Comparison and optimisation of headspace methods for the analyses of oxidation related off-flavour compounds in plant protein concentrates and infant formula

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Abstract

The aim of this study was to develop an effective and fast method for key-marker compound analysis of available oxidation related off-flavours, both in dry protein powder and in protein powder dispersed in water. Four different headspace techniques (static headspace (HS), dynamic headspace (DHS-ITEX), and solid phase microextractions (HS-SPME, HS-Arrow)) were compared under different extraction conditions. HS-Arrow in combination with true capillary cold trapping showed to be most sensitive and an efficient extraction technique for a wide range of oxidation related HRGC-MS analyses. Although HS-Arrow in combination with GC-MS is a relative fast technique for the analyses of the key aroma compounds of plant protein related products and infant formula, still some key aroma compounds could not be detected. Especially 2,4,7-decatrienal, an important key compound to detect "fishy" off-flavours in products with added DHA like infant formula, is missed by these four headspace techniques.

Keywords: headspace extraction, GC-MS, Arrow, off-flavours, oxidation, plant proteins, infant formula

Introduction

The growing world population demands new sustainable sources of protein and a transition from animal to plant based protein foods. Most crop varieties currently available in the market are primarily designed for high yield and other agriculturally important aspects, instead for optimal flavour when used as ingredients in new food applications [1].

Many volatile compounds in plant protein concentrates that lead to sensory off-flavour attributes, such as "beany", "green", "oily", are formed by oxidation, mainly due to the action of lipoxygenases (LOX) [2]. The same group of compounds are found in food and nutritional products such as infant formula with added polyunsaturated fatty acids (PUFAs). In the case of docosahexaenoic acid (DHA) and arachidonic acid (ARA), oxidation leads to "painty", "tallowy", "metallic", and "fishy" off-flavours. Often, the sensory attributes are not caused by one single compound but are the result of a certain balance between different oxidation compounds. Sensory omission experiments with the Composcent (a preparative GC with omission feature) showed that for the typical "cod-liver" / "fishy" off-flavour found in "oxidised" infant formula not one but a balance of four compounds is responsible ((E,E)-2,4-heptadienal, (E,E)-3,5-octadien-2-one, (E,Z,Z)-2,4,7-decatrienal and (E,E)-4,5-epoxy-2-decenal) [3].

The aim of this study was to develop an effective and fast method for key-marker compound analysis of available oxidation related off-flavours, both in dry protein powder and in protein powder dispersed in water.

Four different headspace techniques (static headspace (HS), dynamic headspace (DHS-ITEX), and solid phase microextractions (HS-SPME, HS-Arrow) were compared under different extraction conditions. Beside plant protein concentrates, also an infant formula and a flavour standard were used in this study.

Experimental

Sample preparation

The samples analysed were a flavour mixture (STD), a pea protein concentrate (PPC), a soy protein concentrate (SPC) and a 1st age infant formula (IF). The STD consisted of 26 volatile compounds namely: diacetyl, , hexanal, octanal, nonanal, 2,4-decadienal, 2,6-nonadienal, benzaldehyde, 3-methylbutanal, 2-methylfuran, 2-pentylfuran, hexanol, 3-methylbutanol, 1-octen-3-ol, 2-heptanone, 2-nonanone, pyrrole, 2-ethyl-3-methoxypyrazine, 2-ethylpyrazine, dimethyl sulphide, dimethyl disulphide, δ-decalactone, δ-dodecalactone; internal standards: propyl acetate, undecane, dodecane, d12-hexanal.

The headspaces of the proteins (PPC & SPC) were analysed above the dry powder (2 g) and above a dispersion in water (2.5%). The headspace above the IF was analysed above the dry powder (2 g) and above a dispersion in water (12.5%).

