and 128[c]	0.06	Fatty, waxy
	4,5-Epoxy-(E)-2-decenal	L		<8 and 32[d]		Metallic
	(Z)-4-Heptenal	L		16[c]	0.06	Biscuit-like
	(E)-2-Octenal	L		16[c]	3	Fatty, nutty
	Vanillin			<8 and 16[d]	22	Vanilla-like
Alcohols	3-Methyl-1-butanol	F	17-25[f] and 25-35[e], 18 and 10[d]	128 and 32[d], 32 and 256[c]	250	Balsamic, alcoholic
	1-Octen-3-ol	L	9-13[f]		1	Mushroom
	1-Heptanol	L	2-3[f] and 8-14[e]		3	Green
	2-Methyl-1-propanol	F	0.1-0.3[f] and 0.2-0.8[e]		3200	Glue, alcohol
	2-Phenylethanol	F	0.2-0.4[f] and 0-0.1[e], 12 and 3[d]	512 and 128[d], 4 and 256[c]	1100	Flowery
	1-Propanol	F	0.1-2[e]		6600	Fruity, plastic
Ketones	2,3-Butanedione	F, M	9-33[f] and 19-103[e]	8 and 16[d], 64 and 128[c]	6.5	Buttery, caramel
	6-Methyl-5-hepten-2-one		2-5[e]		50	Herbaceous, green
	3-Hydroxy-2-butanone	F	0.8-1.5[e]		800	Butterscotch
	1-Octen-3-one	L		<8 and 64[d], 128 and 128[c]	0.01	Mushroom-like
	2-Octanone		0.1-0.2[f]		50	
	(Z)-1,5-Octadien-3-one			16[c]	0.0004	Green, geranium
Acids	3-Methylbutanoic acid	F, L, M	5.5 and 15[d]	<8 and 32[d]	120	Rancid, Sweaty
	Acetic acid	F, M		64 and 128[d]	32300	Acidic, pungent
	Butanoic acid	F, L	<1[d]	<8 and 32[d]	240	Rancid, Sweaty
Esters	Ethyl 3-methylbutanoate	F	1.5-11[f]		0.2	Fruity, apple
	Ethyl hexanoate	F	0.2-0.5[f]		1	Fruity, juicy
	Ethyl octanoate	F	0-0.1[e], 19 and 15[d]	512 and 128[d]	92	Sweet, soap, fresh

Chemical group	Volatile compound	Origin	OAV	FD factor	OT in water (µg/kg)	Odour[c,d,e,f]
	Ethyl acetate	F	0-0.1[e]		6200	Sweet, fruity
	Ethyl-2-methyl propanoate	F		16[c]	4.5	Fruity
	Ethyl nonanoate	F		16 and <8[d]		Fruity
Furans	2-Pentylfuran	F, L, M	1-3[f,e]		6	Floral, fruity
	4-Hydroxy-2,5-dimethyl-3(2H)-furanone	M		8 and 64[d]	40	Caramel-like
Phenol	4-Vinyl-2-methoxyphenol	M		16 and 64[d]	3	Spicy
Pyrroline	2-Acetyl-1-pyrroline	M		8[d] and 32[c]	0.053	Roasted
Pyrazine	2-Methoxy-3-isopropylpyrazine	M		16 and 32[c]		Roasty

[a]OAV is defined as the ratio of the volatile compound concentration to the odour threshold in water. [b]FD factor is defined as the ratio of the concentration of the odorant in the initial extract to its concentration in the most dilute extract in which the odour is still detectable by gas chromatography olfactometry. [c]Schieberle and Grosch (1991). [d]Gassenmeier and Schieberle (1995). [e]Birch et al. (2013a). [f]Birch et al. (2013b).

REACTION PATHWAYS FOR AROMA FORMATION IN BREAD

Bread Volatile Compounds Formed by Yeast Activity

Many of the aroma active compounds identified in the crumb of fermented bread are derived from the metabolism of yeast and the dominating compounds are alcohols, aldehydes as well as 2,3-butanedione (diacetyl), 3-hydroxy-2-butanone (acetoin), esters and acids (**Table 2**).

Aldehydes and alcohols. The most aroma-active fermentation aldehydes and alcohols identified in bread crumb are 3-methylbutanal, phenylacetaldehyde, 3-methyl-1-butanol and 2-phenylethanol (**Table 2**). Furthermore, ethanol, the primary product of yeast fermentation together with carbon dioxide, is formed in high concentrations during dough fermentation. The vast majority of the ethanol has however evaporated during baking, as ethanol is a small polar molecule and the concentration in the bread is therefore low. The fermentation aldehydes and their corresponding alcohols are formed inside the yeast cell from degradation of the flour amino acids (**Figure 1**) via the Ehrlich pathway as exemplified for leucine in **Figure 2** (Ehrlich, 1907; Berry and Watson, 1987; Dickinson et al., 2003; Perpète et al., 2006; Hazelwood et al., 2008).

AMINO ACID		ALDEHYDE		ALCOHOL
Valine	→	2-Methylpropanal	→	2-Methyl-1-propanol
Leucine	→	3-Methylbutanal	→	3-Methyl-1-butanol
Isoleucine	→	2-Methylbutanal	→	2-Methylbutanol
Methionine	→	Methional	→	Methionol and Methanthiol
Phenylalanine	→	Phenylacetaldehyde and Benzaldehyde	→	2-Phenylethanol Phenylmethanol

Figure 1. Overview of the aldehydes and alcohols formed during dough fermentation from flour amino acids via the Ehrlich pathway in the yeast cell.

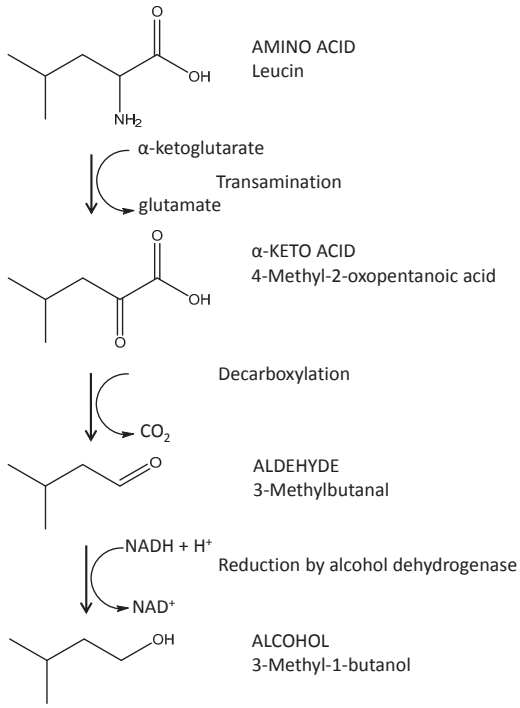

Figure 2. Overview of the formation of 3-methylbutanal and 3-methyl-1-butanol from leucine in the Ehrlich pathway inside the yeast cell.

Acids. The three acids, acetic acid, butanoic acid and 3-methylbutanoic acid can most likely be sensed in bread crumb, because of their high FD factors (**Table 2**). The odour characteristics are acetic, pungent, rancid and sweaty (**Table 2**). Some carboxylic acids (e.g. acetic acid, butanoic acid and hexanoic acid) identified in bread (**Table 1**) are produced in the fatty acid synthase pathway in the yeast cell, with yeast malonyl CoA as the substrate for the fatty acid synthase (Berry and Watson, 1987). Another route is the amino acid biosynthetic pathway, where aldehydes formed during the Ehrlich pathway can be oxidized to their corresponding acids (e.g. 2-methylpropanoic acid and 3-methylbutanoic acid) by aldehyde dehydrogenase (Berry and Watson, 1987).

Esters. The most aroma-active esters in bread crumb were ethyl 3-methylbutanoate, ethyl hexanoate, ethyl octanoate, ethyl acetate and ethyl nonanoate (**Table 2**). Formation of esters in bread crumb is particularly interesting, since they are often characterized as having pleasant and fruity odours (**Table 2**). However, the esters are generally found to have low OAV's and FD factors compared to the other classes of compounds found in bread crumb (**Table 2**), as particularly the small esters (ethyl acetate and ethyl 3-methylbutanoate) are very volatile and evaporates during baking.

