Food Chemistry 174 (2015) 527–537

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Variation of volatile compounds among wheat varieties and landraces

G. Starr *, M.A. Petersen, B.M. Jespersen, Å.S. Hansen

Department of Food Science, Faculty of Science, University of Copenhagen, Rolighedsvej 30, DK1958 Frederiksberg C, Denmark

ARTICLE INFO

Article history:
Received 27 June 2014
Received in revised form 25 October 2014
Accepted 11 November 2014
Available online 18 November 2014

Chemical compounds studied in this article:
Ethyl acetate (PubChem CID: 8857)
2-Butylfuran (PubChem CID: 20534)
Hexanal (PubChem CID: 6184)
Limonene (PubChem CID: 22311)
Octan-2-one (PubChem CID: 8093)
2,3-Dimethylpyrazine (PubChem CID: 22201)
Hexanol (PubChem CID: 8103)
Non-2-enal (PubChem CID: 17166)
2-Methylbutanal (PubChem CID: 7284)
3-Methylbutanal (PubChem CID: 11552)

Keywords:
Wheat varieties
Landraces
Volatile compounds
Dynamic headspace extraction
Gas chromatography–mass spectrometry

ABSTRACT

Analysis of volatile compounds was performed on 81 wheat varieties and landraces, grown under controlled greenhouse conditions, in order to investigate the possibility of differentiating wheat varieties according to their volatile compound profiles. Volatile compounds from wheat samples were extracted by dynamic headspace extraction and analysed by gas chromatography–mass spectrometry. Seventy-two volatile compounds were identified in the wheat samples. Multivariate analysis of the data showed a large diversity in volatile profiles between samples. Differences occurred between samples from Austria compared to British, French and Danish varieties. Landraces were distinguishable from modern varieties and they were characterised by higher averaged peak areas for esters, alcohols, and some furans. Modern varieties were characterised by higher averaged peak areas for terpenes, pyrazines and straight-chained aldehydes. Differences in volatile profiles are demonstrated between wheat samples for the first time, based on variety. These results are significant to plant breeders and commercial users of wheat.

1. Introduction

High-yielding hexaploid wheat varieties are used in current agriculture for bread making, starch production and feed (Belderok, 2000). Adaptations occurred in the tetraploid wheat *Triticum dicoccoides* shortly after domestication resulting in free threshing forms and then an important hybridisation event between these tetraploid wheats and a diploid wild type *Aegilops tauschii*, led to hexaploid bread wheat (Reif et al., 2005). Subsequent haphazard selections of wheat and migrations made by early farmers saw the gradual adaptation of wheat landraces to various local regions and disparate climate zones. Early breeding attempts were conducted by selecting and crossing landraces from different regions (Lupton, 1987). With a greater understanding of Mendelian laws this gradually developed to breeding programs and selection for specific traits, the most important of which has been yield (Belderok, 2000). Little attention has been paid however to odour composition of wheat as a potential quality parameter with influence on the flavour on the corresponding bread. Only few investigations have dealt with variations in volatile profiles between wheat varieties and no studies have attempted to investigate what influence modern wheat breeding may have had on variation of volatile profiles in modern wheat varieties.

Early attempts to investigate wheat volatile composition were undertaken in five wheat samples by Hougen, Quilliam, and Curran (1971). They analysed the volatile headspace composition of four bread-wheat varieties, Manitou, Selkirk, Neepawa and Pembina, all of which had similar pedigrees and the durum wheat Mindum. Of these samples only two: Manitou and Selkirk, were shown to have a similar quantitative composition, while Neepawa, Pembina and Mindum were quantitatively different from each other. Compounds found in the samples which had the same retention time were tentatively regarded as being the same compound.

Chang, Seitz, and Chambers (1995) used a dynamic headspace extraction (DHE) method on bread samples. They identified 74

* Corresponding author. Tel.: +45 23 98 64 16.
E-mail address: starr@life.ku.dk (G. Starr).

http://dx.doi.org/10.1016/j.foodchem.2014.11.077
0308-8146/

compounds and although they found only few differences between their samples they recommended the use of a dynamic headspace method for the extraction of bread flavour as they maintained that this took place in a manner that closely mimics the perception range of the human nose. This method is therefore suitable for gathering information on which volatile compounds can be detected in a food matrix and which are likely to have an influence on its flavour and odour qualities.

Wheat varieties evaluated as cooked grain can be differentiated by a trained sensory panel due to their odour and taste differences (Starr, Bredie, & Hansen, 2013). Differences in some odour and flavour descriptors correlate with darkening grain colour. Liu, Qiu, and Beta (2010) found that there was a link between darker grain colour and higher flavonoid content. This suggests that variation of aroma and flavour in wheat may be a complex interaction between the composition of volatile and non-volatile compounds. These findings merit further investigation using instrumental analysis in order to identify putative variation in the volatile profiles of bread wheat varieties.

The objective of this work is to investigate the content of volatile components of wheat varieties (*Triticum aestivum* ssp. *aestivum*) grown under the same conditions to determine if separation of these varieties can be observed due to their volatile component profiles. This was done by screening 81 varieties and landraces of wheat. Any knowledge acquired of varietal differences among wheat varieties could be directly applicable to future plant breeding efforts. In the short term it could also benefit the food industry where selection of a wheat variety is required. It can also provide a fundament for further research into this area of study.

2. Materials and methods

2.1. Sample material

Eighty-one wheat varieties and landraces were cultivated at the University of Copenhagen research greenhouses at Højbakkegård, Tåstrup, Denmark from autumn 2010 to summer 2011 (Table 1). The wheat varieties and landraces which were screened mostly originated from Germany, Sweden, Denmark, France, Austria and the U.K. However some samples from Australasia, New Zealand, Switzerland, the Netherlands and the U.S. were also represented. The winter wheat samples were first vernalized for 7 weeks at 5 °C and then cultivated for 20 weeks at 20 °C. Artificial lighting was used during the cultivation period to ensure that the samples received 16 h of light per 24 h cycle. The growth substrate used was '*Pindstrup færdigblanding 1*', (Pindstrup A/S, DK), containing 0.650 kg/m^3 NPK fertilizer and 50 g/m^3 micronutrients and the particle size was between 0 and 35 mm. No extra fertiliser and no pesticides were added.

Moisture contents of the grain samples were measured, prior to storage, on a HoH-Express (Pfeuffer GmbH, Kitzingen, Germany). They varied from 12.6% (Wenzel) to 14.7% (Piccollo). The samples were screened for indications of fungal contamination by smell. Each wheat variety and landrace was stored, as grain, in individual paper bags at 5 °C until milling.

2.2. Sample milling

The grain samples were visually assessed against a white paper background and visible impurities were eliminated prior to milling. Milling of the wheat samples was done on a Brabender Quadrumat Junior Mill (Brabender, Duisberg, Germany). The mill was opened and cleaned with a vacuum cleaner between each sample milling. Approximately 20 g grain from the next sample were then milled and the wholemeal from this was discarded, in order to make sure that no residue remained in the mill from the previous sample. Then 250 g sample grain (where available) was milled for each wheat variety or landrace. The particle size of the wheat wholemeal was determined using a jel 200 oscillating sieve series (J. Engelsmann AG, Ludvigshafen, Germany) with different mesh widths. The sieved wholemeal fractions were as follows: less than 75 μm (27%), between 75 μm and 160 μm (35%), between 160 μm and 250 μm (18%), between 250 μm and 500 μm (8%), between 500 μm and 1000 μm (9%) and particle size larger than 1000 μm (3%).

