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Headspace delivery of limonene from the serum and non-serum fractions of orange juice *in-vitro* and *in-vivo*

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ABSTRACT

The impact of orange juice pulp on the physical release of limonene to the headspace of freshly prepared orange juice was evaluated both *in-vitro* and *in-vivo*. Atmospheric pressure chemical ionization mass spectrometry was used to analyse the impact of the matrix on the dynamic release of the volatile aroma compound, limonene, in orange juice. Pulp and aqueous serum was isolated (by centrifugation and filtration) from freshly prepared orange juice and subsequently reconstituted at varying pulp addition levels in serum (0g/100 g–20 g/100 g, wwb). The addition of pulp significantly enhanced the static headspace concentration of limonene with a 210 fold increase with 10 g/100 g pulp addition. In addition, pulp enhanced the ability of the orange juice serum to replenish limonene in the headspace after dynamic headspace dilution. The release of limonene was studied under realistic consumer consumption conditions (In-nose delivery) by atmospheric pressure chemical ionisation-mass spectrometry; pulp significantly enhanced the amount of limonene exhaled in the nasal airflow (retronasal delivery). Surprisingly, given the variations in limonene concentration, naïve consumers did not perceive samples as significantly different on consumption. This is presumably due to the wide range of other aroma compounds not evaluated in this study that contribute to the perceived aroma.

1. Introduction

The aroma of orange juice is one of the most characteristic attributes of all citrus juices (Jordan, Tillman, Mucci, & Laencina, 2001) and fresh orange juice aroma is considered a reference against which all juices are judged (Brat, Rega, Alter, Reynes, & Brillouet, 2003). Orange juice aroma consists of a number of volatile aroma compounds with a variety of physicochemical properties, located in a range of physical structures within the orange juice.

Fresh, hand squeezed orange juice is a heterogeneous multiphase system consisting of serum, a clear aqueous phase containing small oil droplets (cloud), soluble compounds and pulp, a water insoluble phase (Brat et al., 2003). Orange pulp consists of both coarse particles (>2 μm) that tend to settle upon storage and fine particles (<2 μm) (Mizrahi & Berk, 1970), which under favourable conditions remain suspended in the serum (Baker & Bruemmer, 1969). Both the pulp suspension and cloud emulsion enhance the colour, flavour, aroma, and mouthfeel of the orange juice, and are present in many commercial juices (Brat et al., 2003).

Some classes of volatile aroma compounds are distributed unevenly across the matrix with regions of elevated concentration in the pulp or the serum. For example, in citrus fruits monoterpenes and sesquiterpene were shown by Radford, Kawashima, Friedel, Pope, and Gianturco (1974) to be primarily associated with the pulp. Brat et al. (2003) studied the distribution of volatile compounds in the pulp, cloud, and serum and showed that monoterpenes and sesquiterpenes are primarily found in the pulp and cloud; esters and monoterpene alcohols were concentrated in the serum; and long chain aliphatic aldehydes were found to the greatest extent in the pulp. The serum is normally described as a pale yellow liquid that generally has little perceivable juice aroma on its own but acts as the carrier solvent for the distributed cloud emulsion and the macroscopic fragments of pulp (Baker & Cameron, 1999).

The effect of insoluble solids on the composition of aroma of orange juice was studied by Jordan et al. (2001), who showed that a reduction in insoluble solids corresponded to a reduction in the quantities of many volatile components in the headspace. For

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example, they reported that orange juice (containing serum and 3 g/100 g pulp) contained limonene at a concentration of 57 mg/kg, but when pulp was included at 10 g/100 g, the limonene concentration increased to 536 mg/kg (headspace solid phase micro-extraction gas chromatography mass spectrometry). It still remains unclear as to whether aroma compounds are associated with solid cell structures by adsorption of oil droplets onto the particles, physical entrapment inside the cell wall carbohydrate network (Mizrahi & Berk, 1970), or through chemical interactions between volatile compounds and polysaccharides (Dufour & Bayonove, 1999) or glycopeptides (Langourieux & Crouzet, 1997) in the pulp.

Different analytical methods, such as solid-phase micro-extraction (SPME) (Jordan et al., 2001) and liquid–liquid extraction with different organic phases like pentane–diethyl ether (Jella, Rouseff, Goodner, & Widmer, 1998), have been developed to determine the concentration of flavour components in fruit juices. However, to the best of the authors' knowledge, atmospheric pressure chemical ionization mass spectrometry (APCI-MS) has not been used to evaluate the *in-vivo* delivery of volatiles aroma compounds from orange juice as a consequence of pulp fraction. APCI-MS is commonly used for the real time analysis of gas-phases above food samples and in the gas phase within the nasal cavity during consumption (Linforth & Taylor, 2000; Rabe, Linforth, Krings, Taylor, & Berger, 2004; Tsachaki, Linforth, & Taylor, 2005).

Volatile compounds are perceived by consumers in a number of different ways. Prior to consumption, a combination of physico-chemical parameters (such as the partition coefficient (Fisk, Kettle, Hoffmeister, Virdie, & Silanes Kenny, 2012) and the mass transfer coefficient (Fisk, Boyer, & Linforth, 2012)), along with dynamic factors (such as mixing of the phases and airflow), determines the relative distribution of the volatile compounds between the food and its headspace (Marin, Baek, & Taylor, 1999). During consumption the availability of aroma molecules for perception is driven by a volatile's hydrophobicity, volatility, the surface tension of the system and various other interfacial matrix effects.

So far, it is not well known if pulp has any influence on the temporal delivery profile of odour compounds in orange juice even though it is a phase which is well characterised as containing and contributing volatile aroma compounds to the final product.

The objective of this study was to measure the limonene content of pulp and serum fractions of orange juice and to study the effect of pulp on the delivery of limonene to the headspace by APCI-MS in three different situations: equilibrium conditions (static headspace), disturbed headspace conditions (dynamic headspace) and during consumption (In-nose headspace).

