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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