The wheat wholemeal was stored overnight at 5 °C in light-excluding sachets of aluminium foil to minimise odour contamination and light-induced enzymatic reactions. Six wheat samples including replicates were milled and sampled on each sampling day. No more than 18 h elapsed between milling and DHE in order to avoid lipid oxidation of the wheat wholemeal sample.

2.3. Dynamic headspace extraction (DHE)

All analyses were carried out in triplicate or duplicate, depending on the quantity of sample material which was available for a total of 209 samples representing 81 wheat varieties and landraces. The number of sample replicates, for each variety are noted in Table 1. On each sampling day 6 wheat variety samples including replicates and two external standards were purged and extracted making a maximum total of 20 samples per sampling day.

A dry wheat wholemeal per sample of 25 g was placed in a 500-mL purge flask (7.5 cm diameter) together with a magnet for agitation of the wholemeal during volatile extraction. Volatile compounds were collected on Tenax-TA traps. The traps contained 250 mg of Tenax-TA with mesh size 60/80 and a density of 0.37 g mL^{-1} (Buchem bv, Apeldoorn, The Netherlands). The samples were equilibrated to 40 °C ± 1 °C in a circulating water bath and then purged with nitrogen (150 mL min^{-1}) for 40 min. The Tenax-TA trap was then removed from the purge head and dry purged directly with nitrogen gas (50 mL/min) for 10 min to remove excess water from the trap.

The Tenax-TA traps were them capped at each end and stored at 5 °C for a maximum of 3 days before analysis by gas chromatography–mass spectrometry (GC–MS). Duplicate samples of 1 mL 4-methyl-1-pentanol (50 mg L^{-1}) were used as an external standard, one at the beginning of the sampling session and one at the end. They were also sampled on each sampling day and the absolute areas of these samples were compared as a quality control measure in order to control for any daily drift of the system.

2.4. Gas chromatography–mass spectrometry

The trapped volatiles were desorbed using an automatic thermal desorption unit (ATD 400, Perkin Elmer, Norwalk, CT). Primary desorption was carried out by heating the trap to 250 °C with a flow (60 mL min^{-1}) of carrier gas (H_2) for 15.0 min. The stripped volatiles were 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 a gas chromatograph-mass spectrometer (GC–MS, 7890A GC-system interfaced with a 5975C VL MSD with triple-axis detector from Agilent Technologies, Palo Alto, CA) through a heated (225 °C) transfer line.

Separation of volatiles was carried out on a DB-Wax capillary column (30 m long × 0.25 mm internal diameter, 0.50 μm film thickness). The column pressure was held constant at 16,547 Pa resulting in an initial flow rate of approximately 1.4 mL min^{-1} using hydrogen as carrier gas. The column temperature program was: 10 min at 30 °C, from 30 °C to 240 °C at 8 °C min^{-1}, and finally

Table 1
Wheat varieties and landraces used for analysis of volatile compounds.

Wheat sample name	Vernality	Origin	Wheat quality[a]	Released	Remark	Replicates
Alter Deutscher, Unbegrannt, Rot	Winter	DE		Landrace		2
Amaretto	Spring	DE	A	2002		2
Anja	Winter	DK	DK bread list	1980		2
Apache	Winter	FR	BPS	1998		2
Asketis	Winter	DE	A	1998		3
Balaton	Winter	AT	3	2008		3
Barbinger Braun	Winter	DE		Landrace		3
Barbinger Weiß	Winter	DE		Landrace		3
Blasius	Winter	AT	7	2007		3
Caribo	Winter	DE	B	1968		2
Carman Begrannt Rot	Winter	DE		Landrace		3
Charcoal	Winter	US		1977	Purple wheat	3
Complet	Winter	DE	A	1996		3
Dacke	Spring	SE		1990	Organic farming variety	2
Deben	Winter	UK	Nabim grp 3	2000		2
Ellvis	Winter	DE	A	2002		3
Folke	Winter	SE		1981		2
Format	Winter	DE	A	1959		3
Goldblume	Winter	DE	High, soft gluten	1992	Bred from Landrace	2
Goltlandsk Børst	Winter	SE		Landrace		2
Granny	Spring	DE	A	2004		2
Greif	Winter	DE	B	1989		2
Hereford	Winter	DK	B	2007		3
Holger	Winter	SE		1981		3
Igel Unbehaart Rot	Winter	DE		Landrace		2
Indigo	Spring	UK		2000	Purple wheat	3
Inspiration	Winter	DE	B	2007		3
Isengrain	Winter	FR	BPS	1996		3
Jenga	Winter	DE	A	2007		3
Klädener Altmärker Braunweizen	Winter	DE		Landrace		2
Komarom	Winter	AT	A2	2008		3
Konini	Spring	NZ		1981	Purple wheat	3
Kranich	Winter	DE	A	2005		3
Leipziger Begrannt Rot	Winter	DE		Landrace		3
Ludwig	Winter	AT	A	1997		3
Lukullus	Winter	AT	6	2008		3
Luteus	Winter	DE		2000	Yellow wheat	3
Magnifik	Winter	SE	SE bread list	2004		3
Mauerner Unbegrannter Brauner	Winter	DE		Landrace		2
Melbor	Winter	FR		1886		2
Mulan	Winter	DE	B	2006		3
Naxos	Spring	DE	A	1992		3
Nördlinger Roter	Winter	DE		Landrace		2
Nord Desprez	Winter	FR		1945		2
Øland 5	Spring	SE		Landrace		3
Okapi	Winter	NL		1977		3
Opus	Winter	FR	B	2003		2
Paroli	Winter	DE	A	2004		3
Philipp	Winter	AT	E	2005		3
Picolo	Spring	DE		1998		3
Purple Justin	Spring	AU			Purple wheat	2
Purple La Prevision	Winter	UK			Purple wheat	3
Purple Olympic	Spring	AU			Purple wheat	2
Rainer	Winter	AT	6	2006		2
Ralle	Spring	DE		1984		2
Rastatter Roter Breisgauer	Winter	DE		Landrace		3
Rektor	Winter	DE	E	1981		3
Rieggers Schwarzwälder Glatter Braunweizen	Winter	DE		Landrace		3
Ritmo	Winter	NL	B	1990		3
Roter Sächsischer Land	Winter	DE		Landrace		3
Ruppiner Brauner Landweizen	Winter	DE		Landrace		2
Sandomir	Winter	DE		2009		2
Sarah	Winter	DK		1976		3
Skalmeje	Winter	DE	B	2006		3
Skotte	Winter	SE	Very good bread	2004		3
Soissons	Winter	FR	BPS	1988		3
Solstice	Winter	UK	Nabim grp 1	2001		2
Taifun	Spring	DE	E	2003		3
Terra	Winter	DK	A	1992		2
Titlis	Winter	CH		1996		2
Tommi	Winter	DE	A	2002		3
Torrild	Winter	DK	A	2005	Organic farming variety	2
Traunsteiner Braun	Winter	DE		Landrace		3
Trirone	Winter	CH		2001		2

(*continued on next page*)

Table 1 (*continued*)

Wheat sample name	Vernality	Origin	Wheat quality[a]	Released	Remark	Replicates
V. Tschermaks Brauner Moravia	Winter	DE		Landrace		2
Ure	Winter	DK	DK bread list	1990	Organic farming variety	2
Viking	Winter	UK		1962		3
Vilmorin 23	Winter	FR		1923		3
Wenzel	Winter	AT	A2	2008		2
Xenos	Spring	DE		1999	High drought resistance	3
Zebra	Spring	SE		2001		2

[a] *German quality groups:* E-elite wheat, A-quality wheat, B-bread-wheat and C-other use; *French quality group:* BPS (blé panifables supérieurs)- superior bread wheat; *Austrian quality groups:* 7–9 high quality wheat, 3–6 bread-wheat, 1–2 feed/ethanol wheat; *UK and Ireland (Nabim) quality groups:* 1-high quality bread, 2-bread, 3-best suited to cakes and biscuits; *Hungarian quality groups* (based on farinograph values): A1-excellent, A2-good/excellent, B1-fair/good.