2. Materials and methods

2.1. Materials

All chemicals were of analytical grade; chloroform, methanol, n-pentane, and diethyl ether were purchased from Panreac (Barcelona, Spain), and limonene and propyl benzene were purchased from Sigma Aldrich (Poole, United Kingdom). *Citrus sinensis* (L.) Navelina oranges (unwaxed, 50–90 mm diameter, no defects) were purchased locally in a supermarket (Nottingham, United Kingdom). Oranges were stored at 4 °C for no more than 24 h before analysis.

2.2. Sample preparation

Orange juice was obtained using a domestic kitchen juicer. Isolated orange juice was then centrifuged (15 min × 2700× *g*) using a CR3i multifunction centrifuge (Gormley, Canada) to separate the dense pulp and more buoyant supernatant. The isolated supernatant was filtered with filter paper to separate aqueous clouds and serum phase and then reconstituted with different amounts of pulp (0, 5, 10, 15, and 20 g/100 g, wet weight basis). Exact percentages were chosen to be comparable to previous studies and to commercial applications (Stinco et al., 2012).

2.3. Characterization of pulp and serum

2.3.1. Lipid content

Lipid content was analysed by the methodology, as described by Brat et al. (2003). 2 mL of distilled water and 6 mL of chloroform:methanol mixture (2:1) were added to the isolated pulp (5 g). Samples were mixed by vertical shaking for 30 s in a separating funnel and allowed to phase separate for 30 min. The lower organic phase was recovered while the upper phase was extracted a further three times with 6 mL of chloroform:methanol (2:1). Collected organic phase were pooled and dehydrated over anhydrous sodium sulphate and evaporated to dryness in a vacuum rotatory evaporator. All extractions were carried out in triplicate, the extracts weighed and lipid content calculated by gravimetric difference, average results were expressed as g/100 g wwb ± standard deviation.

2.3.2. Water content

Water content of samples was analysed as per Fisk, Linforth, Taylor, and Gray (2011) by drying within a Vacuum oven (Gallenkamp, Fisons, Loughborough, United Kingdom) for 48 h until constant weight.

2.3.3. Limonene content

Limonene was extracted according to the method described by Jella et al. (1998). Briefly, 4 mL of pentane–diethyl ether mixture (1:1) was added to 20 mL serum and 10 g pulp, and mixed on a roller mixer for 12 h. 25 μl of propyl benzene (50 mg/L) was added to the samples prior to extraction as an internal standard. The resulting emulsion was broken by centrifugation (5 min × 7500 RCF). The upper layer was carefully removed, dried and finally concentrated to a final volume of 500 μL. The extractions were carried out in triplicate, and each sample was injected three times, with an injection volume of 1 μL.

The isolated sample was chromatographically separated on a capillary column DB-Wax (60 m × 0.25 mm × 0.25 μm) from Varian (Walnut Creek, CA, USA) using an Agilent Technology 6890N GC-FID system (Palo Alto, CA, USA). The following conditions were used: injector temperature, 250 °C; detector temperature, 250 °C; carrier gas flow (He), 1 mL/min. The injections were made in split mode (split ratio 1:25) and the oven temperature was maintained at 40 °C for 3 min, then increased from 40 to 200 °C at 6 °C/min and eventually held for 6 min. Quantification of the limonene was carried out by the internal standard method (propyl benzene).

Linearity of responses was acceptable ($r^2 > 0.998$) across a standard concentration range that exceeded that of the samples (10–2500 μg/g).

2.3.4. Particle size

The particle size of the suspended pulp in the orange juice was analysed using a laser diffraction particle size analyser (LS 13 320, Beckman Coulter, High Wycombe, UK), capable of measuring particle diameters in the range 0.4 μm–2000 μm. Samples (1 mL) were introduced into the universal liquid module, and obscuration was maintained at 5% by dilution with deionised water (White, Fisk, Makkhun, & Gray, 2009).

2.3.5. Microscopy

Orange juices with pulp concentrations (0, 10, 20, and 100 g/100 g) were observed at the optical microscope Leitz Diaplan Microscope, (Wild Leitz, Heerbrugg, Switzerland) (×10) (Iwanaga et al., 2007).

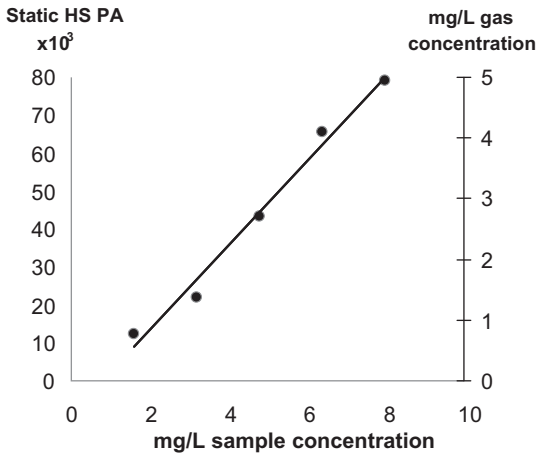

Fig. 1. APCI static headspace analysis calibration curve showing aqueous standard concentration (mg/L) against headspace peak area (HS PA) and predicted gas phase concentration (mg/L), predicted gas phase concentration is calculated from the literature air–water partition coefficient of limonene.

Images analysis was performed using Pixelink Software 2004 (Ottawa, Canada).

2.4. Headspace delivery of limonene

2.4.1. Atmospheric pressure chemical ionization mass spectrometry (APCI-MS)

A Micromass LCZ mass spectrometer was used, fitted with an MS Nose interface (Micromass, Manchester, U.K.) to sample the headspace above the solutions (Linforth & Taylor, 1999). Selected ion monitoring (SIM) analysis was used for all experiments. Cone voltage and ion monitored was 15 V and 137 *m/z*, respectively. MassLynx software (Micromass, Manchester, UK) was used for all data extraction.

2.4.1.1. Static headspace analysis. Orange juice (30 mL) samples (OJ) were placed in Duran graduated laboratory bottles (nominal size = 100 mL, real volume = 123 mL) (Sigma–Aldrich, Poole, U.K.) fitted with a one port lid that allowed headspace sampling. Samples were equilibrated at room temperature and the headspace was sampled through the port into the APCI-MS via a heated transfer line (120 °C), with a gas flow rate of 2.5 mL/min. Each sample was measured in triplicate following a fully randomised design.