The esters identified in bread crumb is produced in the yeast cell by an enzymatic reaction catalyzed by transferases between acetyl coenzym A (acetyl-CoA) derivatives of short to medium chain fatty acids and alcohols (mainly ethanol) as exemplified for ethyl hexanoate in **Figure 3** (Lilly et al., 2000). The reaction requires energy, which is provided by the thioester linkage of the acyl CoA co-substrate (**Figure 3**) (Mason and Dufour, 2000). The majority of acetyl-CoA is formed by the oxidative decarboxylation of pyruvate (Mason and Dufour, 2000). The fatty acids are produced by the fatty acid metabolism in the yeast cell, and it is suggested that ester production is functioning as a way to remove the toxic fatty acids from the yeast cell (Nordstrom, 1964; Lilly et al., 2006). The alcohol acetyltransferase (AAT) encoded with acyltransferase 1 (ATF1) is the yeast enzyme mainly responsible for the syntheses of acetate esters, such as ethyl acetate, 3-methylbutyl acetate (isoamylacetate), hexyl acetate and 2-phenylethyl acetate, which are the major esters produced during dough fermentation (Lilly et al., 2006). Formation of ethyl hexanoate, ethyl octanoate and ethyl decanoate have on the other hand been demonstrated to require activity of the EHT1 encoded ethanol hexanoyl transferase (**Figure 3**) (Lilly et al., 2006).

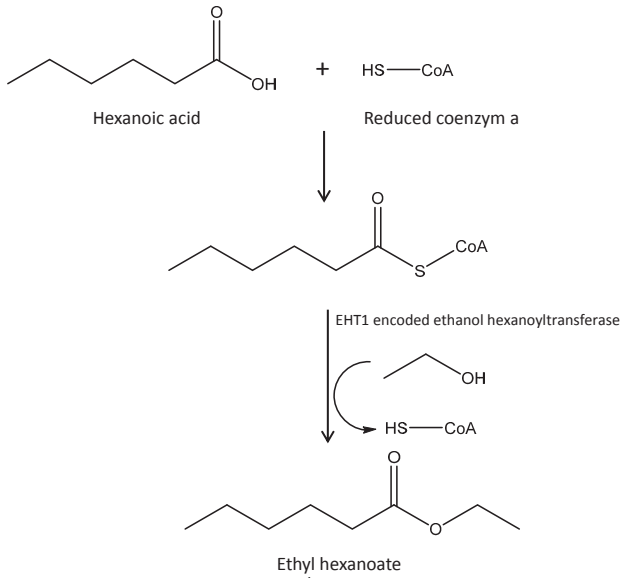

Figure 3. Overview of the formation of ethyl hexanoate through an enzymatic reaction inside the yeast cell (modified from Park et al. (2009)).

2,3-Butanedione and 3-hydroxy-2-butanone. 2,3-Butanedione (diacetyl) is an important aroma compound in bread crumb with high OAV's, low odour threshold value (6.5 µg/kg) and with buttery and caramel odour (**Table 2**). The concentration of the ketone 3-hydroxy-2-butanone in bread crumb was higher than 2,3-butanedione, but due to the high odour threshold value of 3-hydroxy-2-butanone (800µg/kg) the compound was not found to be very aroma active (**Table 2**) (Birch et al., 2013a). The ketone 3-hydroxy-2-butanone can furthermore be enzymatically degraded to 2,3-butanediol (Ehsani et al., 2009).

The ketones 2,3-butanedione and 3-hydroxy-2-butanone are produced from α-acetolactate leaked from the yeast cell by a non-enzymatic reaction outside the yeast cell (**Figure 4**) (Wainwright, 1973; Duong et al., 2011). α-Acetolactate is produced in the yeast cell as an intermediate in the synthesis of valine and leucine in the biosynthetic pathway, which is essential for yeast growth (Wainwright, 1973; Bartowsky and Henschke, 2004) (**Figure 4**). 2,3-Butanedione can be reabsorbed to the yeast cell, where it can be reduced to 3-hydroxy-2-butanone by diacetyl reductase (Dulieu et al., 2000; Duong et al., 2011).

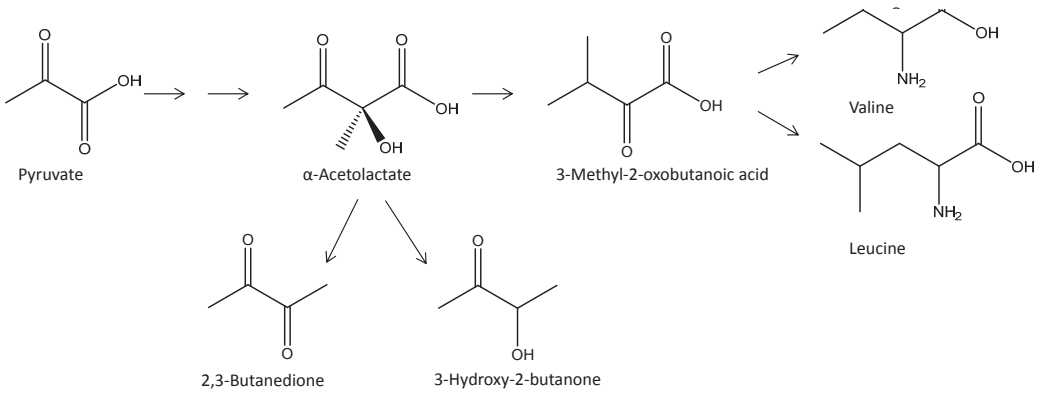

Figure 4. Biosynthetic origin of 2,3-butanedione and 3-hydroxy-2-butanone outside the yeast cell (modified from Wainwright (1973)).

Lactones. Lactones (e.g. γ-nonalactone) identified in wheat bread crumb (**Table 1**) can be formed from the yeast metabolism (Endrizzi et al., 1996; Garbe and Tressl, 2004). Free fatty acids can be catabolised to 4- or 5-hydroxy acids through the peroxisomal β-oxidation system. The 4- or 5-hydroxy acids can then be transformed to the very aroma active γ- and δ lactones, respectively by yeast (Endrizzi et al., 1996; Garbe and Tressl, 2004), through a pathway that is still largely unknown. Lactones are known to have a pleasant, aromatic and often fruity odour (Endrizzi et al., 1996).

Terpenes. The terpenes limonene, linalool, α-terpineol and α-phellandrene have been identified in bread crumb (**Table 1**). The terpenes might be produced from their corresponding glucosidic form by β-glucosidase activity present in the cell walls of some yeast (Mateo and DiStefano, 1997; Sadoudi et al., 2012).

Bread Volatile Compounds Formed from Lipid Oxidation

Aldehydes. Degradation of unsaturated fatty acids plays an important role for the aroma profile of wheat bread crumb. Especially the aldehydes, with low odour threshold values, ((E)-2-nonenal, nonanal, hexanal, heptanal and octanal) account for a large part of the most important bread aroma compounds (**Table 2**). These aldehydes have green, tallowy, mushroom and citrus odours, and they are

often denoted as off-aroma compounds (Zehentbauer and Grosch, 1998).

The polyunsaturated fatty acids in wheat (mainly linoleic and linolenic acid) can undergo peroxidation processes primarily during grinding of the wheat grain, but also during mixing of the dough as long as oxygen is present in the dough (**Figure 5**) (Maraschin et al., 2008). The peroxidation can be an autoxidation process (Frankel, 1980) or an enzymatic process catalysed by lipoxygenase (Drapron and Beaux, 1969). The primary products from both processes are hydroperoxides (**Figure 5**), which are not aroma active. The hydroperoxides produced are unstable and they quickly break down to free radicals, which decompose into several secondary peroxide products. The peroxide products are further degraded into a wide range of volatile compounds during dough mixing, fermentation and baking, with aldehydes (e.g. (E)-2-nonenal and hexanal) as the most aroma active compounds in bread crumb (Frankel, 1980; Ullrich and Grosch, 1987) (**Figure 5**). The hydroperoxides can also be converted to aldehydes by hydroperoxide lyases (Drapron and Beaux, 1969; Gardner, 1996; Gargouri et al., 2004) (**Figure 5**). The two enzyme classes, lipoxygenases and hydroperoxide lyases, are naturally present in wheat endosperm, though the activity is many times higher in the wheat germ and bran (Rani et al., 2001; Every et al., 2006; Lin et al., 2010).