5 min at 240 °C. The mass spectrometer operated in electron ionisation mode at 70 eV. Mass-to-charge ratios between 15 and 300 were scanned. Volatile compounds were identified by probability based matching of their mass spectra with those of a commercial database (Wiley 275.L, HP product No. G1035A). The software program, MSD Chemstation (Version E.02.00, Agilent Technologies, Palo Alto, CA), was used for data analysis. The results from volatile analyses are presented as relative peak areas of the compounds identified.

Volatile compound identification was made by the probability based matching of mass spectra and a correct match in the elution profile. The retention time of each volatile was converted to the Kovats retention index using *n*-alkanes from C_5–C_{22} (Hewlett Packard, Avondale, PA) as references. Retention indices were compared to the retention indices of reference samples which were obtained using the same method and/or Kovats retention indices which were reported in the literature.

An RT/RI curve was obtained by plotting retention times of compounds against Kovats retention indices. Samples for which a reference retention index was either missing or that deviated from the RT/RI curve are claimed to be tentatively identified.

2.5. Data analysis

2.5.1. Multivariate data analysis

Multivariate data analysis was conducted with the peak areas obtained by GC–MS, on the wheat samples in order to assess the variation between them. Principal component analysis (PCA) was performed using Latentix software (LatentiX 2.0, Latent 5, Copenhagen, Denmark, http://www.latentix.com). The analysis was conducted on data representing the peak areas averaged over the sample replicates. The data was auto-scaled and cross validated.

A partial least squares discriminant analysis (PLS-DA) was also performed on the averaged peak area data in order to see if there was any separation of the samples into separate groups. Dummy variables were used to analyse for segregation between modern varieties compared with landraces, between the release dates of modern varieties, between varieties based on geographical origins and on vernality. Only the studies of the criteria which displayed segregation are reported in this paper, PLS-DA was performed using the Latentix software. The data was auto-scaled, and random repeated cross validation was used with 20 repetitions and 10 segments.

2.5.2. Univariate data analysis

Univariate data analysis was conducted as a one-way ANOVA using the software JMP 10 (SAS institute Inc, Cary, NC). The least significant differences (LSD) were determined and post hoc calculations using Tukey HSD were performed to determine any significant differences ($p < 0.05$) between samples.

3. Results and discussion

3.1. Identification of volatile compounds

A total of 72 volatile compounds were identified by DHE/GC–MS analysis of the wheat samples (Table 2). Of that total 62 volatile compounds were identified in the wheat samples and a further 10 volatiles were tentatively identified.

The peak areas of these volatiles varied much between samples for some compounds such as 2,3-dimethylpyrazine, 3-methoxy-2-butanol, δ-3-carene and propanoic acid. Peak areas for other compounds displayed only modest variations between samples such as 1,8-cineole and 4-methylpentan-2-one. The peak areas of the volatile compounds detected varied in order of magnitude from low ($n * 10^2$) for volatiles such as terpinolene, 2-methoxy-3-propylpyrazine and 2-methoxy-3-(2-methylpropyl)pyrazine to high ($n * 10^7$) for volatiles such as hexanol, propanone, pentanal and pentanol. The volatile compounds identified in the wheat samples included 17 alcohols, 14 ketones, 14 aldehydes, 9 terpenes, 6 furans, 4 esters, 4 pyrazines and one aromatic hydrocarbon, one acid, one sulphur compound and one amine.

The Swedish spring wheat Zebra, which has been bred as a high quality baking variety, was identified as an outlier on the basis of having high values for residual variance and Hotellings T^2. This could be attributable to the relatively high values in the data for the compounds decanal, 2-ethylhexan-1-ol and 2-methoxy 3-(2-methylpropyl)pyrazine as high values for most of the late-eluting compounds. It was also seen as an outlier during PLS-DA analysis and was therefore removed from further data analysis.

3.2. Variation between wheat samples

A PCA model was performed with wheat samples as scores and the averaged peak areas of volatile compounds as loadings. The model consisted of 12 principal components (PC). PC1 and PC2 together explained 47% of the variation (Fig. 1). PC 3 accounted for a further 7.8%. PC1–PC3 showed a similar distribution to PC1–PC2 and also showed a tendency towards separation of landraces from modern varieties. The model was robust for this type of data, containing 81 heterogeneous samples, and 72 independent variables with relatively low correlation between them.

The sample scores (Fig. 1a) are distributed across the score plot and some groupings are identified. The largest accumulation of samples are distributed from the top left side of the score plot towards the centre, this grouping contains all of the landraces (red) and some modern varieties (black). The distribution of samples from the centre to the right side of the score plot comprises only modern varieties. In the upper right area of the score plot a small grouping of six samples is distributed and in the upper left side of the score plot a smaller grouping of four samples is distributed. The following discussions will be based on these groupings:

Table 2
List of volatile aroma compounds detected in wheat flour by DHS and GC–MS. Tentatively identified volatiles are written in *italics*.