Resultant detector responses (mV) were converted to Aqueous Standard Equivalents (ASE) by comparing the headspace to that of limonene control samples as analysed by static headspace (APCI-MS). Solutions with different concentration of limonene were measured and coefficient of determination was calculated ($r^2 = 0.985$). The air–water partition coefficient of limonene has previously been documented by Falk, Gullstrand, Löf and Wigaeus-Hjelm (1990) as 0.556 therefore simple calculations of limonene gas concentration can be completed and gas calibration curves can be constructed (mg/L in gas phase) (Fig. 1).

2.4.1.2. Dynamic headspace analysis. Sample headspace was measured by APCI-MS during 5 min of dynamic headspace dilution. 100 mL OJ samples were placed in Duran graduated laboratory bottles (nominal size = 100 mL, real volume = 123 mL) (Sigma–Aldrich, Poole, U.K.) fitted with a two port lid. After equilibration, N_2 was introduced through one port (70 mL/min) to dilute the headspace. Steady flow was achieved prior to analysis. As the gas flowed out of the second port, the exit gas flow was sampled by the APCI-MS (10 mL/min) over a 5 min period (Tsachaki et al., 2005). Each sample was measured in triplicate following a fully randomised design. The profiles were normalized (100%) to the signal intensity at the start of the time course (Fisk et al., 2011).

2.4.1.3. APCI In-nose. Each sample was consumed in triplicate by two panellists using a randomised block design. Each panellist was placed into a separate block to account for individual differences in aroma release caused by differences in physiology and flow rates between panellists. Panellists consumed 10 mL of each sample directly from the sample vial. A small plastic tube, leading to the MS, was immediately inserted into the left nostril. Once in place, the sample was swallowed and the panellist was instructed to breathe normally through the nose, keeping the mouth closed for the duration of the sampling period. Breath was sampled from the panellist (30 mL/min) over a 1 min period after swallowing (dwell time 0.02 s).

All in nose data is calculated relative to the In-nose headspace calibration curve formed through the consumption of a range of limonene calibration samples. Fig. 2 illustrates the response by panellists ($r = 0.996$). Where absolute detector responses (mV), as measured during the consumption of the samples, were converted to Aqueous Standard Equivalents (ASE) by comparing to the absolute detector responses (mV), as measured during the consumption of aqueous standards containing known amounts of limonene.

2.5. Paired comparison analysis

Evaluation of the perceived differences in limonene as defined by orange aroma and consumption flavour by the panellists was

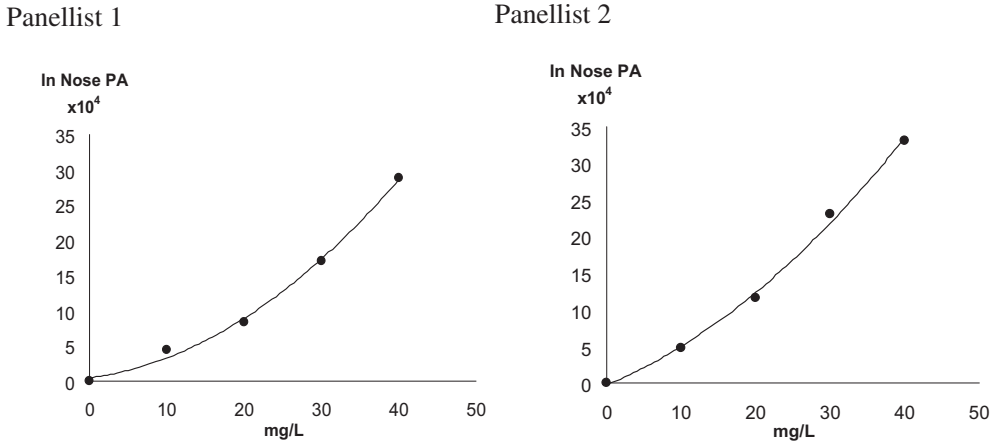

Fig. 2. APCI In-nose analysis calibration curve showing aqueous standard concentration (mg/L) against maximal peak area (PA) for two panellists.

completed by attribute specific difference tests (Paired comparison, ISO 5495, 2005). 30 untrained assessors were recruited from staff and students of University of Nottingham to take part in the study.

Two paired comparison tests were performed; 0 g/100 g versus 10 g/100 g pulp and 0 g/100 g versus 20 g/100 g pulp. For each test, assessors were presented with 2 samples and asked to first smell the sample and determine which one had the strongest orange aroma. Then, they were asked to taste the samples and determine which sample had the strongest orange flavour.

Samples (15 mL) were presented in dark amber glass bottles, labelled with random 3 digit codes, in a randomised order across the panel and under red light conditions to ensure no visual cues were available to panellists. Mineral water (Evian, France) and unsalted crackers (Matzo, U.K.) were provided for palate cleansing and all testing was performed in temperature controlled, individual test booths. Data was collected using Fizz software (Biosystemes, France)

2.6. Data analyses

Analysis of variance, followed, where appropriate by Tukey's post hoc testing, was used to evaluate significant differences within the APCI-MS datasets (Statistica 8 for Windows, StatSoft 2007). Paired comparison tests were analysed as two-tailed tests using Fizz software (Biosystemes, Couternon, France).

2.7. Experimental approach

To further understand the whole study, a flow chart summarizing the complete process is shown in Fig. 3

3. Results and discussion

Our findings show that the delivery of the lipophilic cyclic terpene aroma compound, limonene, is significantly impacted by the pulp and lipid fraction of orange juice, both *in-vivo* and *in-vitro*.

