

# Flavour Science:

## Proceedings of the XIV Weurman Flavour Research Symposium

Edited by

Andrew J Taylor

*Waltham Centre for Pet Nutrition, Mars Petcare*

and

Donald S Mottram

*University of Reading*

**CONTEXT**

Context Products Ltd  
53 Mill Street, Packington  
Leicestershire, LE65 1WN, United Kingdom  
www.contextbookshop.com

First published 2015

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**British Library Cataloguing in Publication Data**

Flavour Science: Proceedings of the XIV Weurman Flavour Research Symposium

ISBN 9781899043705

ISSN 0269-5642

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## Preface

The Weurman Flavour Research Symposia have taken place every three years since 1975, when the idea of a regular European based flavour meeting was proposed by Cornelius Weurman. The location of these meetings was originally determined by a rotation around the Netherlands, the United Kingdom, Germany, France, Scandinavia and Switzerland, which lasted from 1975 to 2008. The development of active flavour research centres in other European countries led to a new plan for the third “rotation” and Spain hosted the 2011 event and the UK was nominated for 2014. The organisation is carried out by volunteers in each country and the style is to present recent research in a location that allows interaction between delegates to facilitate informal discussions and networking. The other key principle of the Weurman symposia is to encourage young flavour researchers to present their work and introduce them to the wider world of flavour research.

For the 2014 event, we chose Queens’ College Cambridge as the venue. Its historical roots and location in the centre of Cambridge, provided an inspiring atmosphere for delegates to immerse themselves in flavour science. Five sessions on Flavour Discovery, Flavour Generation, Flavour Effects on the Body, Flavour Perception and Flavour in Food Products created opportunities for 38 lectures, 17 flash poster presentations and 125 posters. Each session started with a plenary lecture to set the scene for the topic. From the posters submitted for each topic, the Scientific Committee chose around 4 to be presented as “flash presentations” with a maximum of 5 slides and 5 minutes. This allowed wider participation and the short talks complemented the longer lectures.

Organising such an event can only be achieved with support from a team of people and we would like to thank our Scientific Committee for assessing the abstracts and allocating them to the lecture, flash poster or poster categories. The same Scientific Committee was also responsible for organising the refereeing and editing of the written papers. My wife, Helen Taylor, took on the responsibility of organising the social programme for the symposium as well as the programme for partners and spouses of the delegates. Punting on the River Cam, walking tours around Cambridge, an organ recital in the College Chapel plus a barbeque with jazz band on the College lawns all enhanced the event and were much appreciated by the attendees, as was the warm and dry weather. Thanks to generous sponsorship by food and flavour companies, we were able to offer over 30 PhD students a reduced registration fee to fulfil the Weurman principle of encouraging young researchers to take part in the meeting. Finally, we need to thank the team from Mars Petcare who helped with registration of over 200 people, the students who guided delegates to their rooms, the speakers, the chairpersons of the scientific sessions and the staff at Queens College for the excellent meals and support in setting up the meeting.

At the end of the 2014 meeting, we were able to announce that the 2017 symposium would take place in Graz, Austria, organised by Barbara Siegmund and we wish her and her team all success.

**Organising Committee**

Prof A J Taylor  
Prof D S Mottram  
Dr H Taylor

Waltham Centre for Pet Nutrition, Mars Petcare  
University of Reading  
Flavometrix Ltd

**Scientific Committee**

Dr J K Parker  
Dr L Methven  
Dr D A Baines  
B Grainger<sup>§</sup>  
Dr J W Marshall  
Prof J Hort

The Flavour Centre, University of Reading  
University of Reading  
Baines Food Consultancy  
British Society of Flavourists  
Waltham Centre for Pet Nutrition, Mars Petcare  
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<sup>§</sup> It is with regret we report the death of Brian Grainger in March 2015. He was an enthusiastic supporter of the Weurman symposium and had a great passion for flavour science.

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# Characterisation of impact aroma compounds in hop essential oils

Graham T. Eyres<sup>a</sup>, Philip J. Marriott<sup>b</sup>, Michelle Leus<sup>a</sup> and Bridget Lysaght<sup>a</sup>

<sup>a</sup> University of Otago, Department of Food Science, PO Box 56, Dunedin 9054, New Zealand.

<sup>b</sup> Monash University, School of Chemistry, Box 23, Clayton, Victoria 3800, Australia.

Hops (*Humulus lupulus* L.) are a fundamental component in beer, providing desirable aroma characters such as floral, fruity, spicy, woody and herbal notes. However, the compounds responsible for the odour attributes of hop essential oil and hop aroma in beer have yet to be adequately identified. This article presents the results of two studies characterising the aroma profiles of different hop varieties using gas chromatography-olfactometry (GC-O) and identifying the aroma compounds responsible.

## Introduction

Hops (*Humulus lupulus* L.) are an indispensable component of beer, with the essential oil responsible for imparting distinctive hop aroma characteristics. Various hop varieties are used to impart different aroma properties to beer, such as floral, fruity, spicy, woody and herbal characters, where the differences can be attributed to the composition of the volatile aroma compounds in the essential oil. Despite the fact that over 485 compounds have been identified in the literature, not all impact aroma compounds in hops have been identified [1-3]. Furthermore, hop aroma in beer is not well understood due to the complex physical, chemical and biological changes that occur during brewing and fermentation [4].

This paper presents the results of two studies comparing the impact odorants in different hop varieties. The objective of these studies is to identify the compounds responsible for odour attributes using GC-O in combination with mass spectrometry. The long term goal of this research is to understand the aroma compounds responsible for hop derived aroma in beer and to determine how to control it to achieve a desired flavour profile.

## Study 1 – Comparison of liquid CO<sub>2</sub> extracts from four hop varieties

### Background

The origin of the 'spicy' character in commercial fractionated hop oils (Botanix Ltd., Paddock Wood, UK; pure hop aroma) was previously reported [1, 5]. GC-O and two dimensional GC techniques, led to the tentative identification of 14-hydroxycaryophyllene as the compound responsible for a potent 'woody, cedarwood' character. The objective of study 1 was to determine whether this odorant was also important in whole hop essential oils from the corresponding hop varieties.