Compound name	Target ion	RT	Calculated Kovats RI	Kovats RI of Reference compounds	Kovats RI (wax)[a,b] Literature	Organoleptics
Methylsulfanylmethane	62	1.549	748		716	Cabbage, sulphur, corn, asparagus with a dairy creaminess
Propanal	29	1.812	796		801	Solvent, pungent
2-Methylpropanal	43	2.022	814		821	Pungent, malt, green
Propanone	43	2.073	818	815	819	Solvent, ethereal, apple, pear
Oxolane	*42*	*2.583*	*858*			*Butter, caramel*
2-Methylfuran	82	2.803	875		877	Ethereal acetone chocolate
Butanal	72	2.862	879	878	832	Pungent, green
Butan-2-one	43	3.314	906	906	881	Ether
Ethyl acetate	43	3.37	908	895	898	Pineapple
2-Methylbutanal	57	3.565	914		912	Cocao, almond
3-Methylbutanal	44	3.678	918		910	Malt
Propan-2-ol	45	4.267	938	937	917	Ethereal, alcoholic
2-Ethylfuran	81	4.765	954	953	960	Sweet burnt earthy malty
Cyclohept-3-en-1-one	*54*	*4.965*	*961*			
Bicyclo(4,1,0)heptan-2-one	*54*	*5.097*	*965*			
Pentanal	44	5.591	981	983	986	Almond, malt, pungent
2-Methylpentan-3-one	57	6.294	1002		1003	Mint
2,4-Dimethylpentan-3-one	*43*	*6.433*	*1005*			
4-Methylpentan-2-one	43	7.072	1015	1010	1008	Sharp, solvent-like, with green, herbal, fruity and dairy nuances
α-Pinene	93	7.36	1020	1016	1032	Pine, turpentine
Butan-2-ol	45	8.281	1036		1026	Oily wine-like fusel alcoholic note
Propanol	31	9.393	1055		1037	Alcohol pungent
Camphene	93	10.269	1069	1059	1075	Camphor
Hexanal	44	11.492	1090	1089	1084	Grass, tallow, fat
Butyl acetate	43	11.532	1091	1092	1105	Sharp, ethereal, diffusive, fruity banana
2-Methyl-1-propanol	43	12.496	1111	1110	1099	Wine, solvent, bitter
3-Methoxy-2-butanol	*59*	*13.247*	*1132*			
Pentan-2-ol	45	13.717	1145		1118	Green
2-Butylfuran	81	13.83	1148		1140	Mild, fruity, wine, sweet, spicy
δ-3-Carene	93	14.051	1154	1155	1148	Lemon, resin
Butanol	56	14.509	1167	1166	1145	Medicine, fruit
Pent-1-en-3-ol	57	14.999	1180		1157	Bitter pungent
Heptan-2-one	43	15.414	1192	1190	1170	Cheese, fruity, ketonic, green banana, creamy nuances
Heptanal	70	15.489	1194	1192	1174	Fat, citrus, rancid
Limonene	68	15.746	1201		1198	Lemon, orange
1,8-Cineole	43	16.287	1225	1214	1213	Mint, sweet
2-Methylbutan-1-ol	57	16.329	1227	1224	1208	Wine, onion
3-Methylbutan-1-ol	55	16.353	1228		1205	Balsamic
Butyl butanoate	71	16.497	1234	1231		Sweet, fruit, fresh, diffusive, ripe
2-Pentylfuran	81	16.823	1248	1246	1240	Green bean, butter
Pentanol	42	17.451	1275	1274	1255	Pungent, fermented, bready, yeasty, fusel, winey and solvent-like
Octan-3-one	57	17.552	1280	1273	1244	Herbs, musty, mushroom, cheesy
Para-cymene	119	17.697	1286	1284	1277	Solvent, gasoline, citrus
Hexyl acetate	43	17.872	1294	1291	1270	Fruit, herb
Terpinolene	121	18.006	1300	1299	1275	
Octan-2-one	43	18.126	1305	1302	1285	Musty, earthy, dairy, parmesan, blue cheese
Octanal	41	18.212	1308	1306	1280	Fat, soap, lemon, green
Hept-2-enal	41	18.983	1342		1305	Green, fatty
Heptan-2-ol	*45*	*19.017*	*1343*			*Mushroom*
6-Methylhept-5-en-2-one	43	19.302	1356	1354	1365	Mushroom, earthy, vinyl, rubber, woody, blackcurrant, boiled fruit
3-Methylhexan-2-ol	*18*	*19.328*	*1357*			
4-Methylhept-6-en-3-one	*43*	*19.408*	*1360*			
2,3-Dimethylpyrazine	*108*	*19.495*	*1364*			*Musty, nut skins, cocoa powdery and roasted with potato and coffee nuances*
Hexanol	56	19.703	1373	1372	1379	Resin, flower, green
4-Ethylcyclohexan-1-one	*55*	*20.298*	*1399*			
α-Thujone	81	20.345	1402	1411		Cedar leaf
Nonanal	57	20.43	1406	1404	1402	Fat, citrus, green
2-Methoxy-3-propylpyrazine	137	21.072	1441		1434	Pea, earthy, beany (lady bug taint in wine)
2,3,5,6-Tetramethylpyrazine	54	21.932	1487	1493	1474	Nutty, musty and vanilla with dry, brown cocoa nuances
2-Ethylhexan-1-ol	57	22.244	1504	1504	1492	Rose, green
Decanal	43	22.405	1514	1512	1510	Soap, orange peel
1H-pyrrole	67	22.7	1531		1542	Nutty, sweet, warm, ethereal
Nonanol	56	22.731	1533	1535	1532	fat, green
Benzaldehyde	77	22.878	1541	1541		Almond, burnt sugar

(*continued on next page*)

Table 2 (*continued*)

Compound name	Target ion	RT	Calculated Kovats RI	Kovats RI of Reference compounds	Kovats RI (wax)[a,b] Literature	Organoleptics
2-Methoxy-3-(2-methylpropyl)pyrazine	124	23.013	1549	1542	1540	Green pepper-like, green, dry, leaf, spicy
Non-2-enal	41	23.092	1554	1553	1527	Oriis, fat, cucumber
Propanoic acid	74	23.197	1560		1533	Pungent, rancid, soy
Octanol	56	23.433	1574	1573		Chemical, metal, burnt
Junipene	41	23.44	1574		1574	Sweet, woody, rose, medical, fir-needle
Oxolane-2-one	42	24.696	1651		1647	Caramel, sweet
1-Phenylethanone	105	25.042	1673		1645	Must, flower, almond, vanilla- like
1-Methoxy-4-prop-2-enylbenzene	148	27.732	1823	1828		Licorice, anise

[a] www.flavornet.org.
[b] www.pherobase.com.

1. The landraces on the score plot in Fig. 1a have been coloured in red. These are placed on the left side of the plot having negative values of PC1. This means that they have relatively high levels of the compounds, placed on the left and upper side of the loading plot in Fig. 1b, which include mostly esters, furans and alcohols.
2. The modern bred varieties mostly occupy the right and the lower side of the score plot. These varieties are characterised by having higher values for terpenes, pyrazines and straight-chained aldehydes while having lower values for esters.
3. A smaller sample grouping containing Skalmeje, Rektor, Okapi, Luteus, Tommi and Ritmo having rather high values of PC1 can be identified. These samples are characterised by high values for 2,3-dimethylpyrazine; the straight-chained aldehydes heptanal, pentanal, butanal and octanal; 2-methylfuran, and several ketones, i.e., octan-2-one, 2,4-dimethylpenten-3-one, heptan-2-one, 4-methylhept-6-en-3-one, and 6-methylhept-5-en-2-one, some terpenes and pyrazines while having lower values for most esters and most alcohols. The landraces Gotlandske Børst, Leipzigger rot and Reiggers do not form a well separated group but their position on the score plot indicates that they stand in contrast to these six varieties in having a relatively high content of esters and alcohols and lower values for terpenes and straight-chain aldehydes.
4. In the upper part of the plot there are four samples: Rastatter Rot, Igel Unbehart Rot, Dacke and Carmen Begrannt Rot. These samples have higher values for esters, the furans 2-butylfuran, 2-pentylfuran and 2-ethylfuran, the two amino acid derived aldehydes 2-methylbutanal and 3-methylbutanal, as well as many alcohols, while having low values for the pyrazines, the terpenes and the straight-chain aldehydes.

Based on the colouring of compounds in the corresponding loadings plot in Fig. 1b all of the esters (dark blue) and most of the furan derivatives (orange) are shown located in the top left side of the loadings plot. Many of the alcohols (magenta) are also situated in this area. The samples, placed in the corresponding area of the score plot have higher peak areas for these compounds.

In the loadings plot in Fig. 1b, the volatile compounds are distributed so that they form a large group of compounds spreading from top left to the bottom right of the loadings plot. On the mid-right side of the loadings plot in Fig. 1b are the terpenes (green) and the pyrazine derivatives (cyan).

The aldehydes (red) mostly have positive values on PC1 with the exception of two amino acid derived aldehydes, 2-methylbutanal and 3-methylbutanal. They are more evenly dispersed along PC2. The ketones (black) are more evenly dispersed throughout the plot, although mostly on the right side of the plot. The individual compounds (grey) 1H-pyrrole, propanoic acid and methylsulfanylmethane all have positive values for PC1 indicating they are more represented among the modern bred varieties. 1-Methoxy-4-prop-2-enylbenzene occupies the middle of the plot.

In Fig. 2a bar diagram shows 14 wheat samples which are compared for their content of 7 volatile compounds, one from each of the chemical groups found in the wheat samples. The wheat varieties were selected because they either have marked morphological traits or occupy extreme positions in the PCA score plot in Fig. 1a. Thus Purple Justin: a purple wheat, Luteus: a yellow wheat, Format: a German winter wheat, Gotlandske Børst: a Swedish landrace, Torrild: a variety used in organic farming, Melbor: a very old variety, and Øland 5 wheat: a landrace, were chosen in this selection. Carmen Begrannt Rot, Magnifik, Ritmo, Complet, Dacke, and Ure were added because they occupied extreme positions on the PC1–PC2 score plot (Fig. 1a). All of the volatiles selected for comparison had been previously identified in connection with either wheat flour analysis or in bread made from wheat flour.