3.1. Pulp and serum characterization

3.1.1. Lipid concentration

As lipids play a major role in the association of volatiles by pulp, the lipid content of isolated pulp fractions was measured. Total lipids were extracted from wet pulp (pulp water content was

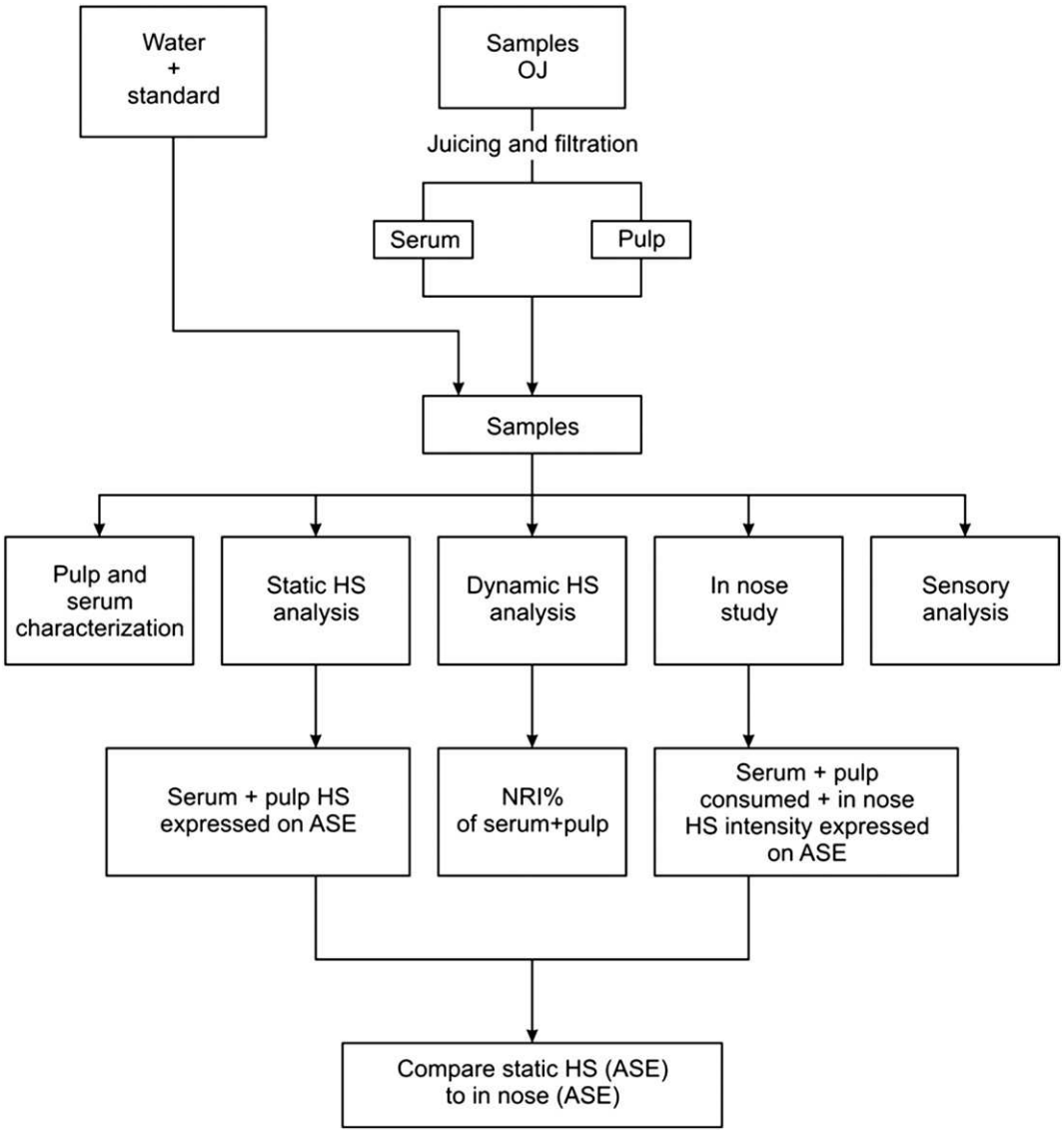

Fig. 3. Schematic flow chart of the experimental process, illustrating the formation of standards and samples, and the analysis series applied to the samples once prepared. (Orange juice, OJ; Headspace, HS; Aqueous standard equivalent, ASE).

86.6 g/100 g) by direct solvent extraction and the total lipid content was 1.8 g/100 g ± 0.125 g/100 g. This is in agreement with Brat et al. (2003), who also reported 1.8 g/100 g lipid content in wet pulp. The implication of lipid on aroma release from aqueous emulsions and colloidal food matrices is widely known both in equilibrium and in disturbed headspace conditions (Hatchwell, 1996). Generally, lipophilic aroma compounds partition into the lipid phase and are therefore present in a lower concentration in the headspace. Hydrophobicity is normally measured as the logarithm of the equilibrium partitioning ratio between two immiscible solvents, octanol and water, and expressed as logP. Guichard states that limonene has a logP of 4.83 (Guichard, 2002), which is hydrophobic, and therefore it can be predicted that the headspace concentration of limonene will be strongly dependent on the concentration of lipid in the product. The lipid and limonene content of the samples containing pulp at 5, 10, 15 and 20 g/100 g were calculated from measured fractions of serum and pulp samples at 0.09, 0.18, 0.27, 0.37 g/100 g and 169, 298, 426, 554 μg/g respectively. Limonene concentrations were at all levels higher than the population odour threshold in an orange juice matrix of 13.7 ug/g (Plotto, Margaria, Goodner, Goodrich, & Baldwin, 2004).

3.1.2. Limonene concentration

The isolated serum contained 40.7 ± 2.5 μg/g limonene and the pulp contained 2609 ± 1033 μg/g (Fig. 1), this means that in a standard 10 g/100 g pulp orange juice 88% of the limonene will originate from the pulp fraction and 12% will originate from the serum phase. Radford et al. (1974) previously showed that the elimination of pulp from fresh orange juice resulted in a significant reduction in terpene concentration and that 2% of limonene was present in the serum and 98% is present in the pulp fraction. Other studies in fresh hand-squeezed orange juice (cv. Naveline) reported concentrations of limonene in pulp and serum of 1630 μg/g and 4.6 μg/g, respectively (Brat et al., 2003) which at a 10 g/100 g add back level would correlate to 0.3% contribution from the serum and 99.7% contribution from the pulp fraction. It is therefore believed that the serum fraction may contain small particulate fractions of cell structures re-suspended from the pulp that contain some limonene, and this therefore has been taken into account in discussions hereinafter.