### Experimental

Samples of whole hop essential oil (liquid CO<sub>2</sub> extracts) were obtained from Botanix Ltd., (varieties Target, Saaz, Hallertauer Hersbrucker (HHE) and Cascade). The odour active compounds were characterised using GC-O and CharmAnalysis with two experienced assessors. Odorants were tentatively identified using comprehensive two-dimensional gas chromatography (GC×GC) combined with time-of-flight mass spectrometry (TOFMS) [1].

### Results

**Table 1** compares the most potent (concentration / threshold) odorants detected in the four hop samples. The most potent odorant in HHE and Saaz samples was isovaleric acid (sweaty, cheesy) and has been identified as a potent odorant in hops [6, 7]. This compound is associated with aged hops and generally is considered to be an undesirable characteristic. The large concentrations observed are a consequence of the intentional hop storage prior to extraction to increase levels of oxygenated compounds. The woody, cedarwood odorant previously identified as 14-hydroxy- $\beta$ -caryophyllene in the spicy fraction of hops [1] was ranked the most potent odorant in Cascade and second in HHE.  $\beta$ -Ionone (floral – violet) was ranked the most potent odorant in Saaz, and linalool (floral – citrus) was the most potent odorant in Target. Other important odorants identified were geraniol,  $\beta$ -damascenone and humulene epoxide III.

These results demonstrated that while these odorants were common between the four hop varieties, the differences in the odour potency (i.e. concentration) are responsible for the differences in their sensory profiles.

**Table 1.** Potent odour regions in four hop varieties determined by CharmAnalysis

| RI*  | Odour Descriptors | Charm Values |             |             |             | Compound                           |
|------|-------------------|--------------|-------------|-------------|-------------|------------------------------------|
|      |                   | Cascade      | Target      | HHE         | Saaz        |                                    |
| 827  | Sweaty, cheesy    | 2421         | 150         | <b>8726</b> | <b>6140</b> | Isovaleric acid <sup>†</sup>       |
| 1683 | Woody, cedarwood  | <b>6909</b>  | 539         | 6140        | 2387        | 14-Hydroxy- $\beta$ -caryophyllene |
| 1497 | Floral – violet   | 2761         | 415         | 1921        | 4029        | $\beta$ -Ionone <sup>†</sup>       |
| 1108 | Floral – citrus   | 1566         | <b>1725</b> | 1028        | 1578        | Linalool <sup>†</sup>              |
| 1260 | Floral – rose     | 3042         | 338         | 1565        | 1779        | Geraniol <sup>†</sup>              |
| 1131 | Vinous            | 1611         | 779         | 643         | 1515        | <i>Exo-Fenchol</i>                 |
| 1122 | Pungent, floral   | 1218         | 53          | 2412        | 2726        | <i>Pentyl isovalerate</i>          |
| 1542 | Medicinal, clove  | 332          | 1069        | 312         | 746         |                                    |
| 1530 | Solvent, vanilla  | 295          | -           | 1346        | 1857        |                                    |
| 1397 | Cooked apple      | 319          | 139         | 1338        | 484         | $\beta$ -Damascenone <sup>†</sup>  |
| 1662 | Woody, cedarwood  | 409          | 203         | 443         | 275         | <i>Humulene epoxide III</i>        |

\* Retention index on BPX5 column (SGE); <sup>†</sup> Odour and RI confirmed with reference compound. Compounds in italic are tentative identifications.

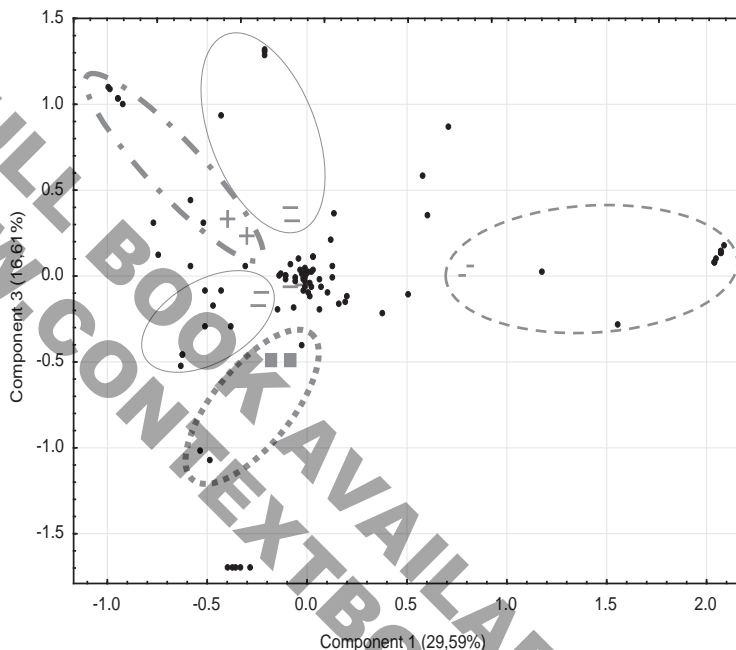
## Study 2 – Characterisation of three New Zealand hop varieties

### Background

Recent growth in the craft beer movement and launch of new hop varieties has led to a renewed interest in the identification of aroma compounds in hop essential oil and beer [4]. Recently developed and released New Zealand hop varieties impart unique aroma characteristics to beer, but the compounds responsible are not well-characterised. The objective of this second study was to identify the important aroma compounds in three NZ hop varieties (Nelson Sauvin (NS), Riwaka and Wai-iti).



weight determination. Esters and alcohols are the two main chemical classes among the odour impact compounds. Particularly, a large amount of ethyl esters may be responsible for the strong fruity notes of spirits. As far as we know, four new compounds have been tentatively identified for the first time in brandy (Table 1). All of them but the furan derivative appear to be discriminant (or “specialist”) compounds.



**Figure 1.** Correspondence Analysis: detection frequencies of OAs (black dots) within samples (grey markers). Constructed ellipses materialise the OAs-sample affiliation. The different sample symbols (+, -, ■ and -) indicate the growth areas.