Ethyl acetate and 1-hexanol have both been reported in wheat flour (Hansen & Hansen, 1994). They have also been reported in bread along with the compounds: hexanal, octan-2-one, 2-butylfuran, 2,3-dimethylpyrazine and limonene (Bianchi, Careri, Chiavaro, Musci, & Vittadini, 2008; Birch, Petersen, & Hansen, 2013; Jensen, Oestdal, Skibsted, Larsen, & Thybo, 2011; Maeda, Kim, Ubukata, & Morita, 2009a; Maeda et al., 2009b; Ruiz et al., 2003; Schieberle & Grosch, 1991; Seitz et al., 1998). Another reason for including 2,3-dimethylpyrazine, which is tentatively identified in this study, is that this compound has a very low odour-threshold value of 2500 ppb (http://www.leffingwell.com/pyrazine.htm) and could potentially be an important odorant.

Fig. 2 shows that there is a considerable and significant variation in the relative peak area between the wheat varieties for all of the seven volatiles. The largest variation occurs for 1-hexanol, ethyl acetate and 2-butylfuran, and the least variation occurs for 2,3-dimethylpyrazine, as only Ritmo had a significantly higher relative peak area compared to the rest of the varieties. Since most of the selected compounds are well known aroma compounds with rather low sensory thresholds, the differences are likely to cause differences in sensory quality between the varieties.

3.3. Grouping of samples by PLS-DA

To direct the analysis and make an even better separation of landraces from modern varieties, PLS-DA was applied. The PLS-DA model, which can be seen as a supplementary figure, resulted in complete separation between landraces and the modern varieties. PLS-DA also showed that the landraces are mostly characterised by having high levels of ethyl acetate, butan-2-one, propanone, propan-2-ol, cyclohept-3-en-1-one, 2-pentylfuran and 2-methylpropanal. There was no clear separation between modern

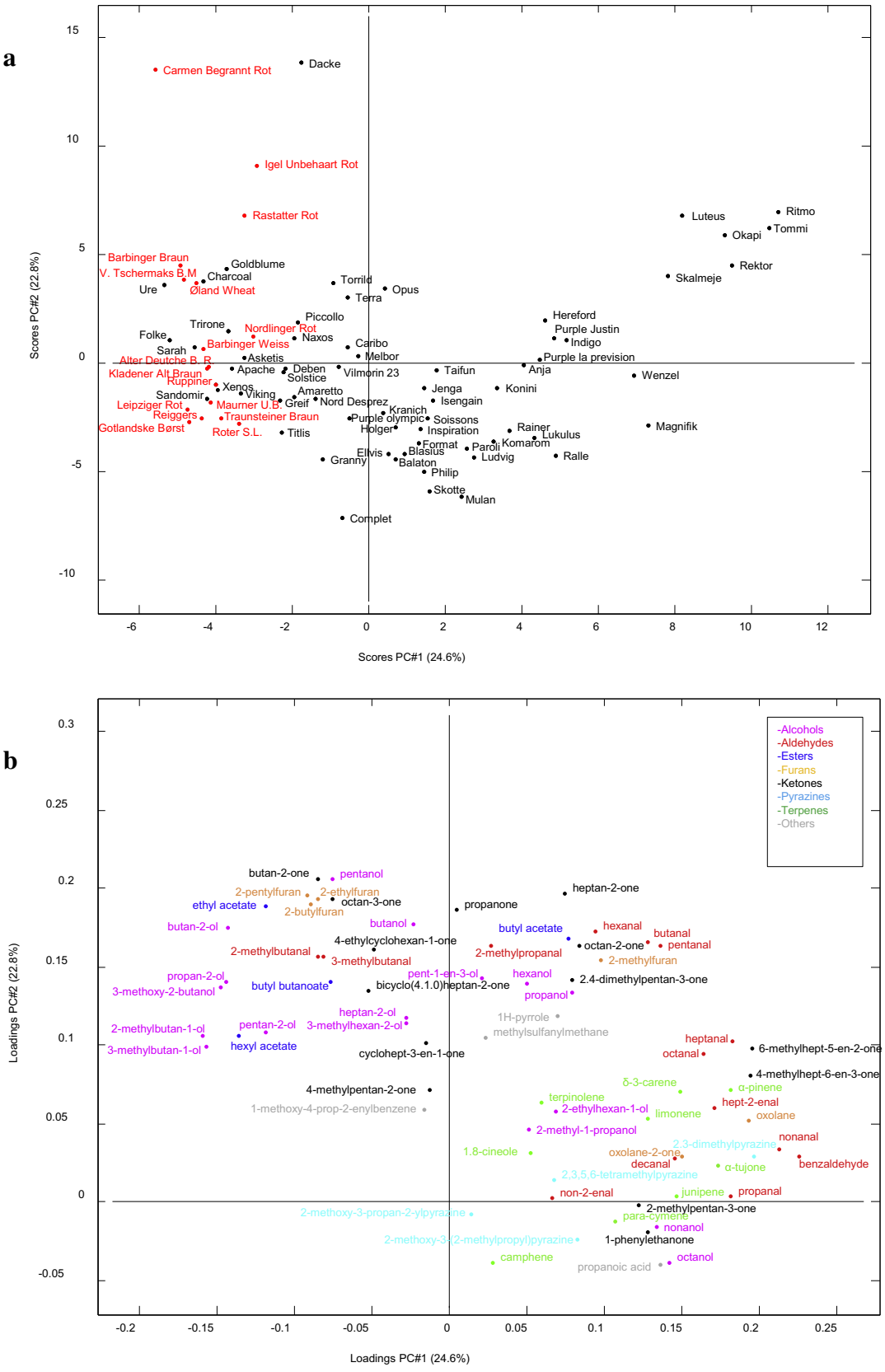
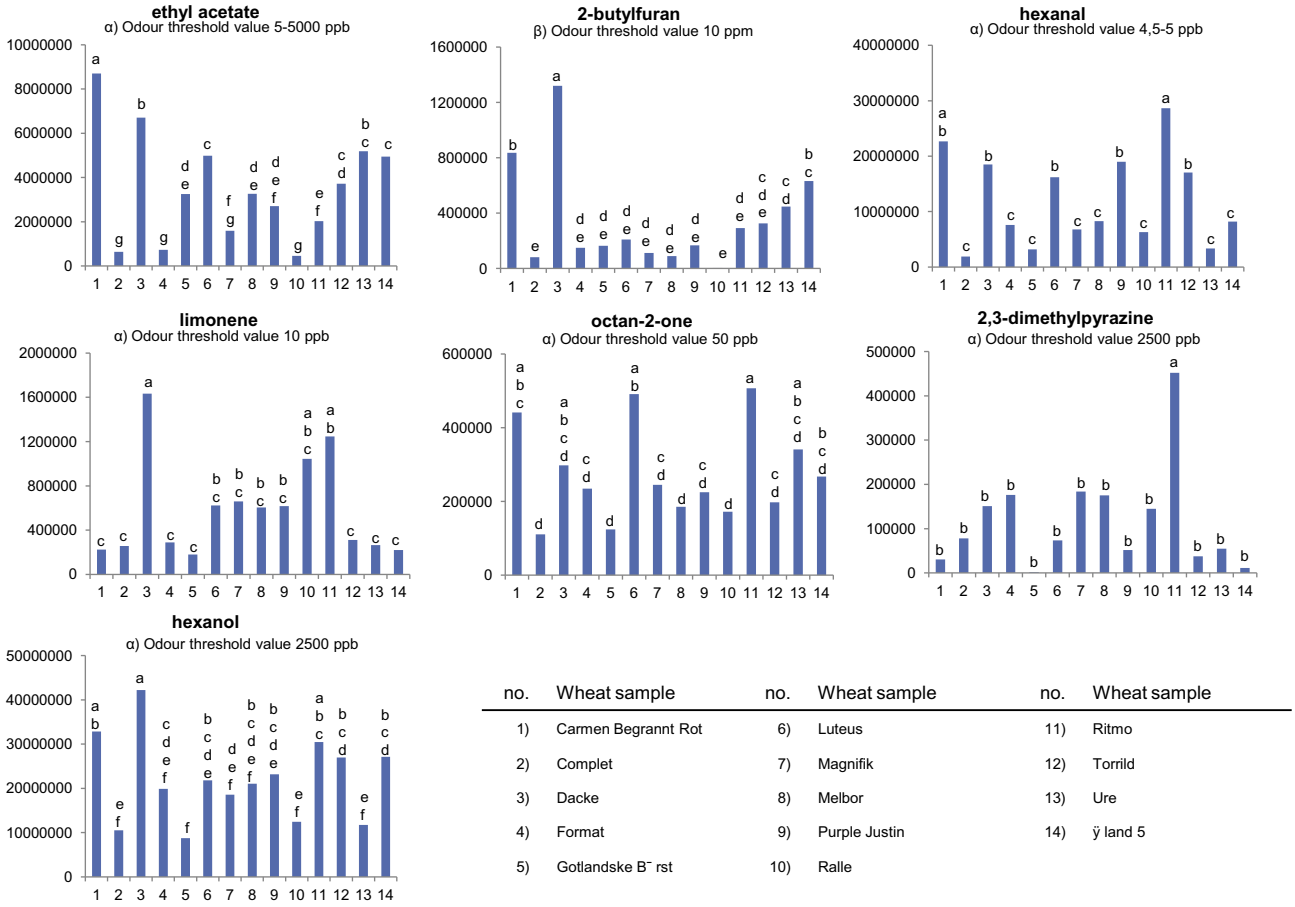