As the major contributor of limonene, it could be suggested that pulp add back would increase the concentration of limonene in the product and therefore potentially impact the headspace availability of limonene.

3.1.3. Particle size distribution

Pulp consists of particulate cellular structures that are dislodged during the juicing process. They are rich in carbohydrates and lipids and form a colloidal dispersion, the size distribution of the colloidal pulp is shown in Fig. 4. Pulp was in the form of clearly defined cell structures which formed larger aggregates as the concentration of pulp increased, in general 90% of the pulp particles were larger than 50 μm and the particle size distribution was mono-modal. Serum contained particles of which 90% were smaller than 50 μm and had a tri-modal particle size distribution; this suggests small cell structures and droplets of emulsified oil are present in the serum phase. The structures are further illustrated by microscopy in Fig. 5.

3.2. Aroma delivery from serum with varying pulp concentrations

3.2.1. APCI-MS static headspace analysis

The headspace concentration of limonene increased with increased pulp concentration; this is illustrated in Fig. 6. The limonene headspace concentration doubled with the addition of 10 g/100 g pulp to the serum fraction, this is especially significant

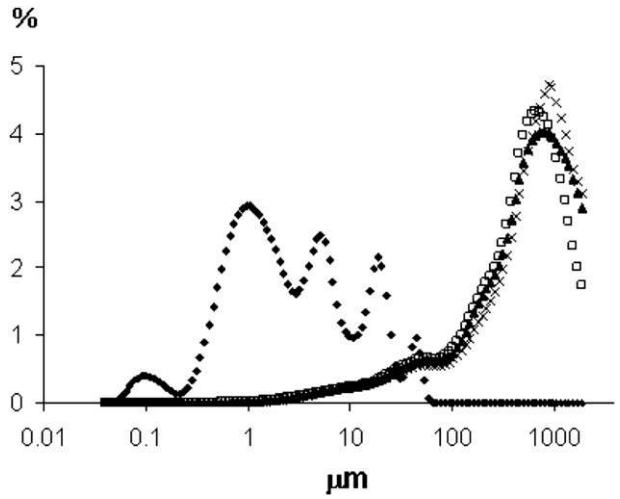

Fig. 4. Particle size distribution of orange juice samples containing 0 g/100 g, 10 g/100 g, 20 g/100 g, and 100 g/100 g pulp (data is represented as a filled diamond, empty square, filled triangle, open cross respectively), particle size is shown on a % volume basis.

considering the additional lipid added to the system from the pulp fraction.

Jordan et al. (2001) concluded that an increase of pulp concentration in orange juice resulted in a significant increase in headspace limonene, and that in general all terpenic compounds were closely associated with the pulp. Brat et al. (2003) has also produced comparable data showing the enhancement of headspace limonene with additional pulp add back.

As has been proposed, the add back of pulp not only increases the concentration of limonene, but also increases the concentration of lipid in the system. Fig. 6 shows that headspace limonene increases with additional pulp, but if non-linear regression is applied, suppression as a consequence of the additional lipid can be seen. When considering the two samples, 5 g/100 g, and 20 g/100 g pulp, the increase in limonene which would lead to an equivalent increase in headspace limonene, if the lipid fraction did not change, would be 328%. In reality the lipid content suppressed the increase in headspace availability and the true change in headspace concentration was 236%.

3.2.2. APCI dynamic headspace analysis

Dynamic dilution of the headspace above the orange juice was used to demonstrate the ability of the matrix to replenish the headspace (headspace persistence). In all cases the addition of pulp enhanced the ability of serum to replenish the headspace. This is illustrated in Fig. 7 and is represented as percentage normalised headspace intensity (% NRI). However it should be noted that among serum samples containing different percentages of pulp (5 g/100 g, 10 g/100 g, 15 g/100 g and 20 g/100 g) there were no significant differences at any time points.

The enhanced ability to replenish a diluting headspace is normally attributed to one of two things, either the equilibrium headspace concentration is low, therefore the mass transfer required to achieve equilibrium is low (Linforth & Taylor, 2010), or there is a reservoir of compounds that are available to partition to the headspace rapidly. In this case it is believed that it is a combination of free oil droplets released from the pulp and the reservoir present in the pulp that together enhances delivery. As the emulsion carries only a relatively small fraction of the limonene, it may allow a rapid replenishment of the headspace and itself be subsequently replenished by the pulp reservoir. Although many authors previously have documented the different reservoirs of hydrophobic compounds in other product, no evidence can be