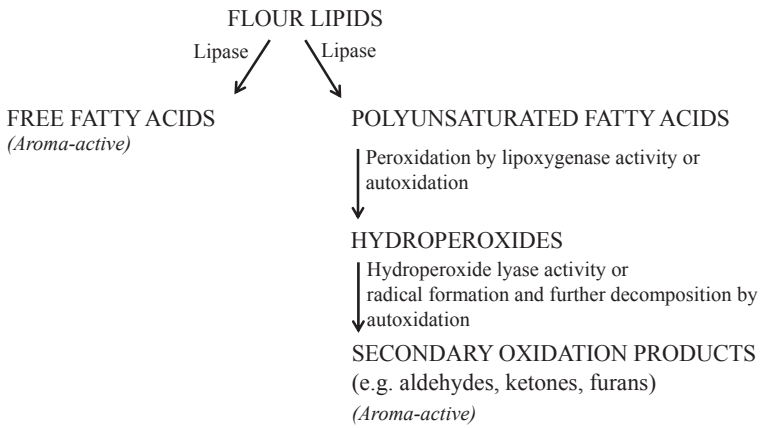

Figure 5. Simplified overview of the volatile compounds formed from degradation of flour lipids.

Maraschin et al. (2008) found that the level of free fatty acids (inclusive polyunsaturated fatty acids) in flour increased and the lipoxygenase activity in flour decreased with higher flour storage temperature (from 22 to 45°C) and longer storage time (from 0 to 16 weeks) as well as with higher water content in the flour (from 10 to 14% of the flour). Furthermore, Delcros et al. (1998) found that mixing markedly decrease the lipoxygenase activity, as two thirds of the activity was lost during the first five minutes of mixing by denaturation of the enzyme.

The volatile compounds formed from peroxidation and further degradation of linoleic acid and linolenic acid were thoroughly investigated by Drapron and Beaux (1969), Frankel (1982), and Ullrich and Grosch (1987), where a more detailed description of the complex reactions can be found.

Furans. Several furans have been identified in wheat bread crumb (**Table 2**). The most aroma active furan in bread crumb is 2-pentylfuran with floral and fruity odour (**Table 2**). 2-Pentylfuran is formed during baking from (E)-2-nonenal, the lipid oxidation product of linoleic acid (Adams et al., 2011).

Alcohols. The majority of the alcohols identified in bread crumb, which are not formed from yeast fermentation (**Table 1**) are formed by reduction of aldehydes from the lipid oxidation by alcohol dehydrogenase (Drapron and Beaux, 1969; Gardner, 1996). Of these alcohols, 1-heptanol (green odour) was the most aroma active identified in bread crumb (**Table 2**).

Bread Volatile Compounds Formed in the Bread Crust

Crust aroma compounds might diffuse into the crumb and hence contribute to the overall aroma profile of bread crumb, therefore the most aroma-active compounds identified in bread crust are presented in this section. The aroma compounds in bread crust has generally attained more focus than the compounds in bread crumb, and the pathways forming the various volatile compounds in bread crust have been widely reviewed in several papers (Pozo-Bayón et al., 2006; Purlis, 2010; Prost et al., 2012).

Aroma-active crust compounds. Schieberle and Grosch (1994) and Grosch and Schieberle (1997) found the most aroma active compounds in bread crust to be 2-acetyl-1-pyrroline, 3-methylbutanal, methional, (E)-2-nonenal, acetic acid, (E,E)-2,4-decadienal, 2-ethyl-3,5-dimethylpyrazine, and 4-hydroxy-2,5-dimethyl-3(2H)-furanone.

Maillard reactions. Volatile compounds, not associated with yeast fermentation or lipid oxidation, such as pyrazines and pyrroles, were identified in the bread crumb (**Table 1**). The majority of these compounds were found in high concentrations in bread crust (Schieberle and Grosch, 1994; Poinot et al., 2010) and they are formed by the heat induced complex Maillard reactions on the dough surface during baking (Hodge, 1953). Since those typical crust compounds are identified in the crumb (**Table 1**), they might have diffused into the crumb.

The brown colour and the typical roasted aroma of bread crust are correlated to both caramelisation and Maillard reactions occurring during baking. Caramelisation of the sugars initiates at temperatures above 120-150°C (Kroh, 1994), and it occurs simultaneously with the Maillard reactions (Wiggins, 1998), however it is widely documented that the overall aroma profile of bread crust mainly origins from Maillard reactions (Pozo-Bayón et al., 2006; Prost et al., 2012).

Hodge (1953) explained the overall reactions in the complex non-enzymatic browning reactions designated the Maillard reactions. Generally, the Maillard reaction is a reaction between carbonyls (most often reducing sugars) and a free amino group of amino acids, peptide or protein (Maillard,

1912). The major end products are melanoidins (brown nitrogenous polymers) and a high number of volatile compounds (Hodge, 1953). Maillard reactions typically require water activities around 0.4 to 0.7 and temperatures above 150°C (Wiggins, 1998; Purlis, 2010)..

The volatiles formed in highest content via the Maillard reaction can be divided into the following groups; furanones, pyranones, pyrroles, aldehydes (e.g. 3-methylbutanal, phenylacetaldehyde and 2-carboxaldehyde), ketones (e.g. 2,3-butanedione and 3-hydroxy-2-butanone) pyridines, pyrazines, oxazoles, thiazoles, pyrroles, and imidazoles (Nursten, 1981; Ravagli et al., 1999; Prost et al., 2012). The relative amount of those volatiles might be small, however they are very important for the overall aroma profile because of their generally low odour threshold.

Compounds formed in both crumb and crust. Some aldehydes (e.g. 3-methylbutanal, acetaldehyde and phenylacetaldehyde), some ketones (2,3-butanedione and 3-hydroxy-2-butanone) and some acids (e.g. acetic acid and 3-methylbutanoic acid) formed during yeast fermentation, can also be formed from the Maillard reaction (Hofmann et al., 2000; Prost et al., 2012).

EFFECT OF THE FERMENTATION CONDITIONS ON BREAD CRUMB AROMA

Effect of Yeast Level on Bread Crumb Aroma

Frasse et al. (1992) investigated the fermentative activity of baker's yeast on the presence of volatile compounds in bread crumb. The authors compared dough containing 25 g inactivated baker's yeast/kg flour with dough containing 25 g active baker's yeast/kg flour (Frasse et al., 1992). As expected the formation of the typical fermentation compounds (such as 3-methyl-1-butanol) was markedly higher in the dough containing the active yeast. The authors found a markedly higher concentration of the volatile compounds formed from the lipid oxidation, such as hexanal and heptanal, in the dough containing the inactivated yeast. The authors suggested that this was due to the substantial consumption of oxygen by the yeast during mixing, which reduces the efficacy of the lipoxygenase reaction in the dough containing the active yeast. Lysed yeast cells might also contribute to the explanation of these results found by Frasse et al. (1992).

Birch et al. (2013b) investigated the aroma profile of doughs fermented at three different yeast concentrations (20, 40 and 60 g/flour). The fermentations were stopped at a predefined equal dough height, therefore the fermentation times changed according to the yeast concentration. Dough fermented with the highest yeast concentration resulted in bread with a higher concentration of the majority of the compounds formed from the fermentative activity of yeast. 2,3-Butanedione and phenylacetaldehyde were the most aroma active of these fermentation compounds (Birch et al., 2013b). Yeast added in levels from 20 to 60 g/kg flour did not influence the formation of the compounds formed from degradation of unsaturated fatty acids (Birch et al., 2013b).

Gassenmeier and Schieberle (1995) investigated how two different yeast levels (15 and 46 g/kg flour) influenced formation of aroma compounds in wheat bread crumb. The author's results are based on wheat dough fermented by the use of pre-ferments (flour, water and yeast) and long fermentation times

(18 hours). The two yeast concentrations investigated is hence the yeast concentration in the final dough. The authors found the lowest content of 3-methyl-1-butanol and 2-phenylethanol and the highest content of phenylacetaldehyde in bread, where the final dough contained 46 g yeast/kg flour compared to 15 g yeast/kg flour (Gassenmeier and Schieberle, 1995). This result is not in agreement with the results from the study by Birch at al. (2013b). The different results are probably explained by the longer fermentation times and the use of pre-ferments in the research work of Gassenmeier and Schieberle (1995) compared to the conditions by Birch at al. (2013b).