Fig. 1. Variation between bread-wheat variety samples based on their profiles of relative volatile contents. (a) Wheat samples of landraces (red) and varieties (black) as scores. (b) Volatile compounds as loadings. Volatiles are coloured after functional group: alcohols (magenta), aldehydes (red), esters (blue), furans (orange), terpenes (green), pyrazines (cyan), ketones (black) and other compounds (grey).



no.	Wheat sample	no.	Wheat sample	no.	Wheat sample
1)	Carmen Begrannt Rot	6)	Luteus	11)	Ritmo
2)	Complet	7)	Magnifik	12)	Torrild
3)	Dacke	8)	Melbor	13)	Ure
4)	Format	9)	Purple Justin	14)	ÿ land 5
5)	Gotlandske B¯ rst	10)	Ralle		

Fig. 2. Averaged peak areas of selected volatile compounds and their odour threshold values in 14 wheat samples. The *x*-axes show wheat sample numbers which are listed in the accompanying table; *y*-axes show average peak area values. Note that they are scaled differently according to levels found. Samples which share a letter are not significantly different for that compound. Odour threshold references: (α) http://www.leffingwell.com/odorthre.htm. (β) Evans, C. D., Moser, H. A., & List, G. R. (1971). Odour and flavour responses to additives in edible oils. *Journal of the American Oil Chemistś Society*, *48*, 495–498.

varieties according to release data. When a PLS-DA model focusing on geographical differences was applied (Fig. 3) it was shown that there is a separation between modern varieties which have originated in Austria (magenta) and modern varieties from the UK (green), Denmark (red) and France (blue), while varieties from Germany and Sweden (black) were distributed more evenly throughout the sample set. The separation between samples from different geographical locations may be explained partly by the pedigree of the varieties in this study and also by the difference in climate zones from the maritime climate experienced in UK, Denmark and France and the more continental climate of Austria to which those samples have been selected for and adapted to. Early UK varieties such as Squarehead wheat were often used in breeding programs in Denmark and France (Belderok, 2000). This could in part explain why the varieties from these three countries are grouped in close proximity to each other in the PLS-DA plot (Fig. 3). Austrian varieties have had a different breeding history which has been influenced more by the introduction of German, Italian, Swiss and Hungarian varieties (Belderok, 2000). The Austrian varieties are more represented by the terpenes δ-3-carene, camphene and junipene and also by 2-methoxy-3-(2-methylpropyl)pyrazine, decanal, propanoic acid, 2-methylpentan-3-one and 1-phenylethanone. In contrast, the French, Danish and UK samples have higher values for 1,8-cineole, para-cymene, 2-methoxy-3-propylpyrazine, 2 methylfuran, methylsulfanylmethane, tetramethylpyrazine and several aldehydes, alcohols and ketones.

These volatile compounds are from chemical groups which are characterised as having marked and powerful odours.

3.4. Pyrazines

Pyrazine compounds were found especially in the modern varieties in the lower right side of the score plot in Fig. 1. Pyrazines are powerful odorants which are often described as having rich, burnt odours like roasted nuts, paprika, chocolate, coffee and potato (Belitz & Grosch, 1999, chap. 5). They are mostly associated with thermal processes. However they have also been found to occur naturally in fresh plants such as peas, green bell peppers and tomatoes (Maga & Sizer, 1973). Pyrazines can also be formed as a reaction between amino acids and sugars during fermentation processes with bacterial catalysts (Müller & Rappert, 2010). Pyrazine compounds might form in wheat grains as they harden during ripening. The drying process could provide conditions similar to those which occur in Maillard reactions. Pyrazines may be mobilised by plants in an aposematic role due to their low odour and taste thresholds and are therefore considered to be responsible for promoting predation-learning in insects (Kaye, Mackintosh, Rothschild, & Moore, 1989).

3.5. Furans

The relative peak areas of furans were mainly found to be highest among the wheat samples at the top of the score plot in Fig. 1a. This includes the samples Dacke, Ritmo, TommI, Luteus, Okapi, Rektor and Skalmeje and the three landraces Carmen Begrannt Rot, Igel Unbehaart Rot and Rastatter Rot. Furans are significant flavour sources in foods and have a wide range of descriptors. They