By observing the attributes description of OAs, the construction of an aroma profile for each growth area was then possible. Similar terms were first grouped into 11 main categories: fruity, floral, empyreumatic, green, undergrowth, chemical, vegetable, nutty, spicy, unpleasant and sweet. For each spirit, the intensity of these categories was based on their citation frequencies of the whole panel. This profile showed that categories may vary a lot within spirits (i.e. intensity of fruity and floral categories may be opposed in some but not all samples). Looking again at fruity and floral notes which are target aromas in brandy, it is not clear whether more of these notes will give a fruitier or more floral spirit, as other odours may interact to produce the final bouquet. This could be seen as a clear illustration of the importance of synergistic effects between the compounds leading to the creation of an overall brandy aroma.

The main conclusion of this work is that no growth-area specific compound was found, meaning that OAs are present in all samples more but it is the difference in odour profile (and potential synergistic effects) that define the sensory properties of a sample. This result suggests that growth area typicity may be due to an equilibrated balance between molecule concentrations rather than the expression of unique aromatic elements.

**Table 1.** Identification of four new compounds. <sup>a</sup> Linear retention indices. <sup>b</sup> Further injections were performed on DB-1701 (30 m x 0.25 mm i.d., 0.25 µm film thickness; J&W Scientific). <sup>c</sup> Main attribute categories cited. <sup>d</sup> Type of identification: MS = mass spectrum, CI = molecular weight confirmed by chemical ionisation (methane and/or ammonia).

| Name                                   | LRI <sup>a</sup><br>(DB-<br>WAX) | LRI <sup>a</sup><br>(DB-<br>1701 <sup>b</sup> ) | Attributes <sup>c</sup> | Identification <sup>d</sup>                 |
|--|----------------------------------|---|-------------------------|---|
| 1-(2,3,6-trimethylphenyl)but-1,3-diene | 1725                             | 1404  | Green &<br>Woody; Spicy | MS  |
| 1,2,4,5-tetramethylbenzene             | 1496                             | 1208  | Undergrowth             | MS, CI (CH <sub>4</sub> )                   |
| 1-(2,3,6-trimethylphenyl)butan-3-one   | 2243                             | 1739  | Sweet                   | MS, CI (CH <sub>4</sub> & NH <sub>3</sub> ) |
| furan-2-ylmethyl formate               | 1289                             | 978   | Medical ;<br>Chemical   | MS, CI (CH <sub>4</sub> & NH <sub>3</sub> ) |

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# Analysis of thiols by dynamic headspace sampling and thermal desorption

Mikael Agerlin Petersen, Inês Oliveira, Shujuan Zhang

University of Copenhagen, Department of Food Science, Rolighedsvej 26, DK-1958, Frederiksberg C, Denmark

Dynamic headspace sampling in combination with thermal desorption is a solvent-free, versatile, sensitive technique. Stainless steel tubes containing Tenax TA are commonly used, and to desorb the tubes, rather high temperatures are applied to avoid band broadening during transfer of volatiles to the GC column. This does, however, result in a high risk of breakdown of thiols. Using a central composite design, the influence of different combinations of temperature in three steps of the thermal desorption procedure was investigated. It was found that the temperatures in the centre point resulted in highest peak areas. Using this combination of temperatures, the effect of using glass traps instead of steel traps was investigated, and it was shown that areas obtained using steel traps could be reduced by up to 89% compared to glass traps.

## Introduction

The thiols include several potent odorants that are found in a variety of food products, for example wine. Thiols are very reactive and are therefore easily degraded during analytical procedures. Thiols are degraded at high temperatures and the presence of metals like iron can accelerate breakdown. Andersen et al. [1] demonstrated that methanethiol reacted to form dimethyl disulfide during dynamic headspace sampling and almost half a century ago Wallace [2] showed that metal oxides, including ferric oxide, could react with thiols to form sulfides.

In dynamic headspace sampling, traps consisting of stainless steel tubes with Tenax TA as trapping material are commonly used. Furthermore, rather high temperatures are applied to desorb the traps rapidly to avoid band broadening in the GC column. This gives a high risk of breakdown of thiols.

In the current study, the influence of temperature in different steps of the desorption procedure was investigated and glass traps were compared to stainless steel traps to determine whether this affected thiol breakdown.

## Materials and methods

### *Materials*

An artificial wine model system was used (12% ethanol in water with 3.5 g/L tartaric acid and pH adjusted to 3.4 by addition of 1M NaOH). A variety of thiols representing both negative and positive notes in wine were added in known amounts.

### *Dynamic headspace sampling*

The wine model (20 ml) was purged using purified nitrogen at a flow rate of 100 ml/min for 20 min at 37°C. The thiols were trapped using 200 mg of Tenax TA in either stainless steel tubes or glass tubes.

### Thermal desorption

The tubes were thermally desorbed using a Perkin Elmer Turbomatrix ATD350 with internal sample lines and valve surfaces coated to eliminate interaction especially between sulfur analytes and steel surfaces. The thermal desorber uses a two-stage procedure, where the first stage is a desorption of the Tenax tube followed by a re-trapping on a smaller Peltier-cooled (5°C) trap made of glass, containing 30 mg of Tenax TA. The second stage is desorption of the trap to transfer volatiles to the GC column. A combination of flash-heating and an outlet split (10:1) ensures rapid transfer, thus avoiding band broadening.

The effect of temperature in three parts of the analytical procedure was investigated:

Primary desorption (transfer of trapped components from glass tube to the internal cold trap): 120°C - 160°C - 200°C.

Secondary desorption (transfer of trapped components from internal cold trap to transfer line): 150°C - 200°C - 250°C.

Transfer of trapped components to GC through a heated transfer line: 80°C - 152°C - 225°C.

A central composite design with 16 combinations of temperatures was created using the software package JMP ver. 11.1.1 (SAS Institute, Cary, NC).

Finally the effect of exchanging glass tubes with steel tubes was investigated, and limits of detection were estimated. The steel tubes were analysed using centre point temperatures (primary desorption 160°C; secondary desorption 200°C; transfer line 152°C). LOD was estimated as the concentration where signal-to-noise ratio = 3 assuming a linear relationship between peak area and concentration.

### Results

Table 1 shows p-values from ANOVA on the central composite design data. It is seen that only primary desorption temperature and transfer line temperature significantly influenced the peak areas obtained. 2-Methyl-3-furanthiol and 2-mercapto-ethanol were not significantly influenced by temperature in the ranges tested.