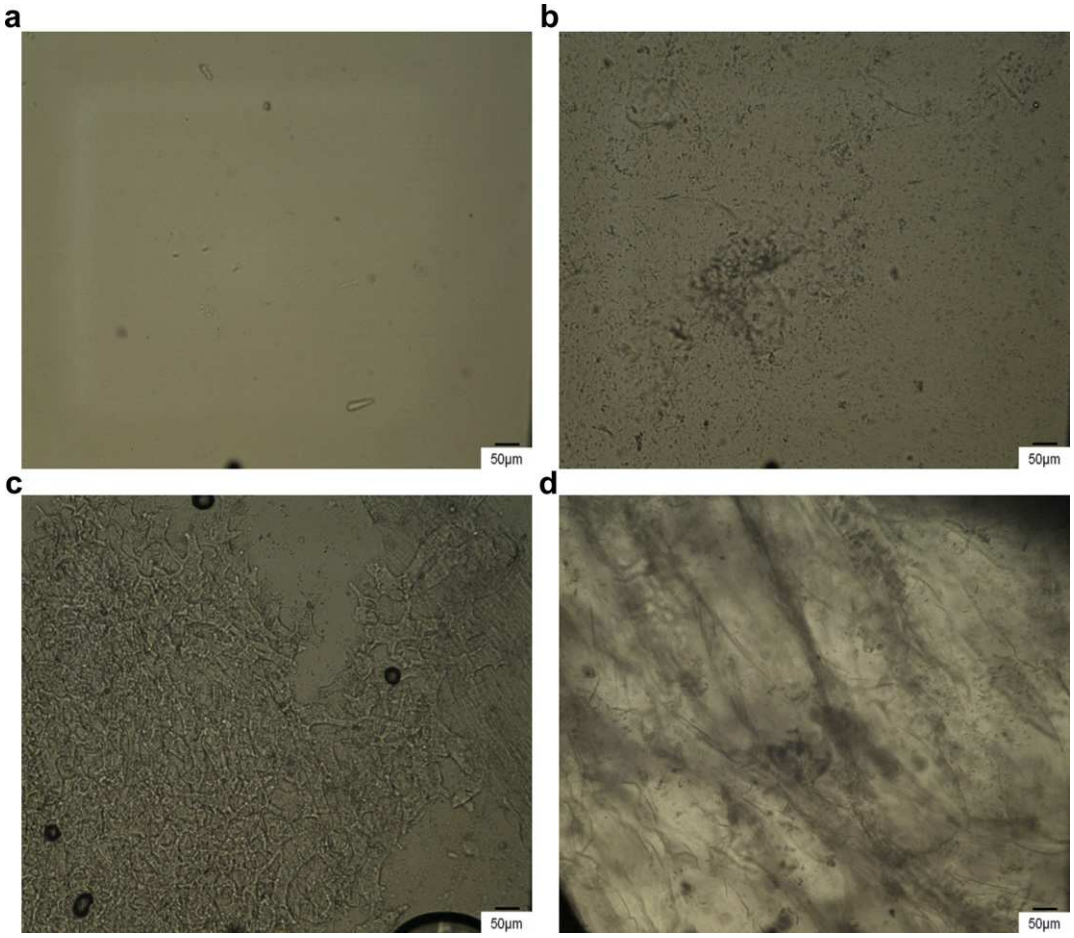

Fig. 5. Optical microscope images of serum containing 0 g/100 g (a), 10 g/100 g (b), 20 g/100 g (c), and 100 g/100 g (d) pulp (magnification ×10), images are characteristic of the samples and are chosen from multiple datasets ($n = 20$).

found that the rate release kinetics have been explained by such a phenomenon.

3.2.3. APCI In-nose

Ultimately consumers will drink orange juice, therefore the delivery rates of aroma to regions close to the point of perception,

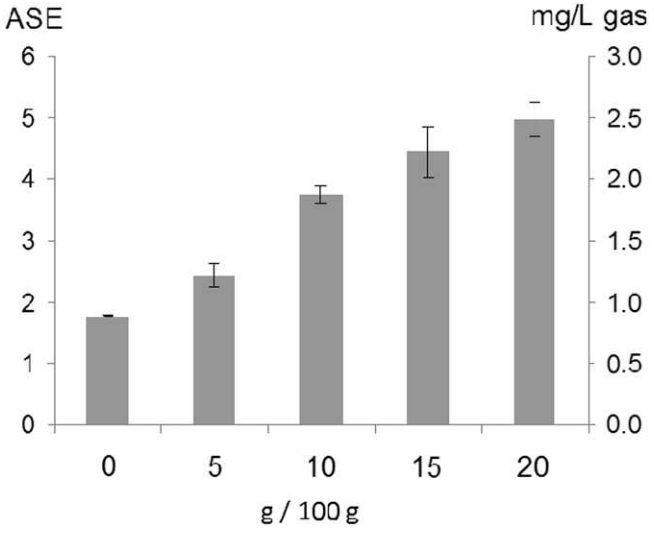

Fig. 6. APCI static headspace analysis. Limonene concentration for each sample in aqueous standard equivalents (ASE) and gas phase concentration (mg/L).

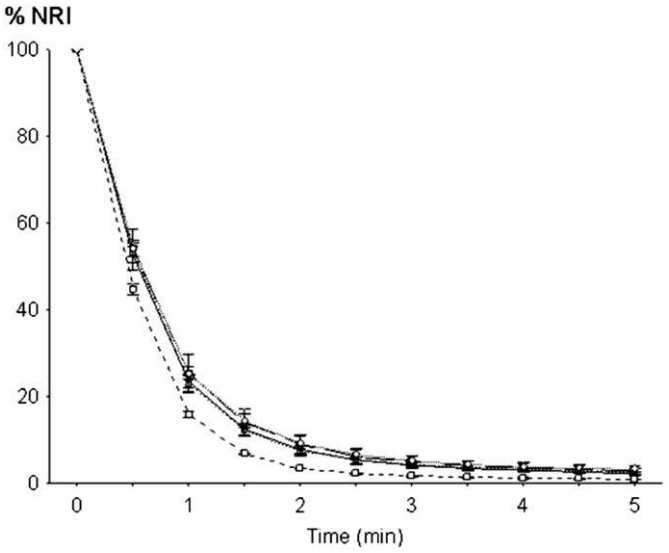

Fig. 7. Average dynamic headspace profiles (Normalized relative intensity, NRI) of limonene in OJs (0 g/100 g, 5 g/100 g, 10 g/100 g, 15 g/100 g, and 20 g/100 g pulp). Data is represented as an open square, filled circle, open triangle, filled square and open diamond respectively, and each point is the mean of three replicates with error bars showing standard deviation.