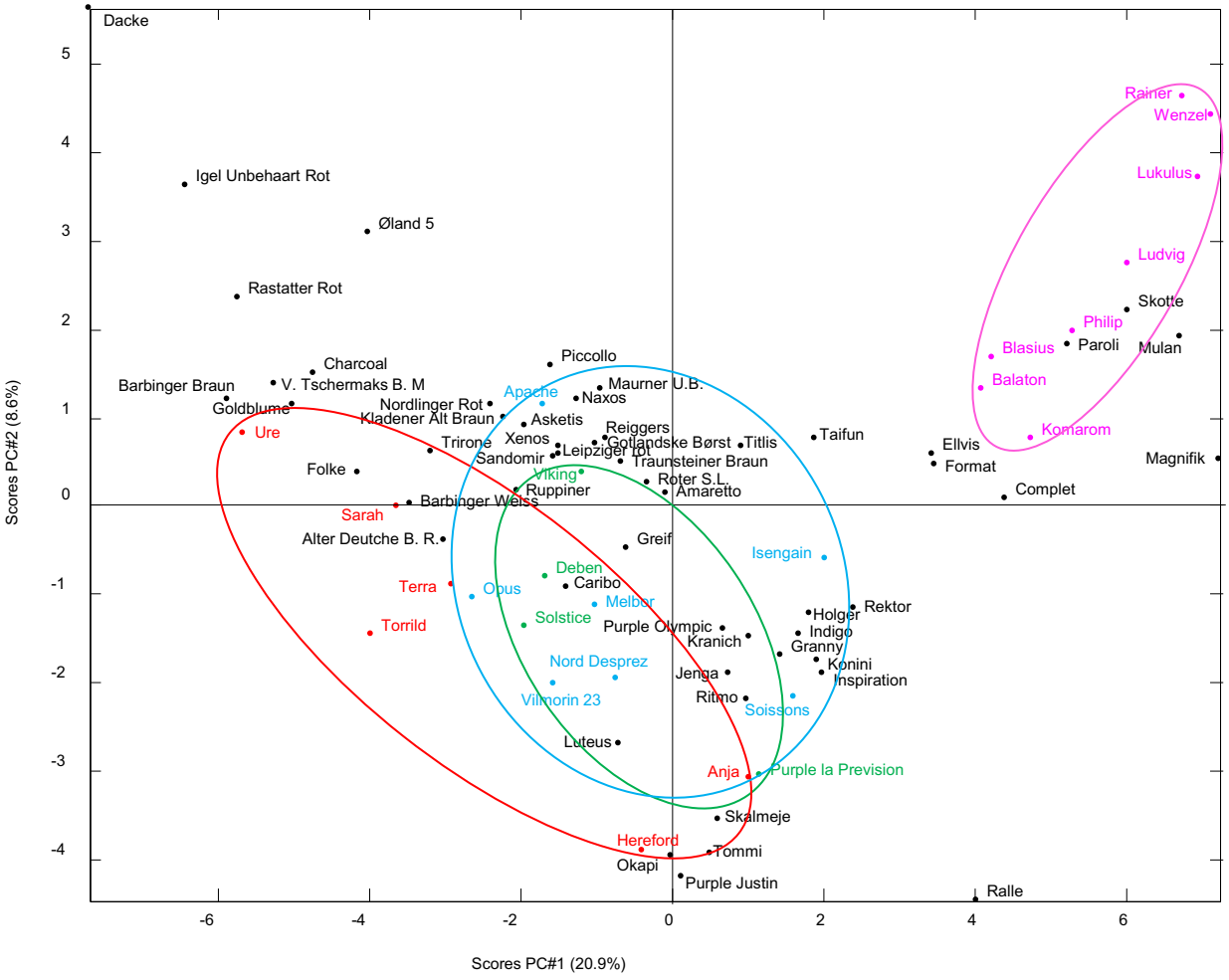


Fig. 3. The score plot for a PLS-DA model showing segregation between varieties from Austria (magenta) and varieties from France (blue), UK (green) and Denmark (red).

are often described as having green-beany, meaty, burnt, sweet, roasted, caramel, malty and chocolate-like odours (Flavornet, 2014; Pherobase, 2014). Furan compounds have been reported in unprocessed foods such as green coffee, tomato and orange juice where they have been detected by DHS/GC–MS analysis (Senyuva & Gökmen, 2005). Furans in avocado have been shown to have growth-inhibiting and insecticidal properties (Rodriguez-Saona, Maynard, Phillips, & Trumble, 2000).

3.6. Terpenes

The highest peak values for terpenes were found among those samples which occupied the upper right part of the score plot (Fig. 1a), such as the samples Ritmo, Tommi, Okapi, Luteus, Rektor, and Skalmeje. Meanwhile the samples which had the lowest values for terpenes were mostly the German landraces which occupied the lower left of the score plot (Fig. 1a). Terpenes and terpenoids are the main constituents of essential oils found in plants and flowers. They often have penetrating, plant-like and powerful odours, such as citrus, mint, camphor or pine resin. The understanding of the role of terpenes in nature is still very small in comparison to the approximately 25,000 terpene structures which have been reported so far; however, it is likely that their primary role is that of defence (Gershenzon & Dudareva, 2007). Monoterpenes (C_{10}) have been reported to be toxic to insects (Lee, Peterson, & Coats, 2003), to fungi (Hammer, Carson, & Riley, 2003) and to bacteria (Friedman, Henika, & Mandrell, 2002). Another role which particularly the volatile terpenes have, such as mono- and sesquiterpenes, is as chemical messengers involved in plant- insect communication (Gershenzon & Dudareva, 2007). An example of this is how β-farnesene functions as an insect pheromone and can cause insects to cease feeding behaviour and even attract aphid enemies (Beale et al., 2006). Modern breeding for increased resistance to pathogens and predators could possibly be affecting the volatile composition of wheat and leading to increasing levels of volatiles, such as terpenes and pyrazines.

Figs. 1 and 2 show that the volatile profiles of samples used in this study appear to be a diverging between modern varieties and landraces, after little over a century of plant breeding. This sample group has been composed randomly based on important European wheat varieties and is sufficiently large enough be representative of a wider trend and merit further investigation. The practice of breeding successive generations of new wheat varieties on the basis of earlier successful varieties has previously been brought into question (Reif et al., 2005; Tanksley & McCouch, 1997) on the grounds that it leads to a narrowing of the genetic base of newer varieties. However the modern varieties in this study continue to display a large variation in volatile profile. Wider studies profiling more varieties and landraces from different regions would help to gain a more thorough understanding of this.

3.7. Potency of volatiles in wheat flour

While the question of odour potency of individual volatiles found in this study cannot be resolved here, there are inferences which can be made. For example, Czerny and Schieberle (2002) listed odour-active compounds, mostly aldehydes, which they analysed by aroma extract dilution analysis and by quantitative studies using stable isotope dilution assays in whole wheat and white wheat flour and they concluded that hexanal, heptanal, non-2-enal, 2-methylbutanal and 3-methylbutanal were important odorants. These volatile compounds have also been detected in this study using DHE and GC–MS. Hexanal has an odour threshold value of 4.5–5 ppb (Table 2); however, other compounds listed there, such as limonene, octan-2-one, ethylacetate, hexanol and 2,3 dimethylpyrazine, have comparable odour thresholds and there is also quite large variation in their peak areas (Table 2). More generally there are likely to be odour contribution from the terpenes, pyrazines and also furans, as well as ketones and esters but it is the interaction of several odorants which would characterise the flavours and odours of different varieties and therefore the variations seen between wheat varieties for different volatile compounds promises so much potential for flavour and odour variations. However it ought to be considered that hexanal, heptanal and non-2-enal are lipid oxidation products, which would, in high concentrations, be characterised by harsh, oxidised or rancid notes, yet these notes were not described in sensory evaluations of cooked wheat grains (Starr et al., 2013). So the role played by individual volatile compounds may be a subtle one. Investigation of which volatile compounds are potent aroma contributors in wheat wholemeal ought to be conducted following further studies.