Richard-Molard et al. (1979) investigated the effect of yeast level from 5 to 20 g/kg flour on formation of acetic acid in bread crumb, when the fermentation was done for 5h at 22°C. They found an increase in acetic acid from 123 to 195 mg/kg when the yeast level was increased from 5 to 15 g/kg flour. A yeast level above 15 g/kg flour did not further increase the level of acetic acid in bread crumb.

A high yeast concentration is used to decrease the fermentation time, which is an economical benefit for the bread industry. However, it is possible that non-volatile compounds from the yeast are unwanted from a sensory point of view. Very few research studies have investigated the influence of non-volatile compounds on bread flavour (Jensen et al., 2011; Bin et al., 2012). Research within identification and quantification of non-volatile compounds in bread could therefore be relevant in order to provide a more detailed flavour profile of bread fermented at different yeast levels. Analysis of non-volatile compounds could be done by high performance liquid chromatography (HPLC) analysis (Jensen et al., 2011; Jayaram et al., 2013) or by applying different derivatisation methods followed by GC-MS analysis (Proestos et al., 2006; Sha et al., 2010) in combination with sensory analysis.

Effect of Yeast Strain on Bread Crumb Aroma

Little attention has generally been paid towards the influence of the yeast strain on bread aroma, however research within alcoholic fermentation shows that the strain of yeast is very important for the aroma profile of the final fermented beverage (Procopio et al., 2011; Suárez-Lepe and Morata, 2012). Different commercial baker's yeasts may consist of different strains of *Saccharomyces cerevisiae*. Birch et al. (2013a) investigated the influence of fermentation by seven European commercial baker's yeasts on bread aroma. The seven baker's yeasts were added to the dough in different amounts to obtain equal number of yeast cells in the doughs. They found significantly different aroma profiles between bread fermented by seven commercial baker's yeasts. Fermentation by the two baker´s yeast from Belgian resulted in bread having a higher concentration of the typical fermentation compounds. However, two of the fermentation compounds (2-phenylethanol and phenylacetaldehyde) were found in a higher concentration in bread fermented with two organic baker´s yeast, hence the aroma formation during fermentation was different for some of the yeasts. The compounds formed from the lipid oxidation were also influenced by the type of yeast and particularly the breads fermented with the two organic yeasts were having a higher concentration of these oxidation compounds (e.g. hexanal). The authors suggested that the different aroma profiles were due to different yeast strains in some of the commercial baker's yeasts. The differences in the aroma profiles may therefore be due to differences in the gene-regulated mechanisms and biosynthetic pathways of aroma compound formation between

these different yeast strains during dough fermentation.

Additional research is needed to improve the understanding of aroma formation during dough fermentation with different yeast strains. Research within selection of yeast strains with improved aroma formation could be of industrial interest. Particularly selection of yeast strains with increased ester formation could be interesting. Studies have recently been carried out on alcohol acetyltransferases from *S. cerevisiae* and their influence on ester formation in alcoholic fermentation (Verstrepen et al., 2003; Procopio et al., 2011; Suárez-Lepe and Morata, 2012). The results from these studies might be successfully applied to dough fermentation in the search for novel ways of optimising the aroma of bread.

Further experiments with the use of non-*Saccharomyces* yeast strains producing high concentrations of esters in the dough could also be interesting in order to develop bread with a more fruity and appealing aroma. The choice of strain could be inspired from research studies within alcoholic fermentation, where strains of *Pichia anomala*, *Hanseniospora guillermondii* and *Hanseniospora osmophila* were found to significantly increase formation of some esters (Rojas et al., 2001; Rojas et al., 2003; Viana et al., 2008). Non-*Saccharomyces* yeasts are normally used in mixed cultures combined with *S. cerevisae* in alcoholic fermentation. Ciani et al. (2006) and Andorrá et al. (2012) found that pure cultures of some non-*Saccharomyces* wine yeasts (*Hanseniaspora uvarum*, *Torulaspora delbrueckii*, *Candida zemplinina* and *Kluyveromyces thermotolerans*) had reduced fermentation rate compared with pure cultures of *S. cerevisae*. Use of non-*Saccharomyces* yeast combined with *S. cerevisae* is therefore also assumed to be the best choice in future experiments within dough fermentation. Several non-saccharomyces yeast strains are common in natural sourdoughs (Hansen, 2012).

Effect of Fermentation Temperature on Bread Crumb Aroma

Gassenmeier and Schieberle (1995) found the significantly highest concentrations of 3-methyl-1-butanol and 2-phenylethanol in wheat bread when fermentations of liquid pre-ferments (flour, water and yeast) were done at 35ºC, compared to 25, 30 and 40ºC. Another study of the effect of fermentation temperature (5, 15 and 35°C) on the aroma profile of bread crumb found that a low fermentation temperature (5°C) increased formation of the three esters ethyl acetate, ethyl hexanoate, and ethyl octanoate (Birch et al., 2013b). Increasing formation of these esters by decreasing the fermentation temperature has also been found during wine fermentation (Molina et al., 2007; Beltran et al., 2008). Furthermore, 3-methylbutanal was found in a significant higher concentration in breads fermented at 15°C compared to breads fermented at 5 and 35°C (Birch et al., 2013b).

Fermentation temperatures of 15 and 35°C was found to increase formation of the majority of the compounds formed from degradation of unsaturated fatty acids compared to fermentation at 5°C (Birch et al., 2013b). It is well known that the rate of lipid oxidation increases at higher temperature. Many lipid oxidation compounds have low odour thresholds and are often characterised as off-aroma compounds with green odours (Zehentbauer and Grosch, 1998).

In future experiments sensory evaluation of bread fermented at different fermentation temperatures would be relevant in order to elucidate if the increased ester formation and decreased formation of lipid oxidation compounds by lowering the fermentation temperature to e.g. 5°C can actually be sensed in bread. Furthermore, it should be investigated, whether these breads are preferred over breads fermented at higher temperatures. It should be noted that bread fermentation at 5°C is problematic, as industrial bakeries do typically not have the facilities to store large amounts of dough for several hours. Furthermore, cooling is expensive and therefore dough fermentation at 5°C might only be relevant in production of "high quality" specialty breads.

Effect of Fermentation Time on Bread Crumb Aroma

Yeast level, fermentation temperature, and fermentation time are factors that are indistinguishably linked with each other and changes within yeast level or fermentation temperature will have a great impact on the fermentation time (Birch et al., 2013b). This is important to consider when investigating the effect of fermentation time on bread aroma. Maeda et al. (2009) investigated the influence of fermentation time (15min, 1h and 2h) on the aroma profile of bread crumb. The doughs were fermented by 20g yeast/kg flour at 27°C. The authors found that a 3h dough fermentation period resulted in the highest content of alcohols, esters and aromatic compounds in bread crumb, compared to fermentation for shorter periods. They found on the other hand that the levels of aldehydes, carboxylic acids, ketones and diketones were reduced during 3h fermentation, compared to the shorter fermentation times. The levels of alkenals, sulphuric and furanic compounds were not found to be influenced by fermentation times from 15min to 3h (Maeda et al., 2009). Schieberle and Grosch (1991) found that the concentration of the fermentation compounds 2,3-butanedione, 3-methyl-1-butanol and 2-phenylethanol increased by 2, 8, and 64 times, respectively, in long-time fermented bread (3h) compared to short-time fermented bread (time not stated). The doughs were fermented with 60g yeast/kg flour at 35°C. The result from Schieberle and Grosch (1991) is in agreement with Maeda et al. (2009), however not for 2,3-butanedione.

Richard-Molard et al. (1979) investigated the effect of fermentation time from 3 to 10h on formation of acetic acid in bread crumb. The fermentations were done with 20 g yeast/kg flour (temperature not stated in the paper). They found an increasing content of acetic acid from 30 to 75 mg/kg when the fermentation time was increased from 3 to 6 hrs, where after the concentration of acetic acid was stable. Acetic acid is described as sour and pungent (Gassenmeier and Schieberle, 1995), however it is also suggested to act as an aroma enhancer in the levels of 100-200 mg/kg (Richard-Molard et al., 1979).

Birch et al (2013b) found that the fermentation time for an optimally developed dough varied greatly from 15min to 21h when doughs were fermented at different fermentation temperatures (5, 15 and 35°C) and yeast levels (20, 40 and 60 g/kg flour). However, no direct correlation was found between fermentation time and formation of volatile compounds by their experimental design.