**Table 1.** Significance of temperature effect on GC peak areas (central composite design).

| Compound                               | p-values           |                      |               |
|--|--------------------|----------------------|---------------|
|  | Primary desorption | Secondary desorption | Transfer line |
| 2-methyl-3-furanthiol                  | 0.34               | 0.41                 | 0.43          |
| 4-mercapto-4-methylpentan-2-one (4MMP) | 0.02               | 0.17                 | 0.37          |
| furfuryl mercaptan                     | 0.78               | 0.55                 | 0.05          |
| 2-mercaptoethanol                      | 0.31               | 0.41                 | 0.44          |
| 3-mercaptohexyl acetate (3MHA)         | 0.01               | 0.88                 | 0.003         |
| 3-mercaptohexanol (3MH)                | 0.17               | 0.43                 | 0.005         |
| p-mentha-8-thiol-3-one                 | 0.002              | 0.66                 | 0.48          |

From Figure 1 it can be seen that the lowest primary desorption temperature (120°C) resulted in reduced peak areas of three of the sulfur compounds investigated, probably due to incomplete desorption of the primary trap. There was no significant difference between 160°C and 200°C.

# Volatile flavours through enzyme catalysis

**Ralf G. Berger**

*Institute of Food Chemistry, Gottfried Wilhelm Leibniz Universität, Callinstrasse 5, D-30167 Hannover, lci.uni-hannover.de*

Biocatalysts will be indispensable to implement the principles of green chemistry in the field of flavour generation. Lipases, used since the 1980s to synthesize fruit esters, pioneered the field. Oxidoreductases form volatile aldehydes, such as vanillin or piperonal, through the cleavage of double bonds, convert vanillin to divanillin forming carbon-carbon bonds through a radical mechanism, form smoke flavour by carbon-carbon cleavage, and terpenoids by oxidizing non-activated carbons of terpene hydrocarbons. Enzymes without co-factor requirement, such as lipoxygenases and laccases, are desired for industrial application. QTOF-MS-MS for *ab initio* sequencing accelerates the screening for a target enzyme through data base aided homology searches. Heterologous hosts are then used to create pure, tailored catalysts and to generate their volatile reaction products.

## Natural flavours from biocatalysis

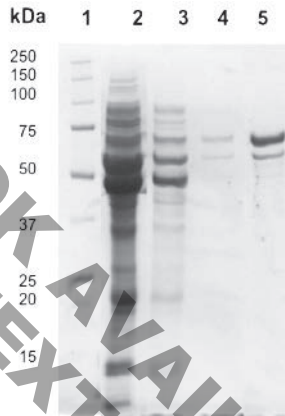
The transition from petroleum-based to a more sustainable, biomass-based production of chemicals is in full operation. In the field of food flavours, the effective European (EG 1334/2008) and US laws (CFR - Title 21 of the FDA) reflect the consumer preference and the superior properties of bioprocesses defining a 'natural flavouring substance' as a compound 'obtained by appropriate physical, enzymatic or microbiological processes from material of vegetable, animal or microbiological origin ....' or derived from 'enzymolysis' and 'fermentation' (CFR), respectively [1]. There are, however, critical consumers expressing concerns when it comes to recombinant strains and enzymes, and sometimes towards biotechnology in general.

## Enzymes are superior catalysts

Enzymes are edible, globular proteins with catalytic properties built from amino acid modules. They act in a reproducible and selective manner at ambient conditions without the need of solvents and toxic metals. Hundreds of enzymes and the matching co-substrates occur in food and in every microbial, plant and animal cell as endogenous constituents. Enzymes of yeasts change fruit musts, like enzymes of lactic bacteria acidify and flavour moist cereal flour, left-over milk, or minced meat and vegetables. Modern biotechnology originates from these empirical processes applied since millennia. All of these fermented foods possess a long history of safe use, a high level of chemical and microbial safety, and some of them are even supposed to maintain a balanced intestinal flora. Food enzymes improve the digestibility and lower health risks of food. Prominent examples are  $\beta$ -galactosidases (lactose intolerance), asparaginase (acrylamide reduction) or prolyl peptidases (coeliac disease).

Because enzymes are highly efficient, nature-invented mechanisms to limit their activity, factors, such as substrate and product inhibition, co-factor deficiency, and

inactivation by specific inhibitors and non-specific chemical or physical means need to be mentioned. Previously quoted scientific concerns on enzymes from recombinant sources, such as remaining marker genes coding for antibiotics resistance, have been overcome by food grade vectors and production hosts. Genetic engineering protocols can be performed successfully only after knowing the exact amino acid sequence of the enzyme and the coding nucleotide sequence. The over-producing genetically modified microorganism accumulates an excess of the pure target enzyme as the inducer controlled gene product. One example is the commercial recombinant chymosin from camel [2], another is a recombinant dioxygenase used for the peroxidation of terpene hydrocarbons to flavours [3] (Figure 1).



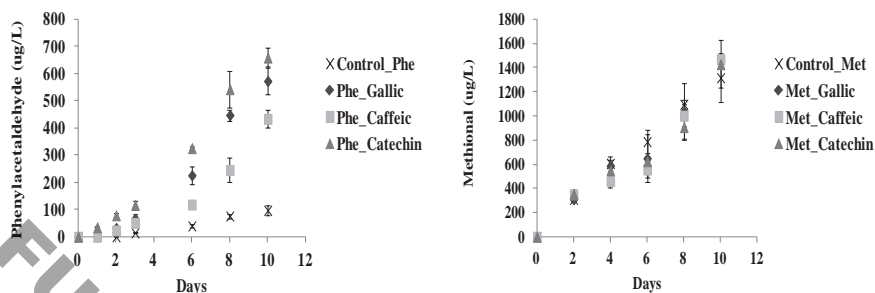
**Figure 1.** Sodium dodecyl sulfate-polyacryl-amide gel electrophoresis (SDS-PAGE) of a dioxygenase from *Pleurotus sapidus* expressed in *Escherichia coli*. 1: mass marker; 2: crude lysate; 3 and 4: wash fractions; 5: recombinant enzyme and below a co-expressed chaperone [3].