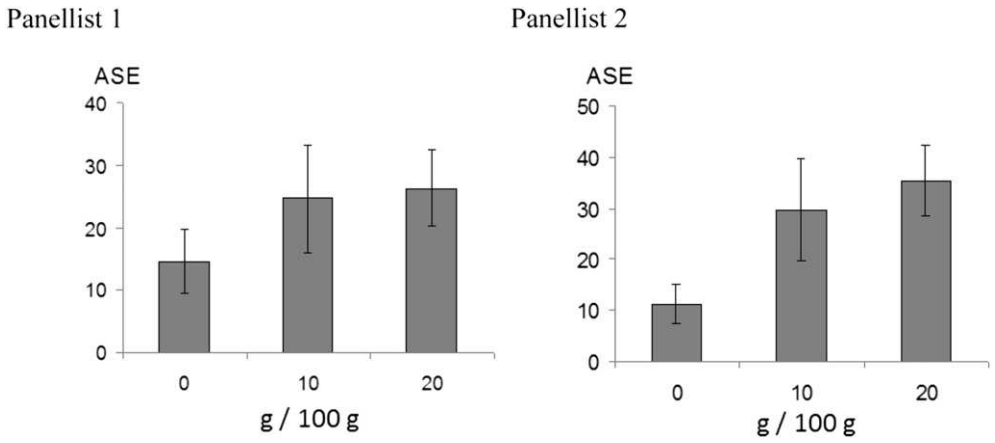

Fig. 8. Delivery of limonene to exhaled breath by APCI-In nose analysis and represented as ASE. Data is illustrated for two separate panellists ($n = 3$).

i.e. in the nose, are the most important to consider. Samples with different pulp concentrations (serum, 10 g/100 g, and 20 g/100 g) were therefore analysed by APCI In-nose to study the release of limonene under realistic consumer consumption conditions.

In all panellists, an increase in the pulp fraction resulted in an increase in the limonene concentration (Fig. 8) in the exhaled air; exhaled air was calibrated against a standard curve generated by each panellist consuming a series of standards of limonene in water. Interestingly the calibration curve was not linear (Fig. 2) and there was no significant difference between the 10 g/100 g and 20 g/100 g samples.

This clearly suggests that addition of 10 g/100 g pulp significantly enhances the delivery of limonene to the nasal cavity. Further additions did not result in significantly enhanced delivery of limonene to the nasal cavity.

3.2.4. Comparison of APCI static headspace analysis and APCI In-nose

In order to compare results from the APCI-MS static headspace analysis and that of the APCI-MS In-nose analysis, the ASE for both datasets are represented in Fig. 9. The addition of pulp facilitates a more efficient delivery of limonene from the food to the nasal cavity than when in a static state. This is presumably due to the combination of oral processing, destabilisation of the colloidal emulsion by salivary proteins (Vingerhoeds, Blijdenstein, Zoet, & van Aken, 2005), lipid coating of the buccal cavity and interaction of the lipophilic compounds and lipids within the food with the hydrophobic surfaces of the mucosal cavity (Dresselhuis, de Hoog, Cohen Stuart, & van Aken, 2008) which combined would increase surface area, enhance mass transfer kinetics and In-nose delivery significantly.

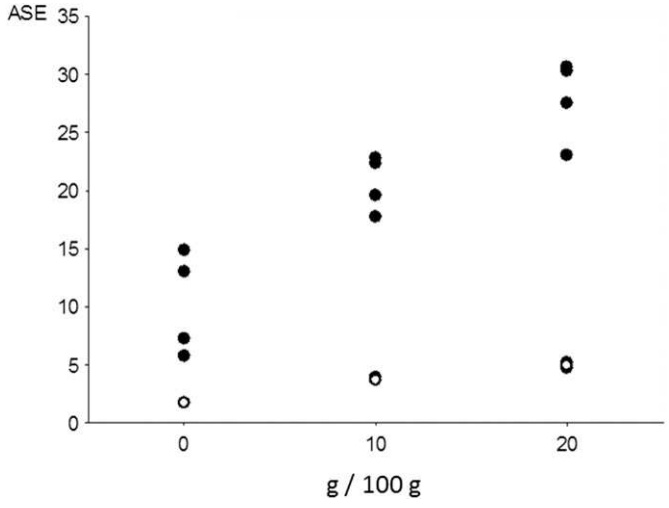

Fig. 9. Concentration of limonene (ASE) analysed by APCI static (empty circle) versus APCI In nose (filled circle) of varying pulp concentrations (0 g/100 g, 10 g/100 g, 20 g/100 g).

3.3. Sensory evaluation

Results from paired comparison testing did not provide sufficient evidence to conclude a significant difference existed between either the serum sample and the sample containing 10 g/100 g pulp ($P > 0.05$), or between the serum samples and the sample containing 20 g/100 g pulp when orange aroma and flavour ($P = 0.05$) was evaluated. Pulp clearly increased the delivery of limonene both in an *in-vivo* and an *in-vitro* situation when analysed instrumentally, but this increase was not reflected by an increase in orange aroma or orange flavour on consumption when assessed by panellists. This may be due to the fact that limonene is one of many key aroma compounds present within an orange juice but is not necessarily the most important or overriding compound when evaluating flavour quality (Jia, Zhang, & Min, 1998). Moreover Radford et al. (1974) found that low concentrations of aroma compounds in the serum played a significant role in the flavour of orange juice, this may further explain why panellists did not identify significant differences between the samples when asked to differentiate by paired comparison analysis.

The main limitation of this study is that it addressed only one aroma compound of the larger number which are present within the real food system, orange juice. Future research in this area using a series of known aroma compounds in a model system would significantly add value in this area.

4. Conclusion

In conclusion, we systematically evaluated the impact of pulp addition on the delivery of orange juice limonene to the headspace during headspace equilibrium, during disturbed headspace conditions and further investigated the impact on delivery of limonene in the exhaled breath (In-nose) by APCI-MS. Pulp addition significantly increased the equilibrium headspace concentration and increased the persistence of limonene to headspace disturbance, which is proposed to be due to the addition of the lipid fraction of the pulp. Addition of pulp enhanced limonene delivery to the nasal

exhaled air, but further additions of pulp to serum above 10 g/100 g pulp did not result in further increases in APCI In-nose delivery. This finding addresses the commercial impact of pulp addition and identifies the need for further research in this area to detail the impact of other aroma compounds, surface tension, cloud emulsions and other matrix effects on the release kinetics of aroma from orange juice.

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References

Baker, R. A., & Bruemmer, J. H. (1969). Cloud stability in the absence of various orange juice soluble components. *Proceedings of the Florida State Horticultural Society, 82*, 215–220.

Baker, R. A., & Cameron, R. G. (1999). Clouds of citrus juices and juice drinks. *Food Technology, 53*, 64–69.