CONCLUSIONS

More than 150 compounds were identified in wheat bread crumb originating mainly from the fermentative activity of yeast, from oxidation of flour lipids and from Maillard reactions (in the crust). The most aroma-active compounds in wheat bread crumb were (E)-2-nonanal (green, tallow), 3-methyl-1-butanol (balsamic, alcoholic), 3-methylbutanal (malty), hexanal (green), 2,3-butanedione (buttery, caramel), nonanal (citrus), 1-octen-3-ol (mushroom), phenylacetaldehyde (honey-like), (E, E)-2,4-decadienal (fatty, waxy) and *trans*-4,5-epoxy-(E)-2-decenal (metallic).

Yeast level and strain, as well as fermentation temperature and time, are important factors significantly influencing bread crumb aroma. Yeast level and strain primarily influenced formation of compounds formed from the yeast activity and the yeast strain furthermore affected the level of hexanal in the bread crumb. A high fermentation temperature was found to increase the concentration of compounds formed from the lipid oxidation, whereas a low temperature was found to increase the concentration of some esters. Disagreeing results were found regarding the effect of fermentation temperature on formation of volatile compounds formed from yeast fermentation. A long fermentation time (3h) was found to increase formation of some compounds (such as 3-methyl-1-butanol and 2-phenylethanol) formed from the yeast activity, however contradictory results were found regarding the effect of fermentation time on formation of 2,3-butanedione.

Based on this review the conditions for the most optimal bread aroma might be to ferment the dough at a fermentation temperature of 5°C, as these conditions result in bread with a slightly fruity odour from the esters. However, it is recommended to include sensory analysis combined with analysis of non-volatile compounds in future research studies of bread crumb aroma in order to improve the understanding of the effect of the fermentation conditions on the overall flavour (taste and odour) of bread crumb.

Furthermore, research within the effect of yeast strains on bread aroma has only just been initiated and it is suggested that investigation of yeast strains with improved aroma formation, as e.g. increased ester formation, might have great potential for the bread industry. Additionally, the effect of mixed cultures of *S. cerevisiae* and non-*Saccharomyces* strains on bread aroma should be included in future studies of bread aroma.

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REFERENCES

Adams, A., Bouckaert, C., Van Lancker, F., De Meulenaer, B., De Kimpe, N., 2011. Amino Acid Catalysis of 2-Alkylfuran Formation from Lipid Oxidation-Derived alpha,beta-Unsaturated Aldehydes. Journal of Agricultural and Food Chemistry 59, 11058-11062.

Andorrá, I., Berradre, M., Mas, A., Esteve-Zarzoso, B., Guillamón, J. M., 2012. Effect of mixed culture fermentations on yeast populations and aroma profile. LWT - Food Science and Technology 49, 8-13.

Bartowsky, E. J., Henschke, P. A., 2004. The 'buttery' attribute of wine-diacetyl-desirability, spoilage and beyond. International Journal of Food Microbiology 96, 235-252.

Beltran, G., Novo, M., Guillamon, J. M., Mas, A., Rozes, N., 2008. Effect of fermentation temperature and culture media on the yeast lipid composition and wine volatile compounds. International Journal of Food Microbiology 121, 169-177.

Berry, D. R., Watson, D. C., 1987. Production of organoleptic compounds. In: Berry, D R, Russell, I, Stewart, G G (Eds.), Yeast biotechnology. Allen and Unwin, London, pp. 345-368.

Bianchi, F., Careri, M., Chiavaro, E., Musci, M., Vittadini, E., 2008. Gas chromatographic-mass spectrometric characterisation of the Italian protected designation of origin "Altamura" bread volatile profile. Food Chemistry 110, 787-793.

Bin, Q., Jiang, D. S., Cho, I. H., Peterson, D. G., 2012. Chemical markers for bitterness in wheat bread. Flavour and Fragrance Journal 27, 454-458.

Birch, A. N., Petersen, M. A., Arneborg, N., Hansen, Å. S., 2013a. Influence of commercial baker's yeasts on bread aroma profiles. Food Research International 52, 160-166.

Birch, A. N., Petersen, M. A., Hansen, Å. S., 2013b. The aroma profile of wheat bread crumb influenced by yeast concentration and fermentation temperature. LWT - Food Science and Technology 50, 480-488.

Chang, C.-Y., Seitz, L. M., Chambers, E., 1995. Volatile flavor components of breads made from hard red winter wheat and hard white winter wheat. Cereal Chemistry 72, 237-242.

Chiavaro, E., Vittadini, E., Musci, M., Bianchi, F., Curti, E., 2008. Shelf-life stability of artisanally and industrially produced durum wheat sourdough bread ("Altamura bread"). Lwt-Food Science and Technology 41, 58-70.

Ciani, M., Beco, L., Comitini, F., 2006. Fermentation behaviour and metabolic interactions of multistarter wine yeast fermentations. International Journal of Food Microbiology 108, 239-245.

Delcros, J. F., Rakotozafy, L., Boussard, A., Davidou, S., Porte, C., Potus, J., Nicolas, J., 1998. Effect of mixing conditions on the behavior of lipoxygenase, peroxidase, and catalase in wheat flour doughs. Cereal Chemistry 75, 85-93.

Dickinson, J. R., Eshantha, L., Salgado, J., Hewlins, M. J. E., 2003. The catabolism of amino acids to long chain and complex alcohols in *Saccharomyces cerevisiae*. Journal of Biological Chemistry 278, 8028-8034.

Drapron, R., Beaux, Y., 1969. Oxidation of Essential Fatty Acids by Lipoxygenase (E.C.1.13.1.13) in Panification and Formation of Volatile Compounds in Bread Aroma. Comptes Rendus Hebdomadaires des Seances de l Academie des Sciences Serie D 268, 2598.

Dulieu, C., Moll, M., Boudrant, J., Poncelet, D., 2000. Improved performances and control of beer fermentation using encapsulated alpha-acetolactate decarboxylase and modeling. Biotechnology Progress 16, 958-965.

Duong, C. T., Strack, L., Futschik, M., Katou, Y., Nakao, Y., Fujimura, T., Shirahige, K., Kodama, Y., Nevoigt, E., 2011. Identification of Sc-type ILV6 as a target to reduce diacetyl formation in lager brewers' yeast. Metabolic Engineering 13, 638-647.

Ehrlich, F., 1907. Über die Bedingungen der Fuselölbildung und über ihren Zusammenhang mit dem Eiweissaufbau der Hefe. Berichte der Deutschen Chemischen Gesellschaft 40, 1047.

Ehsani, M., Fernandez, M. R., Biosca, J. A., Julien, A., Dequin, S., 2009. Engineering of 2,3-Butanediol Dehydrogenase To Reduce Acetoin Formation by Glycerol-Overproducing, Low-Alcohol Saccharomyces cerevisiae. Applied and Environmental Microbiology 75, 3196-3205.

Endrizzi, A., Pagot, Y., LeClainche, A., Nicaud, J. M., Belin, J. M., 1996. Production of lactones and peroxisomal beta-oxidation in yeasts. Critical Reviews in Biotechnology 16, 301-329.

Every, D., Simmons, L. D., Ross, M. P., 2006. Distribution of redox enzymes in millstreams and relationships to chemical and baking properties of flour. Cereal Chemistry 83, 62-68.

Frankel, E. N., 1980. Lipid oxidation. Progress in Lipid Research 19, 1-22.

Frankel, E. N., 1982. Volatile Lipid Oxidation-Products. Journal of the American Oil Chemists Society 59, A269.

Frasse, P., Lambert, S., Levesque, C., Melcion, D., Richard-Molard, D., Chiron, H., 1992. The influence of fermentation on volatile compounds in french bread crumb. Food Science and Technology-Lebensmittel-Wissenschaft and Technologie 25, 66-70.

Garbe, L. A., Tressl, R., 2004. Metabolism of deuterated threo-dihydroxy fatty acids in *Saccharomyces cerevisiae*: Enantioselective formation and characterization of hydroxylactones and gamma-lactones. Helvetica Chimica Acta 87, 180-196.

Gardner, H. W., 1996. Lipoxygenase as a versatile biocatalyst. Journal of the American Oil Chemists Society 73, 1347-1357.

Gargouri, M., Ben Akacha, N., Legoy, M. D., 2004. Coupled hydroperoxide lyase and alcohol dehydrogenase for selective synthesis of aldehyde or alcohol. Applied Biochemistry and Biotechnology 119, 171-180.