### Enzymes from food for food flavours

*Basidiomycota* and *Ascomycota* are referred to as 'higher fungi' (Subkingdom *Dikarya* in the Kingdom *Fungi*). Saprotrophic basidiomycetes are commonly found on forest detritus, leaf litter, and fallen trees. Accordingly, agro-industrial side-streams, such as peels, husks, and straw, molasses, pomace and press cakes support their growth in surface or submerged culture. The vegetative cells spread out in the subterranean sphere. Others grow symbiotically with trees (mycorrhizae). More than 950 species of the basidiomycota are regarded as edible; in others words, they are food in one part of the world or another.

To thrive on lignin (a recalcitrant network of polycondensates and phenyl-propanoid polymers) fungi secrete an impressive diversity of hydrolases and oxidoreductases, coded by divergent gene families. These unique enzymes are not only a functional component of complex communities in soil and forest and part of the global carbon cycle, but they are also attractive candidates for future industrial biotechnologies including the generation of flavour compounds. Particular advantages of these enzymes are substrate inducibility, operational stability, good water solubility, and a multitude of reactions catalysed (<http://www.basidionet.uni-hannover.de>).

Enzymes are typically screened according to substrate and reaction specificity using colour reagents or photometric assays. A sequence-based search is possible if reference data are available. Once the amino acid sequence is known, the concerted (directed

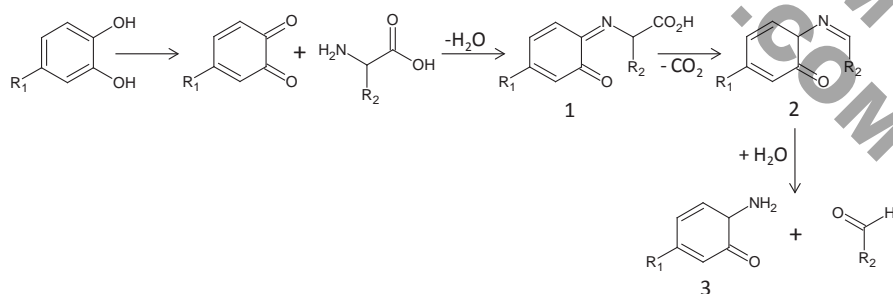


**Figure 1.** Evolution of phenylacetaldehyde and methional in the wine-model system used for the study of Strecker aldehydes;  $n=2$ .

The analogous reaction with methionine also produced the Strecker aldehyde methional (Figure 1), but a similar increase in this aldehyde was observed by the direct degradation of methionine (Control\_Met) in the presence of ROS (Figure 1). Methionine is very sensitive to oxygen, and readily oxidised to the sulfoxide and sulfone. This could have limited the reaction with quinones. These results suggest that Strecker aldehydes can be formed by quinone intermediates at wine pH. The reaction products between phenylalanine and the *ortho*-quinones formed from either gallic acid, caffeic acid or (+)-catechin, under these model-wine conditions, were tentatively determined by MS analysis. Samples were analysed after 1 hour of reaction.

#### Reaction products of phenylalanine with gallic acid, caffeic acid, and (+)-catechin *ortho*-quinones

The proposed initial Michael addition of a  $\alpha$ -amino acid with an *ortho*-quinone to form the  $\alpha$ -aminobenzoquinones were screened by full MS for phenylalanine with gallic acid, caffeic acid, and (+)-catechin *ortho*-quinones. The ions  $m/z$  332.3, 342.3, and 452.5 (negative mode) corresponding to phenylalanine adducts to gallic acid, caffeic acid, and (+)-catechin quinones moieties ( $C_{16}H_{15}NO_7$ ,  $C_{18}H_{17}NO_6$ , and  $C_{24}H_{23}NO_8$ ), respectively were not observed. This result suggests that these compounds did not result from the Michael addition of phenylalanine onto the quinones. Nevertheless, a similar reaction as reported by Strecker [7] to effect decarboxylation/deamination of  $\alpha$ -amino acids is proposed (Figure 2).



**Figure 2.** Formation of Strecker aldehydes from *ortho*-quinone assisted decarboxylation/deamination of  $\alpha$ -amino acids.

Figure 2 and Table 1 illustrate the formation of Strecker aldehydes from *ortho*-quinones and the corresponding mass-selected precursor ions (negative mode) of the proposed reaction products. Results showed that for gallic acid, structures 2 and 3 were proposed with  $m/z$  270.3 and 168.1, whereas for caffeic acid, structures 1, 2 and 3 were proposed with  $m/z$  of 324.3, 280.3, and 178.1. Finally, for (+)-catechin, the corresponding  $m/z$  434.4, 390.4, and 288.3 were not observed. These results could be explained by the different phenolic reactivities. (+)-Catechin was the phenolic compound which produced the highest levels of phenylacetaldehyde (Figure 1), suggesting that the formation of Strecker aldehydes from this phenolic is faster than that of gallic or caffeic acids, and so the intermediate products were not observed. This could be related with a faster quinone formation, i.e., a faster phenolic oxidation.

**Table 1.** Mass-selected precursor ions (negative mode) of the proposed Strecker aldehyde reaction products formation through assisted *ortho*-quinones.

| Structure type | [M-H] <sup>-</sup> $m/z$    |                              |                               |
|----------------|-----------------------------|------------------------------|-------------------------------|
|                | 0.6 mM Gallic<br>2.4 mM Phe | 0.6 mM Caffeic<br>2.4 mM Phe | 0.6 mM Catechin<br>2.4 mM Phe |
| 1              | 314.3                       | 324.3                        | 434.4                         |
| 2              | 270.3                       | 280.3                        | 390.4                         |
| 3              | 168.1                       | 178.1                        | 288.3                         |

Following (+)-catechin, gallic acid promoted higher levels of phenylacetaldehyde (Figure 1). In the same way, only structures 2 and 3 were observed with this phenolic (Figure 2). The reason for not finding the structure 1 may be related to its faster reactivity as well. Finally, caffeic acid was the phenolic that generated the least phenylacetaldehyde (Figure 1). All the three structures 1, 2 and 3 (Figure 2) were tentatively identified in the reaction medium with caffeic acid, transition metal ions, and phenylalanine. Furthermore, all reactions moieties have a product ion at  $m/z = 163$  ([M-H]<sup>-</sup>). This compound is phenylpyruvic acid, the  $\alpha$ -keto acid derived from phenylalanine oxidative deamination. This compound was quantified, at the end of the protocol (after 10 days) in all four reaction media (Control\_Phe, Phe\_Gallic, Phe\_Caffeic, and Phe\_Catechin). Results showed similar concentrations of phenylpyruvic acid in the four reaction media, indicating that this compound is a result of phenylalanine oxidative deamination and had contributed to certain level of phenylacetaldehyde in all samples (Figure 1).