3.8. Link to sensory observations

One interesting observation was made while comparing the data in this study to a previous sensory study of cooked wheat grains of different varieties and landraces (Starr et al., 2013). According to the PCA scores the varieties Konini and Purple Justin are located on the right side of the PCA plot in Fig. 1, while Complet and Solstice is located in the middle and Ure, Goldblume and Øland wheat, are located on the left side of the PCA plot. Starr et al. (2013) found that these varieties were positioned in a similar configuration in a bi-plot based on sensory evaluation of the cooked grains showing sample values for significant odours and flavours. Starr et al. (2013) also observed that the greatest variation in samples of cooked grain occurred between 7 varieties which had been cultivated at the same location. Although these samples were exposed to a thermal process by being cooked they seem to have retained a similar differentiation to each other that they have in their raw and natural state. The samples in this study which were cultivated under greenhouse conditions show a large variation for peak areas of volatile compounds and pattern of distribution based on volatile compound content. These two findings suggest that aroma differences found in wheat samples and differences in the volatile fraction between wheat samples might be linked. By eliminating variations in growing conditions between the wheat samples, the remaining differentiating factor is the variety of bread-wheat used. This would indicate that the content of volatile compounds in each variety might be under genetic control. This would have implications for future wheat breeding programs as it could be possible to breed future wheat varieties for enhanced or specific gastronomic qualities. It could potentially mean that GC–MS screening of wheat samples could stand in lieu of laborious and expensive sensory testing for new varieties for flavour and odour variation. However, more work needs to be conducted to investigate the relationship between volatile profiles and sensory profiles of wheat wholemeal and how much volatile compounds found in wheat can influence the sensory properties of products, such as bread.

4. Conclusion

This is the first time such a broad sample group of wheat varieties has been studied for variations in volatile compound profiles. A total of 81 wheat varieties and landraces were found to harbour large variation across 72 volatile compounds using extraction by DHE and GC–MS analysis of wheat wholemeal. Among the trends observed in the sample set was that modern wheat varieties were diverging from landraces based on their volatile profiles, as landraces have higher levels of esters, alcohols and some furans, while modern varieties are characterised by higher levels of terpenes, pyrazines and straight-chain aldehydes. A differentiation between wheat growing regions was observed in the separation of Austrian wheat samples from those from Britain, Denmark and France, using PLS DA on the volatile profile data. The data analysis revealed that Austrian samples have higher volatile peak areas for most of the terpenes compared to wheat samples from Britain, Denmark, and France. These findings will be useful to plant breeders, the baking industry and other manufacturers of wheat-based products, by bringing to their attention the potential diversity of wheat. Those with a gastronomic interest in wheat may also find these results useful. Future studies of wheat volatile profiles can use these results as a reference.

Acknowledgements

The authors would like to express their gratitude to Jihad Orabi for cultivating and supplying the wheat samples and to the lab technicians Abdelrhani Mourhrib, Anna Petersen, and Christina Aunsbak for their assistance with DHE and GC–MS work. They would also like to thank the Danish ministry of Food, Agriculture and Fisheries for their financial support for this project.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.11.077.

References

Beale, M. H., Birkett, M. A., Bruce, T. J. A., Chamberlain, K., Field, L. M., Huttly, A. K., et al. (2006). Aphid alarm pheromone produced by transgenic plants affects aphid and parasitoid behavior. *Proceedings of the National academy of Sciences of the United States of America, 103*, 10509–10513.

Belderok, B. (2000). Developments in bread-making processes. In D. A. Donner (Ed.), *Bread-making quality of wheat: A century of breeding in Europe* (1st ed., pp. 3–86). Dordrecht: Kluwer Academic Publishers.

Belitz, H. D., & Grosch, W. (1999). *Food chemistry* (2nd ed.). Berlin: Springer.

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.

Birch, A. N., Petersen, M. A., & Hansen, Å. (2013). 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.

Czerny, M., & Schieberle, P. (2002). Important aroma compounds in freshly ground wholemeal and white wheat flour-identification and quantitative changes during sourdough fermentation. *Journal of Agriculture and Food Chemistry, 50*, 6835–6840.

Flavornet. (2014). Cornell University, NYSAES, Geneva, NY. Available from <http://flavornet.org/flavornet.html>. Accessed 25.05.14.

Friedman, M., Henika, P. R., & Mandrell, R. E. (2002). Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. *Journal of Food Protection, 65*, 1545–1560.

Gershenzon, J., & Dudareva, N. (2007). The function of terpene natural products in the natural world. *Nature Chemical Biology, 3*(7), 408–414.
Hammer, K. A., Carson, C. F., & Riley, T. V. (2003). Antifungal activity of the components of *Melaleuca alternifolia* (tea tree) oil. *Journal of Applied Microbiology, 95*, 853–860.
Hansen, Å., & Hansen, B. (1994). Influence of wheat flour type on the production of flavour compounds in the wheat sourdoughs. *Journal of Cereal Science, 19*, 185–190.
Hougen, F. W., Quilliam, M. A., & Curran, W. A. (1971). Headspace vapors from cereal grains. *Journal of Agricultural and Food Chemistry, 19*, 182–183.
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.
Kaye, H., Mackintosh, N. J., Rothschild, M., & Moore, B. P. (1989). Odour of pyrazine potentiates an association between environmental cues and unpalatable taste. *Animal Behaviour, 37*, 563–568.
Lee, S., Peterson, C. J., & Coats, J. R. (2003). Fumigation toxicity of monoterpenoids to several stored product insects. *Journal of Stored Products Research, 39*, 77–85.
Liu, Q., Qiu, Y., & Beta, T. (2010). Comparison of antioxidant activities of different colored wheat grains and analysis of phenolic compounds. *Journal of Agricultural & Food Chemistry, 58*, 9235–9241.
Lupton, F. G. H. (1987). *Wheat breeding: Its scientific basis* (1st ed.). London: Chapman & Hall.
Maeda, T., Kikuma, S., Araki, T., Ikeda, G., Takeya, K., & Sagara, Y. (2009b). 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.
Maeda, T., Kim, J. H., Ubukata, Y., & Morita, N. (2009a). Analysis of volatile compounds in polished-graded wheat flour bread using headspace sorptive extraction. *European Food Research and Technology, 228*, 457–465.
Maga, J. A., & Sizer, C. E. (1973). Pyrazines in foods. A review. *Journal of Agricultural and Food Chemistry, 21*, 22–30.
Müller, R., & Rappert, S. (2010). Pyrazines: Occurrence, formation and biodegradation. *Applied Microbiology and Biotechnology, 85*, 1315–1320.
Pherobase. (2014). El-Sayed AM 2014. The pherobase: Database of pheromones and semiochemicals. Available from <http://www.pherobase.com/database/floral-compounds/floral-taxa-compounds-fg-Furans.php> Accessed 25.05.14.
Reif, J. C., Zhang, P., Dreisigacker, S., Warburton, M. L., van Ginkel, M., Hoisington, D., et al. (2005). Wheat genetic diversity trends during domestication and breeding. *Theoretical and Applied Genetics, 110*, 859–864.
Rodriguez-Saona, C., Maynard, D. F., Phillips, S., & Trumble, J. T. (2000). Avocado furans and their tetrahydrofuran analogues: Comparison of growth inhibitory and insecticidal activity. *Journal of Agriculture and Food Chemistry, 48*, 3642–3645.
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.
Schieberle, P., & Grosch, W. (1991). Potent odorants of the wheat bread crumb. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 192*, 130–135.
Seitz, L. M., Chung, O. K., & Rengarajan, R. (1998). Volatiles in selected commercial breads. *Cereal Chemistry, 75*, 847–853.
Senyuva, H. Z., & Gökmen, V. (2005). Analysis of furan in foods. Is headspace sampling a fit-for-purpose technique? *Food Additives & Contaminants, 22*(12), 1198–1202.
Starr, G., Bredie, W. L. P., & Hansen, Å. S. (2013). Sensory profiles of cooked grains from wheat species and varieties. *Journal of Cereal Science, 57*, 295–303.
Tanksley, S. D., & McCouch, S. R. (1997). Seed banks and molecular maps: Unlocking genetic potential from the wild. *Science, 277*, 1063–1066.