Gassenmeier, K., Schieberle, P., 1995. Potent aromatic compounds in the crumb of wheat bread (French-type) - influence of pre-ferments and studies on the formation of key odorants during dough processing. Zeitschrift für Lebensmittel-Untersuchung und - Forschung 201, 241-248.

Grosch, W., 1993. Detection of potent odorants in foods by aroma extract dilution analysis. Trends in Food Science and Technology 4, 68-73.

Grosch, W., Schieberle, P., 1997. Flavor of cereal products - A review. Cereal Chemistry 74, 91-97.

Hansen, Å. S., 2012. Sourdough Bread. In: Hui, Y H (Ed.), Handbook of Plant-Based Fermented Food and Beverages. Tailor & Francis Books, Inc.,

Hazelwood, L. A., Daran, J. M., van Maris, A. J. A., Pronk, J. T., Dickinson, J. R., 2008. The ehrlich pathway for fusel alcohol production: a century of research on *Saccharomyces cerevisiae* metabolism. Applied and Environmental Microbiology 74, 2259-2266.

Hodge, J. E., 1953. Dehydrated foods - Chemistry of browning reactions in model systems. Journal of Agricultural and Food Chemistry 1, 928-943.

Hofmann, T., Münch, P., Schieberle, P., 2000. Quantitative model studies on the formation of aroma-active aldehydes and acids be Strecker-type reactions. Journal of Agricultural and Food Chemistry 48, 434-440.

Jacob, H. E., 1997. Six thousand years of bread. Its holy and unholy history, The Lyons Press,

Jayaram, V. B., Cuyvers, S., Lagrain, B., Verstrepen, K. J., Delcour, J. A., Courtin, C. M., 2013. Mapping of *Saccharomyces cerevisiae* metabolites in fermenting wheat straight-dough reveals succinic acid as pH-determining factor. Food Chemistry 136, 301-308.

Jensen, S., Oestdal, H., Skibsted, L. H., Larsen, E., Thybo, A. K., 2011. Chemical changes in wheat pan bread during storage and how it affects the sensory perception of aroma, flavour, and taste. Journal of Cereal Science 53, 259-268.

Kroh, L. W., 1994. Caramelisation in food and beverages. Food Chemistry 51, 373-379.

Lilly, M., Bauer, F. F., Lambrechts, M. G., Swiegers, J. H., Cozzolino, D., Pretorius, I. S., 2006. The effect of increased yeast alcohol acetyltransferase and esterase activity on the flavour profiles of wine and distillates. Yeast 23, 641-659.

Lilly, M., Lambrechts, M. G., Pretorius, I. S., 2000. Effect of increased yeast alcohol acetyltransferase activity on flavor profiles of wine and distillates. Applied and Environmental Microbiology 66, 744-753.

Lin, L. Y., Peng, C. C., Wu, T. H., Yu, T. H., Chen, C. C., Wang, H. E., Peng, R. Y., 2010. Flour Blending Based on Crude Fat Is More Crucial Than Ash Content in View of Prolonged Quality Assurrance. Journal of Food Processing and Preservation 34, 104-126.

Maeda, T., Kikuma, S., Araki, T., Ikeda, G., Takeya, K., Sagara, Y., 2009. The effects of mixing stage and fermentation time on the quantity of flavor compounds and sensory intensity of flavor in white bread. Food Science and Technology Research 15, 117-126.

Mahattanatawee, K., Rouseff, R., 2011. Gas Chromatography/Olfactometry (GC/O). In: Goodner, K, Rouseff, R (Eds.), Practical Analysis of Flavor and Fragrance Materials. Blackwell Publishing Ltd, pp. 69-90.

Maillard, L. C., 1912. The action of amino acids on sugar; The formation of melanoidin by a methodic route. Comptes Rendus Hebdomadaires des Seances de l Academie des Sciences 154, 66-68.

Maraschin, C., Robert, H., Boussard, A., Potus, J., Baret, J. L., Nicolas, J., 2008. Effect of storage temperature and flour water content on lipids, lipoxygenase activity, and oxygen uptake during dough mixing. Cereal Chemistry 85, 372-378.

Mason, A. B., Dufour, J. P., 2000. Alcohol acetyltransferases and the significance of ester synthesis in yeast. Yeast 16, 1287-1298.

Mateo, J. J., DiStefano, R., 1997. Description of the beta-glucosidase activity of wine yeasts. Food Microbiology 14, 583-591.

Molina, A. M., Swiegers, J. H., Varela, C., Pretorius, I. S., Agosin, E., 2007. Influence of wine fermentation temperature on the synthesis of yeast-derived volatile aroma compounds. Applied Microbiology and Biotechnology 77, 675-687.

Nordstrom, K., 1964. Studies on the Formation of Volatile Esters in Fermentations with Brewer´s Yeast. Svensk Kemisk Tidsskrift 76, 510-543.

Nursten, H. E., 1981. Recent developments in studies of the maillard reaction. Food Chemistry 6, 263-277.

Park, Y. C., Shaffer, C. E. H., Bennett, G. N., 2009. Microbial formation of esters. Applied Microbiology and Biotechnology 85, 13-25.

Perpète, P., Duthoit, O., De Maeyer, S., Imray, L., Lawton, A. I., Stavropoulos, K. E., Gitonga, V. W., Hewlins, M. J. E., Dickinson, J. R., 2006. Methionine catabolism in *Saccharomyces cerevisiae*. Federation of European Microbiological Societies (FEMS) Yeast Research 6, 48-56.

Poinot, P., Arvisenet, G., Grua-Priol, J., Fillonneau, C., Le-Bail, A., Prost, C., 2010. Influence of inulin on bread: Kinetics and physico-chemical indicators of the formation of volatile compounds during baking. Food Chemistry 119, 1474-1484.

Pozo-Bayón, M. A., Guichard, E., Cayot, N., 2006. Flavor control in baked cereal products. Food Reviews International 22, 335-379.

Procopio, S., Qian, F., Becker, T., 2011. Review Paper: Function and regulation of yeast genes involved in higher alcohol and ester metabolism during beverage fermentation. European Food Research and Technology 233, 721-729.

Proestos, C., Sereli, D., Komaitis, M., 2006. Determination of phenolic compounds in aromatic plants by RP-HPLC and GC-MS. Food Chemistry 95, 44-52.

Prost, C., Poinot, P., Rannou, C., Arvisenet, G., 2012. Bread aroma. In: Cauvain, S P (Ed.), Breadmaking: Improving quality. Woodhead Publishing ltd, Cambridge, pp. 523-561.

Purlis, E., 2010. Browning development in bakery products - A review. Journal of Food Engineering 99, 239-249.

Rani, K. U., Prasada Rao, U. J. S., Leelavathi, K., Haridas Rao, P., 2001. Distribution of Enzymes in Wheat Flour Mill Streams. Journal of Cereal Science 34, 233-242.

Ravagli, A., Boschin, G., Scaglioni, L., Arnoldi, A., 1999. Reinvestigation of the reaction between 2-furancarboxaldehyde and 4-hydroxy-5-methyl-3(2H)-furanone. Journal of Agricultural and Food Chemistry 47, 4962-4969.

Richard-Molard, D., Nago, M. C., Drapron, R., 1979. Influence of the bread making method on french bread flavor. Bakers Digest 53, 34-38.

Rojas, V., Gil, J. V., Piñaga, F., Manzanares, P., 2001. Studies on acetate ester production by non-Saccharomyces wine yeasts. International Journal of Food Microbiology 70, 283-289.

Rojas, V., Gil, J. V., Piñaga, F., Manzanares, P., 2003. Acetate ester formation in wine by mixed cultures in laboratory fermentations. International Journal of Food Microbiology 86, 181-188.

Ruiz, J. A., Quilez, J., Mestres, M., Guasch, J., 2003. Solid-Phase Microextraction Method for Headspace Analysis of Volatile Compounds in Bread Crumb. Cereal Chemistry 80, 255-259.

Sadoudi, M., Tourdot-Marechal, R., Rousseaux, S., Steyer, D., Gallardo-Chacon, J. J., Ballester, J., Vichi, S., Guerin-Schneider, R., Caixach, J., Alexandre, H., 2012. Yeast-yeast interactions revealed by aromatic profile analysis of Sauvignon Blanc wine fermented by single or co-culture of non-*Saccharomyces* and *Saccharomyces* yeasts. Food Microbiology 32, 243-253.