MS/MS fragments were found for the precursor ions of the caffeic and gallic acids quinone moieties. Figure 3a and Figure 3b represents the MS/MS fragments occurred for caffeic acid and gallic acid quinone products respectively, with phenylalanine.

In conclusion, Strecker aldehydes can be formed by quinone intermediates at wine pH. (+)-Catechin was the phenolic that had produced the highest amounts of phenylacetaldehyde, followed by gallic acid and then caffeic acid. A similar reaction to that reported by Strecker [7] to effect decarboxylation/deamination of  $\alpha$ -amino acids is proposed to explain Strecker aldehydes formation.



# Sensory omission studies: Application of a novel research method to a commercial environment

Perrine Delime<sup>a</sup>, Neil Desforges<sup>b</sup> and Joanne Hort<sup>a</sup>.

<sup>a</sup> Division of Food Sciences, University of Nottingham, LE12 5RD, UK.

<sup>b</sup> WALTHAM® Centre for Pet Nutrition, Mars Petcare, Waltham-on-the-Wolds, LE14 4RT UK

Sensory omission is an experimental method used to identify the key compounds contributing to a flavour. A novel method for omission testing involving the Same-Different test and the Thurstonian measure,  $d'$ , was applied to a model savoury flavour. Results from separate omission experiments determined the orthonasal impact of removing different fractions of each individual volatile and the retronasal impact of the presence and absence of tastants on volatile omission. All omission tests were performed by one hundred assessors and  $d'$  values were calculated using Receiver Operating Characteristic (ROC) curve fitting software. Orthonasal and retronasal results indicate that 2-furanmethanethiol was the most important compound contributing to the savoury flavour. Our new approach to sensory omission provides insight into the perception of flavour mixtures with  $d'$  indicating the relative contribution of an odorant to the overall perceived aroma. It can also be used to increase our understanding of inter- and intra-modal interactions in flavour perception.

## Introduction

Often, the selection of the key volatiles contributing to a food flavour is based on the idea that the higher the perceived intensity of a volatile, the higher its contribution to the flavour. Odour Activity Values (OAV) or dilution techniques such as Aroma Extract Dilution Analysis (AEDA) or Charm<sup>TM</sup> analysis [1] are commonly used to determine key volatiles contributing to the flavour of food products [2]. However, due to interactions between volatiles, volatiles with higher OAV can actually be of only minor importance, whereas volatiles with lower OAV can be essential for the flavour [3]. In this case, sensory omission experiments have successfully been applied to identify the key volatiles of recombinants [4-6].

Sensory omission experiments involve omitting one volatile from a flavour mixture and comparing the new sample to the original flavour [3]. Omission experiments have been used to assess the contribution of individual volatiles to a flavour model [7, 8] and to show interactions between volatiles [9,10]. Recently, a new approach has been introduced in sensory omission testing, using the same-different test and Thurstonian modelling [11]. This approach has proved to be more discriminating compared to the approach using the more common discrimination test, the triangle test.

The aim of this study was to (1) measure the relative importance of each individual volatile within a flavour model, (2) investigate intra-modal interactions between volatiles and (3) investigate cross-modal interactions between volatiles and tastants.

## Materials

*Savoury flavour model preparation.* The savoury flavour (Table 1) was developed from a boiled beef flavour [12]. 12-Methyltridecanal was supplied by Symrise (UK), and all other chemicals were purchased from Sigma-Aldrich (UK). It was prepared using the concentrations in Table 1. Samples were prepared by pipetting the volatiles into Propylene Glycol (PG) using a calibrated balance. Samples were sealed in Duran® GL 45 laboratory glass bottles (SCHOTT, USA) and mixed on a roller bed for 30 minutes. The savoury flavour was diluted in Evian® mineral water (DANONE Group, France) at 0.1% w/w. Samples were kept at 4°C and used within 24 h. All flavour samples were removed from the refrigerator at least one hour prior to testing to ensure aromas were at room temperature (20 °C +/- 2°C).

**Table 1:** Concentrations of volatiles in the savoury flavour, and their respective detection thresholds and Odour Activity Values (OAVs).

| Flavour block | Volatile                          | Concentration in PG (mg/kg) | Detection threshold (µg/kg) | OAV*  |
|---------------|-----------------------------------|-----------------------------|-----------------------------|-------|
| Top note      | 2-Methylpropanal                  | 23.4                        | 0.7                         | 32    |
| Meaty block   | 2-Furanmethanethiol               | 43.5                        | 0.01                        | 4,263 |
|               | 4-Hydroxy-2,5-dimethyl-3-furanone | 13,600                      | 10                          | 1,334 |
|               | 3-Mercapto-2-butanone             | 104                         | 0.7                         | 28    |
|               | 2-Methyl-3-furanthiol             | 36.0                        | 0.007                       | 4,885 |
|               | 3-Methylthiopropional             | 54.0                        | 0.2                         | 262   |
| Fatty block   | E,E-2,4-Decadienal                | 27.0                        | 0.2                         | 135   |
|               | 12-Methyltridecanal               | 962                         | 0.1                         | 962   |
|               | 1-Octen-3-one                     | 9.40                        | 0.05                        | 94    |

\* OAV 
$$\frac{\text{Concentration}_{\text{odorant}}}{\text{Detection threshold}_{\text{odorant}}}$$

## Methods

*Sensory omission testing.* For each experiment, 100 volunteers (~70% female, aged between 18 and 25) were recruited from students at the University of Nottingham. All subjects gave informed consent to participate in the study. Assessors were instructed to fast (except water) at least one hour prior to the sessions.