Brat, P., Rega, B., Alter, P., Reynes, M., & Brillouet, J. M. (2003). Distribution of volatile compounds in the pulp, cloud, and serum of freshly squeezed orange juice. *Journal of Agricultural and Food Chemistry, 51*, 3442–3447.

Dresselhuis, D. M., de Hoog, E. H. A., Cohen Stuart, M. A., & van Aken, G. A. (2008). Application of oral tissue in tribological measurements in an emulsion perception context. *Food Hydrocolloids, 22*, 323–335.

Dufour, C., & Bayonove, C. L. (1999). Influence of wine structurally different polysaccharides on the volatility of aroma substances in a model system. *Journal of Agricultural and Food Chemistry, 47*, 671–677.

Falk, A., Gullstrand, E., Löf, A., & Wigaeus-Hjelm, E. (1990). Liquid/air partition coefficients of four terpenes. *British Journal of Industrial Medicine, 47*, 62–64.

Fisk, I. D., Boyer, M., & Linforth, R. (2012). Impact of protein, lipid and carbohydrate on the headspace delivery of volatile compounds from hydrating powders. *European Food Research and Technology, 235*, 517–525.

Fisk, I. D., Kettle, A., Hoffmeister, S., Virdie, A., & Silanes Kenny, J. (2012). Discrimination of roast and ground coffee aroma. *Flavour, 14*.

Fisk, I., Linforth, R., Taylor, A., & Gray, D. (2011). Aroma encapsulation and aroma delivery by oil body suspensions derived from sunflower seeds (*Helianthus annus*). *European Food Research and Technology, 232*, 905–910.

Guichard, E. (2002). Interactions between flavor compounds and food ingredients and their influence on flavor perception. *Food Reviews International, 18*, 49–70.

Hatchwell, L. C. (1996). *Flavor–food interactions* (633 ed.). American Chemical Society.

ISO 5495. (2005). *Sensory analysis. Methodology. Paired comparison test*. International Organization for Standardization.

Iwanaga, D., Gray, D. A., Fisk, I. D., Decker, E. A., Weiss, J., & McClements, D. J. (2007). Extraction and characterization of oil bodies from soy beans: a natural source of pre-emulsified soybean oil. *Journal of Agricultural and Food Chemistry, 55*, 8711–8716.

Jella, P., Rouseff, R., Goodner, K., & Widmer, W. (1998). Determination of key flavor components in methylene chloride extracts from processed grapefruit juice. *Journal of Agricultural and Food Chemistry, 46*, 242–247.

Jia, M., Zhang, Q. H., & Min, D. B. (1998). Optimization of solid-phase microextraction analysis for headspace flavor compounds of orange juice. *Journal of Agricultural and Food Chemistry, 46*, 2744–2747.

Jordan, M. J., Tillman, T. N., Mucci, B., & Laencina, J. (2001). Using HS-SPME to determine the effects of reducing insoluble solids on aromatic composition of orange juice. *LWT - Food Science and Technology, 34*, 244–250.

Langourieux, S., & Crouzet, J. C. (1997). Study of interactions between aroma compounds and glycopeptides by a model system. *Journal of Agricultural and Food Chemistry, 45*, 1873–1877.

Linforth, R. S. T. & Taylor, A. J. (1999). Apparatus and methods for the analysis of trace constituents of gases. 5,869,334. US. 1999.

Linforth, R., & Taylor, A. J. (2000). Persistence of volatile compounds in the breath after their consumption in aqueous solutions. *Journal of Agricultural and Food Chemistry, 48*, 5419–5423.

Linforth, R. S. T., & Taylor, A. J. (2010). Expression of multidisciplinary favour science. Flavour delivery from the oral cavity to the nose. In I. Blank, M. Wust, & C. Yeretzian (Eds.), *Expression of multidisciplinary favour science* (pp. 101–104).

Marin, M., Baek, I., & Taylor, A. J. (1999). Volatile release from aqueous solutions under dynamic headspace dilution conditions. *Journal of Agricultural and Food Chemistry, 47*, 4750–4755.

Mizrahi, S., & Berk, Z. (1970). Physico-chemical characteristics of orange juice cloud. *Journal of the Science of Food and Agriculture, 21*, 250–253.

Plotto, A., Margaria, C. A., Goodner, K. L., Goodrich, R., & Baldwin, E. A. (2004). Odour and flavour thresholds for key aroma components in an orange juice matrix: terpenes and aldehydes. *Flavour and Fragrance Journal, 19*, 491–498.

Rabe, S., Linforth, R. S. T., Krings, U., Taylor, A. J., & Berger, R. G. (2004). Volatile release from liquids: a comparison of *in-vivo* APCI-MS, in-mouth headspace trapping and *in vitro* mouth model data. *Chemical Senses, 29*, 163–173.

Radford, T., Kawashima, K., Friedel, P. K., Pope, L. E., & Gianturco, M. A. (1974). Distribution of volatile compounds between the pulp and serum of some fruit juices. *Journal of Agricultural and Food Chemistry, 22*, 1066–1070.

Stinco, C., Fernández-Vázquez, R., Escudero-Gilete, M. L., Heredia, F. J., Melendez-Martinez, A. J., & Vicario, I. M. (2012). Effect of orange juice's processing on the color, particle size, and bioaccessibility of carotenoids. *Journal of Agricultural and Food Chemistry, 60*, 1447–1455.

Tsachaki, M., Linforth, R. S. T., & Taylor, A. J. (2005). Dynamic headspace analysis of the release of volatile organic compounds from ethanolic systems by direct APCI-MS. *Journal of Agricultural and Food Chemistry, 53*, 8328–8333.

Vingerhoeds, M. H., Blijdenstein, T. B. J., Zoet, F. D., & van Aken, G. A. (2005). Emulsion flocculation induced by saliva and mucin. *Food Hydrocolloids, 19*, 915–922.

White, D. A., Fisk, I. D., Makkhun, S., & Gray, D. A. (2009). In vitro assessment of the bioaccessibility of tocopherol and fatty acids from sunflower seed oil bodies. *Journal of Agricultural and Food Chemistry, 57*, 5720–5726.