Schieberle, P., Grosch, W., 1987. Evaluation of the flavour of wheat and rye bread crusts by aroma extract dilution analysis. Zeitschrift für Lebensmittel-Untersuchung und - Forschung 185, 111-113.

Schieberle, P., Grosch, W., 1991. Potent odorants of the wheat bread crumb. Zeitschrift für Lebensmittel-Untersuchung und - Forschung 192, 130-135.

Schieberle, P., Grosch, W., 1994. Potent odorants of rye bread crust - differences from the crumb and from wheat bread crust. Zeitschrift für Lebensmittel-Untersuchung und - Forschung 198, 292-296.

Seitz, L. M., Chung, O. K., Rengarajan, R., 1998. Volatiles in selected commercial breads. Cereal Chemistry 75, 847-853.

Sha, Y. F., Meng, J. R., Zhang, Y. C., Deng, C. H., Wu, D., 2010. Determination of volatile organic acids in tobacco by single-drop microextraction with in-syringe derivatization followed by GC-MS. Journal of Separation Science 33, 212-217.

Suárez-Lepe, J. A., Morata, A., 2012. New trends in yeast selection for winemaking. Trends in Food Science and Technology 23, 39-50.

Ullrich, F., Grosch, W., 1987. Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. Zeitschrift für Lebensmittel-Untersuchung und -Forschung 184, 277-282.

Verstrepen, K. J., Van Laere, S. D. M., Vanderhaegen, B. M. P., Derdelinckx, G., Dufour, J. P., Pretorius, I. S., Winderickx, J., Thevelein, J. M., Delvaux, F. R., 2003. Expression levels of the yeast alcohol acetyltransferase genes ATF1, Lg-ATF1, and ATF2 control the formation of a broad range of volatile esters. Applied and Environmental Microbiology 69, 5228-5237.

Viana, F., Gil, J. V., Genovés, S., Vallés, S., Manzanares, P., 2008. Rational selection of non-Saccharomyces wine yeasts for mixed starters based on ester formation and enological traits. Food Microbiology 25, 778-785.

Wainwright, T., 1973. Diacetyl a review. Part I - Analytical and biochemical considerations: Part II - Brewing experience. Journal of the Institute of Brewing 79, 451-470.

Wiggins, C., 1998. Proving, baking and cooling. In: Cauvain, S P, Young, L S (Eds.), Technology of breadmaking. Blackie Academic and Professional, London, pp. 120-148.

Young, L. S., 2012. Applications of texture analysis to dough and bread. In: Cauvain, S P (Ed.), Breadmaking. Improving Quality. Woodhead Publishing Ltd., Cambridge, pp. 562-579.

Zehentbauer, G., Grosch, W., 1998. Crust aroma of baguettes II. Dependence of the concentrations of key odorants on yeast level and dough processing. Journal of Cereal Science 28, 93-96.

PAPER V

Birch, A. N., Hansen, Å. S and Petersen, M. A.

Multiple headspace extraction - an effective method to quantify aroma compounds in bread crumb.

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Multiple Headspace Extraction – an Effective Method to Quantify Aroma Compounds in Bread Crumb

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ABSTRACT

Quantification of aroma compounds in a solid food product as bread can be difficult and time-consuming. Multiple headspace extraction (MHE) was performed on wheat bread crumb by dynamic headspace sampling followed by gas chromatography mass spectrometry analysis. MHE was found to be a successful, relatively simple and reproducible method for quantification of aroma compounds in bread, since addition of standards directly into the solid bread sample is not necessary. Furthermore, two different purge conditions within dynamic headspace sampling were successfully used for quantification of volatiles with low and high breakthrough volumes.

1. INTRODUCTION

Quantification of the aroma compounds in solid food matrices as bread can be difficult, because uniform dispersion of internal standards is practically impossible. An effective quantification method "Multiple Headspace Extraction" (MHE) was developed by Kolb and Pospisil in 1977*[1]*, but the method has only rarely been applied within food science *[2]*. Extraction and further quantification of aroma compounds in food products are today primarily done with solid phase micro extraction (SPME), dynamic headspace extraction, static headspace extraction, Likens-Nickerson or stable isotope dilution assay (SIDA). However, many of these methods are time-consuming and/or require internal standards added directly to the food sample. The MHE method is particularly interesting when extracting aroma compounds from a solid or semi-solid matrix, since addition of standards directly into the food sample for quantification is not necessary *[3]*. MHE was originally developed for static headspace sampling. The purpose of this study has been to investigate the possibility of using MHE combined with dynamic headspace extraction and gas chromatography mass spectrometry (GC-MS) in order to quantify the aroma compounds in bread crumb.

2. MATERIALS AND METHODS

Bread making. 300 g of flour, 190 mL water (30°C), 4 g saccharose, 4 g NaCl and 12 g baker´s yeast (4% of the flour) were mixed and the dough was left for fermentation for 60 minutes at 15°C before baking for 31 minutes at 130°C.
MHE. Seven consecutive dynamic headspace extractions were performed from the same sample. Two different combinations of purge flow and time were used; 1) purging seven times with nitrogen (50 mL/min) for 5 minutes to extract the very volatile aroma compounds and 2) purging seven times with nitrogen (150 mL/min) for 60 minutes to extract the less volatile compounds. All extractions were performed in triplicates. To determine the relationship between peak areas and absolute amounts of compounds sampled on traps, standard series of pure aroma compound in heptane solution were analyzed for the identified aroma compounds (Table 1) in concentrations of 10, 100, 500 and 1000 µL/L. 2 µL of each solution was injected to Tenax-TA traps. Standard series were performed in triplicates.
Gas Chromatography Mass Spectrometry (GC-MS): Separation of aroma compounds was performed by GC-MS.
Data analysis: The principle of MHE is that stepwise headspace extractions of aroma compounds are performed, each successive extraction resulting in a lower area of the aroma compound. After an infinite number of headspace extractions all aroma compounds are extracted from the sample. In practice, the peak area corresponding to a complete dynamic headspace extraction is calculated assuming that the decline in peak area during MHE follows first order kinetics, i.e.: $\sum_{i=1}^{i=\infty} A_i = \frac{A_1}{1-e^{-q}}$, where A_i is the sum of all extractions from A_1 to A_∞, A_1 represents the area of an aroma compound in a the first extraction and q represents the slope of the regression curve of the natural logarithm to the peak area vs. number of extractions *[1]*. q is determined for each aroma compound by running a limited number of consecutive extractions of a selected sample, where after A_1 for each aroma compound can be used to calculate the area representing the total amount of aroma compound in other similar samples. Finally, areas can be converted into absolute amounts, using the standard curves obtained from pure standards injected directly on traps.

3. RESULTS

The MHE method for quantification of the identified aroma compounds was found to perform well with generally low standard deviations. The coefficient of variation (CV%) of q was from 2.2 to 13.3% and the CV% of the slope of the standard curves for the authentic pure standards was from 1.2 to 7.3%. A total of 39 aroma compounds identified in wheat bread crumb were quantified by MHE (Table 1).

Table 1: Concentrations of aroma compounds identified in bread crumb, their q values, Odor Activity Value's (OAV's) [a] and odor characteristics. Aroma compounds having an OAV of 0.1 or higher[b] are highlighted with bold.