*Orthonasal delivery.* Screw top 20-mL glass bottles containing 10mL of aroma sample were presented to the assessors. Assessors were instructed to sniff the samples and replace the lid immediately to prevent the aroma dispersal.

*Retronasal delivery.* Assessors were instructed to sip from a 20-mL sample through the straw of a lidded pot. Mineral water and crackers were provided as a palate cleanser between samples to minimize carry over effect.

*Same-different testing.* For each same-different test, assessors were presented with two samples and were instructed to assess samples from left to right. They were allowed to re-evaluate the samples if necessary. Assessors were asked if they thought the samples were the same or different and then asked to rate the sureness of their decision, using a four point scale ('very unsure', 'unsure', 'sure', 'very sure').

**Table 1.** Ethanol, glucose and tannin contents in samples

| Sample | Ethanol (% v/v) | Glucose (g.L <sup>-1</sup> ) | Tannins (mg L <sup>-1</sup> ) |
|--------|-----------------|------------------------------|-------------------------------|
| c      | 12              | 4.0                          | 73.0                          |
| b      | 12              | 9.0                          | 7.3                           |
| bc     | 12              | 9.0                          | 73.0                          |
| cp01   | 13              | 6.5                          | 40.2                          |
| a      | 14              | 4.0                          | 7.3                           |
| ac     | 14              | 4.0                          | 73.0                          |
| ab     | 14              | 9.0                          | 7.3                           |
| abc    | 14              | 9.0                          | 73.0                          |

**Table 2.** Composition of the wine volatile mixture with the corresponding mass ion ( $m/z$ ) measured for each compound by PTR-MS

| Flavour compounds       | Concentration (mg.L <sup>-1</sup> ) | Measured $m/z$ |
|-------------------------|-------------------------------------|----------------|
| <i>trans</i> -2-Hexenol | 2.0                                 | 83             |
| 2-Phenyl ethanol        | 12.0                                | 105            |
| Ethyl acetate           | 74.0                                | 62             |
| Ethyl butyrate          | 40.0                                | 117            |
| Ethyl octanoate         | 2.0                                 | 173            |
| Isoamyl acetate         | 8.0                                 | 71             |
| Linalool                | 2.0                                 | 81             |
| Octanoic acid           | 10                                  | 145            |
| Hexyl acetate           | 15                                  | 79             |

#### *Nose space analysis by PTR-MS*

For each sample the assessors (n=6) consumed 15 ml with 15 min between samples. Volatile release of mass ions associated with each VOC (Table 2), were measured from the nose space over 80 cycles. A free consumption protocol was used, while assessors' breathing patterns were controlled. The time of sample ingestion and subsequent time of swallowing were recorded. The resulting PTR-MS cps data was normalised to H<sub>3</sub>O<sup>+</sup> cps. Nose-space release profiles of all samples for all assessors were collated separately for each compound and analysed by GPA.

#### *Sensory analysis*

The effect of ethanol, glucose and tannin concentrations on perceived wine intensity was determined by a trained panel (n=10) measuring a single attribute, wine flavour intensity and, in separate sessions, by free choice profiling to examine any flavour quality changes.

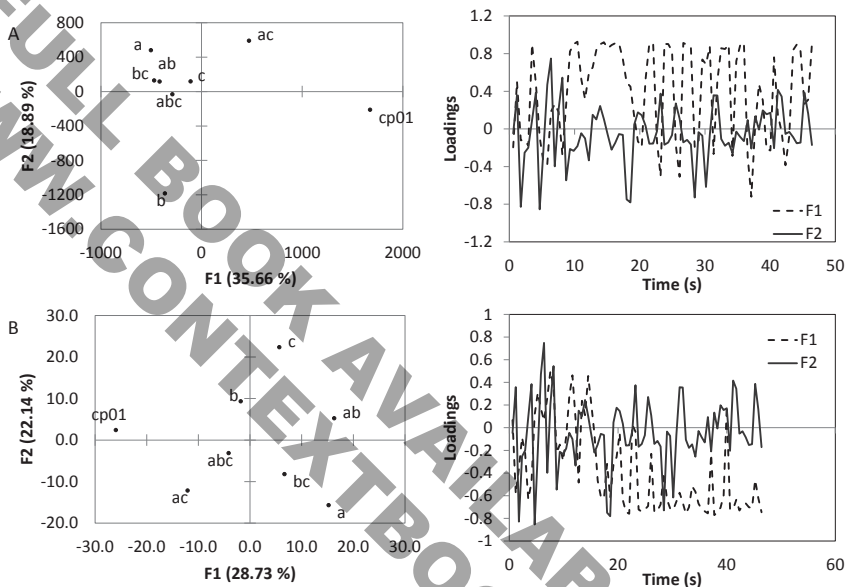
## **Results and discussion**

#### *Nose space analysis*

Release profiles of each VOC varied between compositions and between assessors. Use of a free swallowing profile and other physiological factors resulted in misalignment of the consumption profiles between samples (i.e. both between assessors and within each assessor).

The use of GPA allowed analysis of the data without alignment, and produced loading plots that were typical of nose-space profiles (Figure 1 A & B). The loadings across each factor attributed variation in the data to pre-swallow, swallow and post-swallow. It is thought that the baseline is a feature in the loadings due to relative changes in the nose space concentrations between samples.

In Figure 1 B, variation between samples cp01 and ab was depicted along F1, while differences between a and c were shown along F2 in the release of 2-phenyl ethanol.



**Figure 1.** GPA scores and loadings plots for ethyl butyrate (A) and 2-phenyl ethanol (B). Scores plots depict sample differences in response to nose space release of compounds; the loadings plots show the release of compounds over time (0-50 s)

No clear effect of sample composition on sample differences due to nose space release was observed (Figure 2). Though for isoamyl acetate, hexyl acetate and phenyl ethanol, separation on F2 appeared related to ethanol content. The distribution of wines in the scores plots, were similar between esters, and also between alcohols. However, a shift in the positioning of the samples CP01, ac and b relative to the other samples, can be observed as the ester hydrophobicity changes. Overall, GPA provided a useful means to examine the nose space data without requiring data alignment or calculation of secondary parameters. Use of other multivariate data analysis techniques like PARAFAC2 analysis may enable similar data projection [4], which may overcome the loss of the concentration data, a disadvantage associated with GPA.