Aroma compound	Concentration (µg/kg)	q[c]	OAV	Odor
1-propanol[d]	269 ± 24	-0.45	0.040	fruity, plastic
2-methyl-1-propanol[d]	**3160 ± 37**	-0.58	**0.99**	glue, alcohol
1-butanol[d]	28.0 ± 3.5	-0.46	0.060	medicinal
1-penten-3-ol[e]	6.36 ± 1.0	-0.98	0.020	paint-like, chemical
3-methyl-1-butanol[e]	**232000 ± 42000**	-0.47	**928**	balsamic, alcohol
3-methyl-3-buten-1-ol[e]	0.89 ± 0.26	-0.36	-	herbaceous
1-pentanol[e]	26.8 ± 4.0	-0.39	0.010	fruity
2-penten-1-ol[e]	3.34 ± 0.67	-0.29	-	mushroom
1-hexanol[e]	42.3 ± 6.6	-0.26	0.020	green grass
3-hexenol[e]	**1.28 ± 0.36**	-0.25	**0.10**	green
1-octen-3-ol[e]	**9.85 ± 2.2**	-0.29	**9.9**	mushroom
1-heptanol[e]	**8.08 ± 1.3**	-0.22	**2.7**	green
2-ethyl-1-hexanol[e]	6.80 ± 0.84	-0.16	0.000025	green, alcohol
1-octanol[e]	4.95 ± 1.4	-0.072	0.050	citrus
3-nonen-1-ol[e]	11.7 ± 1.2	-0.24	-	-
2-phenylethanol[e]	**812 ± 55**	-0.016	**0.74**	flowery
1-dodecanol[e]	5.64 ± 3.7	-0.59	-	-
3-methylbutanal[d]	**22.3 ± 2.4**	-0.13	**112**	malty
hexanal[e]	**88.5 ± 10**	-0.55	**20**	green
heptanal[e]	**34.9 ± 2.3**	-0.37	**12**	fatty, rancid
octanal[e]	**2.45 ± 0.93**	-0.21	3.5	citrus
nonanal[e]	**18.6 ± 6.7**	-0.24	**19**	citrus
2-furancarboxaldehyde[e]	7.34 ± 0.74	-0.36	0.00032	woody, almond
decanal[e]	**5.37 ± 3.2**	-0.26	**2.7**	citrus
benzaldehyde[e]	56.6 ± 5.3	-0.39	0.16	almond
2-nonenal[e]	**5.82 ± 0.67**	-0.34	**73**	beans, cucumber
phenylacetaldehyde[e]	**41.1 ± 8.**	-0.14	**10**	honey-like
2,3-butanedione[d]	**133 ± 29**	-0.058	**20**	buttery, caramel
2-heptanone[e]	3.36 ± 0.27	-0.56	0.020	fruity, sweet
3-hydroxy-2-butanone	nq		-	butterscotch
2-octanone[e]	4.15 ± 0.52	-0.32	0.080	-
3-octen-2-one[e]	1.82 ± 0.60	-0.44	-	rose
ethyl acetate[d]	147 ± 8.1	-0.22	0.020	sweet, fruity
ethyl-3-methyl butanoate[e]	**0.79 ± 0.19**	-0.45	**4.0**	fruity, apple
3-methylbutyl acetate[f]	nq	-0.75	-	banana
ethyl hexanoate[e]	**0.26 ± 0.044**	-0.80	**0.26**	fruity-juicy
ethyl octanoate[e]	0.70 ± 0.072	-0.35	0.010	sweet, soap, fresh
phenethyl acetate[e]	3.26 ± 0.56	-0.19	0.010	flowery
gamma-nonalactone[e]	1.33 ± 0.40	-0.13	0.010	sweet, creamy
2-pentylfuran[e]	**15.1 ± 2.7**	-0.45	**2.5**	floral, fruit
trimethylpyrazine[e]	0.83 ± 0.11	-0.11	0.0013	earthy

[a]OAV is the ratio of the concentration of aroma compound and the literature OT of the compound in water [4]. [b]An OAV of 0.1 or higher and not above 1 is chosen to limit the risk of missing aroma compounds, that can be detected in bread crumb, since the OAV's are based on OT in water and not in bread crumb. [c]q represents the slope of the regression curve of the natural logarithm to the peak area vs. number of extractions. [d]Quantified from the samples purged with nitrogen (50 mL/min) for 5 minutes. [e]Quantified from the samples purged with nitrogen (150 mL/min) for 60 minutes. [f]No standard available. nq, not quantitated.

3-Methyl-1-butanol, 3-methylbutanal, (E)-2-nonenal and 2,3-butanedione were found to be the sensorily most important aroma compounds identified in bread crumb based on their odour activity values (OAV's). OAV's were calculated as the ratio of each aroma compound concentration found in bread crumb to the literature odor threshold (OT) in water [4]. Bread is, however, a complex food matrix and therefore it should be underlined that an aqueous OT value of an aroma compound will only give an approximation of the

OAV in bread crumb. It would have been more appropriate to calculate the OAV´s from OT in starch or cellulose. They were however, not available from the literature for the majority of the compounds. To take the uncertainty of the calculated OAV's into account it was assumed that aroma compounds having an OAV of 0.1 or higher might be sensorily important compounds. This was the case for 18 compounds (highlighted with bold in Table 1).

DISCUSSION AND CONCLUSION

Dynamic headspace sampling with two different purge conditions resulted in successful quantification of aroma compounds even with very high volatility (and therefore low breakthrough volumes) e.g. 2,3-butanedione and 3-methylbutanal. MHE was originally developed for static headspace extraction, however this study showed that dynamic headspace sampling is also a well suited extraction method for quantification by MHE.

In conclusion MHE was found to be a relatively simple and reproducible method for quantification of aroma compounds in bread crumb. The great advantage is that it is possible to quantify the aroma compounds from a large sample set with similar texture, by performing MHE only on one sample (in triplicate). The method is anticipated to be well suited for quantification of aroma compounds from solid or semi-solid food products, since addition of standards directly into the food sample for quantification is not necessary.

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References

1. Kolb, B.; Pospisil, P. Chromatographia. 1977; *10*; 705-711
2. Soria, A. C.; Martinez-Castro, I.; Sanz, J. J of Chromatogr A. 2007**;** *1157*; 430-436
3. Kolb, B. Chromatographia. 1982; 15; 587-594
4. Birch, A.N; Hansen, Å.S.;Petersen, M.A.. LWT - Food Science and Technology, 50 480-488.

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AROMA OF WHEAT BREAD CRUMB
Effect of fermentation temperature and baker's yeast

Understanding how the dough fermentation conditions influence the wheat bread production time and the bread aroma is important for the bread industry. The overall purpose of this PhD project was to investigate the effects of commercial baker's yeast (level and type) and fermentation temperature on dough expansion and aroma in bread crumb.

In Paper I the effects of commercial baker's yeast (level and type) and fermentation temperature on dough expansion were investigated. Wheat doughs were fermented by seven commercial baker's yeasts at three yeast concentrations ($2.88 \cdot 10^{11}$, $5.76 \cdot 10^{11}$ and $8.64 \cdot 10^{11}$ CFU/kg flour) and four fermentation temperatures (5, 15, 25 and 35°C). Dough expansion was investigated by monitoring the dough height and the expansion profile was found to be described well by a first order kinetic model. The highest kinetic rate constants corresponding to the shortest fermentation times were found for doughs fermented at 25°C and the highest yeast concentration. Doughs fermented by four of the seven baker's yeasts had significantly shortest fermentation times. The longest fermentation times were generally found for doughs fermented with all baker's yeasts at 5°C and the lowest yeast concentration ($2.88 \cdot 10^{11}$ CFU/kg flour).

In Paper II, III and V wheat breads were produced for volatile analysis. The dough samples were fermented to equal height and baked and the volatile compounds from the bread crumb were extracted by dynamic headspace sampling and analysed by gas chromatography mass spectrometry. A wide range of volatile compounds was identified in bread crumb, mainly originating from the activity of yeast. The dominating fermentation compounds were alcohols, aldehydes as well as 2,3-butanedione (diacetyl), 3-hydroxy-2-butanone (acetoin), esters and acids. Furthermore, oxidation of flour lipids was generating primarily aldehydes and ketones.

In Paper II the effects of yeast level (20, 40 and 60 g baker's yeast/kg flour) and fermentation temperature (5, 15 and 35°C) on aroma in bread crumb were investigated. Fermentation with the highest yeast concentration (60 g/kg flour) resulted in bread containing the highest concentration of the majority of the compounds formed from yeast activity. A fermentation temperature at 5°C resulted in breads with the highest concentration of esters having fruity odours. Fermentation at 15 and 35°C resulted in breads with the highest concentration of many lipid oxidation compounds (sometimes characterised as off-odours).

In Paper III the effect of the type of commercial baker's yeast ($2.88 \cdot 10^{11}$ yeast CFU/kg flour) on bread aroma was investigated. Significantly different aroma profiles between bread fermented by the seven commercial baker's yeasts were found. The differences in the aroma profiles may be due to differences in the gene-regulated mechanisms and biosynthetic pathways of aroma compound formation between different yeast strains during dough fermentation.

Commercial baker's yeast (level and type) and fermentation temperature are concluded to significantly influence the fermentation time and the aroma profile of bread crumb. Sensory analysis and analysis of acids and non-volatile compounds in breads fermented at the same conditions as in this PhD thesis could be relevant in future research studies to elucidate the importance of the different chemical components for the flavour and taste of bread.

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