### Sensory results

An ethanol effect and ethanol-tannin interaction ( $p < 0.05$ ) were observed on the perceived wine flavour intensity of the model wines. Wine flavour intensity tended to increase as ethanol concentration increased, The Free Choice Profiling suggested that, in addition to an intensity change, the flavour quality also changed.

# Understanding flavour as a major driver of product quality

**Imre Blank**

*Nestlé Product Technology Centre York, Haxby Road, York, YO91 1XY, UK*

Food products usually deliver several quality attributes such as aroma, taste, colour, and texture. Raw materials and ingredients play a key role in conjunction with adequate food processes in developing palatable products with high nutritional value. Major food constituents, such as carbohydrates, proteins, lipids, and polyphenols are transformed into food products using bio-assisted and/or thermal approaches. They trigger reactions and changes at molecular level that finally will determine sensory attributes and the overall food quality. Understanding the changes in molecular composition during food processing and storage has been one of the major challenges in academia and food industry resulting in valuable knowledge to ensure product quality. Phenomena such as aroma freshness and authentic taste can be described to a great extent by well-defined molecules and changes in molecular composition that is illustrated using coffee as an example.

## Introduction

Besides the stimulating effect of coffee, the main drivers for its consumption are the complex aroma and the powerful taste of the beverage. Scientific knowledge of coffee has advanced considerably during recent decades. In the headspace of coffee, hundreds of substances have been identified, and the ones mainly responsible for the aroma, the so-called key impact compounds, have been elucidated by gas chromatography–olfactometry and omission experiments [1-5]. In addition, significant progress has been made in the identification of key taste compounds. Caffeoyl quinic acid lactones [6-7], 4-vinylcatechol oligomers [8], diketopiperazines [9], and (furan-2-yl)methylated benzene diols and triols [10] have been identified as compounds with a major impact on coffee bitterness.

Many of these impact flavour components derive from multiple reactions, including Maillard-type reactions, caramelisation, polyphenol degradation, polymerisation, lipid oxidation, and pyrolysis. Polyphenols lead on the one hand to the generation of guaiacols and cresols that contribute to coffee flavour. On the other hand, they are precursors of phenolic compounds that can trap thiols and thus induce aroma degradation, which can be a particular concern when coffee is stored as a bottled beverage or as a liquid concentrate. Coffee aroma staling is mainly due to the decrease of the coffee-like smelling compound 2-furfurylthiol (FFT) trapped by the polyphenol degradation products di- and trihydroxybenzenes, and in particular hydroxyhydroquinone [11]. This oxidative coupling reaction may also affect other odour-active thiols such as methanethiol that are present in freshly brewed coffee.

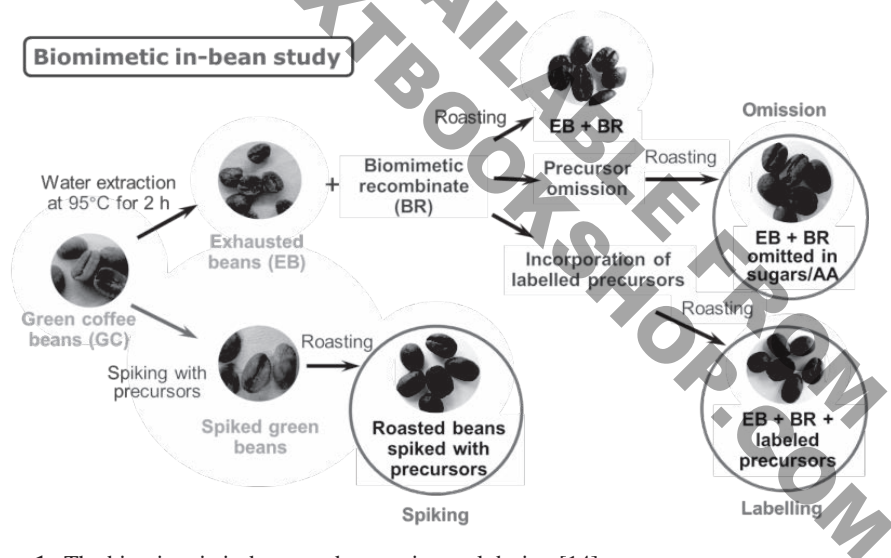
Although the flavour qualities of the identified single compounds are known, their individual contribution to a complex mixture such as coffee flavour remains unclear. Until today, sensory profiling remains the most accurate method for describing coffee flavour. However, it would be desirable to statistically link sensory descriptors to the concentration

of flavour compounds [12] in a statistical model for the prediction of coffee aroma based on sensory profiling and analytical headspace measurements. To better understand the link between sensory perception of coffee and quantitative analytical data, coffee blends have recently been assessed [13] by instrumental analysis and sensory profiling, and the two resulting datasets were statistically correlated. Several flavour compounds analysed in the study exhibited a good correlation with specific sensory descriptors and may therefore be used as chemical markers for the characterisation of flavour profiles.

## Results

### *Coffee aroma and freshness*

The formation of coffee flavour is a highly complex phenomenon. Aroma- and taste-active components are formed from sugars, amino acids, trigonelline, chlorogenic acids, and organic acids by Maillard-type reactions, caramelisation, and fragmentation. An elegant way to elucidate the formation pathways of these components is to work in model systems which represent well the actual product. In the coffee area, Poisson et al. [14] developed a few years ago the so called 'biomimetic in-bean' approach (Figure 1). The coffee bean is used as a reactor as it is very solid and can work under high pressure. The approach consists of extracting the water soluble precursors from the green coffee bean and using then this depleted coffee bean as a reactor by reintroducing flavour precursors into the bean and then roasting it under defined conditions. Then the sensory profile is evaluated and analytical composition of the modified bean characterised to understand how flavour components are generated upon roasting.



**Figure 1.** The biomimetic in-bean study experimental design [14].

Adding a certain amount of precursor compounds is one approach to study formation pathways. The idea is to learn how key components are formed, from which precursors, and in what amounts. The second idea is then to add just some of the water soluble precursor components. In that case the role of those components can be evaluated which were omitted from the recombine fraction and this will indicate their importance in flavour formation. Because a component can be generated by several pathways, the use of labelled

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