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Gas chromatography-mass spectrometry (GC-MS)

The gas chromatograph (HRGC) consisted of a Thermo Trace 1300 GC equipped with a SSL injector (250°C) in combination with true capillary (in column) cryogen cold trapping (-120°C) and fitted with a capillary column Rxi-5Sil MS, 30 m × 0.25 mm I.D. × 1.0 µm df. The HRGC was connected with a Single quad-MS ISQ7000 (Thermo Scientific™) using Advanced Electron Ionisation (AEI) and robotics to execute the different extraction and HRGC-injection techniques.

The operating conditions were:

- GC Tx program: 40°C (3 min)—8°C/min—140°C –25°C/min—240° (5 min)
- GC flow: 1.5 ml/min (constant flow)
- Split mode: SSL; 6 min; 30 ml/min
- Cold trap: -120°C/260°C, 0.53 mm fused silica capillary
- MS scan: 35-250 amu

Extraction techniques

1. Static Headspace (HS)

Parameter	Setting
Analysis time	35 min
Sample draw	1.5 mL
Agitator temp	40-60°C
Incubation time	10-40 min
Agitator on	10 sec
Agitator off	2 sec
Syringe temp	70°C
Filling volume	1-2 mL
Pre-inj. syringe flush	5 sec
Post-inj. syringe flush	30 sec
Filling speed	20 mL/min
Injection speed	5 mL/min
Injection depth	45 mm
Penetration speed	25 mm/s
Pre-inj. Delay	0 sec
Post inj. Delay	0 sec

2. Dynamic Headspace (DHS-ITEX)

Parameter	Setting
Adsorbent	Tenax TA 80/100 mesh
Analysis time	35 min
Sampling volume	1000 µL
Agitator temp	40-60°C
Incubation time	10-40 min
Agitator on	10 sec
Agitator off	2 sec
Sampling depth mode	Standard
Extraction volume	1000 µL
Aspirate/Dispense flow	100 µL/s
Number of strokes	5-25
Prefill ratio	40%
Syringe temp	65°C
Trap pre cleaning temp	250°C
Trap pre cleaning time	120 sec
Trap extraction temp	35°C
Trap purge time	10 sec
Trap post cleaning temp	250°C
Trap post cleaning time	120 sec
Injection depth	35 mm
Injection speed	80 µL/s
Injection temp	250°C

3. Headspace Solid Phase Micro Extraction (SPME & Arrow)

Parameter	Setting	Parameter	Setting
Fibre SPME	50/30 µm, 1 cm, DVB/CAR/PDMS	Analysis time	35 min
Fibre Arrow	120µm, 2cm, DVB/CWR/PDMS	Injection depth	70 mm
Temp incubation/extraction	40-60°C	Penetration speed	40 mm/sec
Agitator speed	750 -1200rpm	Desorption time	2 min
Incubation time	2- 5 min	Start mode	At injection
Extraction stirrer speed	700-1200 rpm	Pre desorb time	1.0 min
Extract time	10-40 min	Post desorb time	1.0 min
Needle speed /depth in vial	20 mm/s / 20mm	Conditioning temp	250°C

Results and discussion

Several analysis experiments were carried out with varying parameters regarding sample incubation (volume, temperature, time, mixing) and injection/desorption (temperature, time, strokes, speed). The results were compared in order to find optimum conditions for the different analysis techniques in which as much as possible (PPC, SPC

and IF) key aroma could be analysed with a simple and fast method. Special attention was paid to the oxidation related compounds. A summary of the outcome of this large analysis experiment is shown in Table 1.

Table 1: Optimal extraction and injection conditions for the HRGC-MS analyses of oxidation compounds in PPC, SPC and IF.

Extraction technique	HS	DHS	SPME	ARROW
Extraction mode	HS	HS	HS	HS
Incubation time (min)	20	20	12	12
Incubation temp (°C)	60	60	40	40
Extraction volume (ml)	2	1	x	x
Strokes (#)	x	15	x	x
Agitator on fixed/Arrow (rpm)	on	on	on	750
Desorption time (min)	x	12.5	2	2

In Figure 1, as an example, a part of the GC-MS chromatogram of a pea protein concentrate is shown, obtained with a HS-Arrow extraction executed with the parameters shown in Table 1.

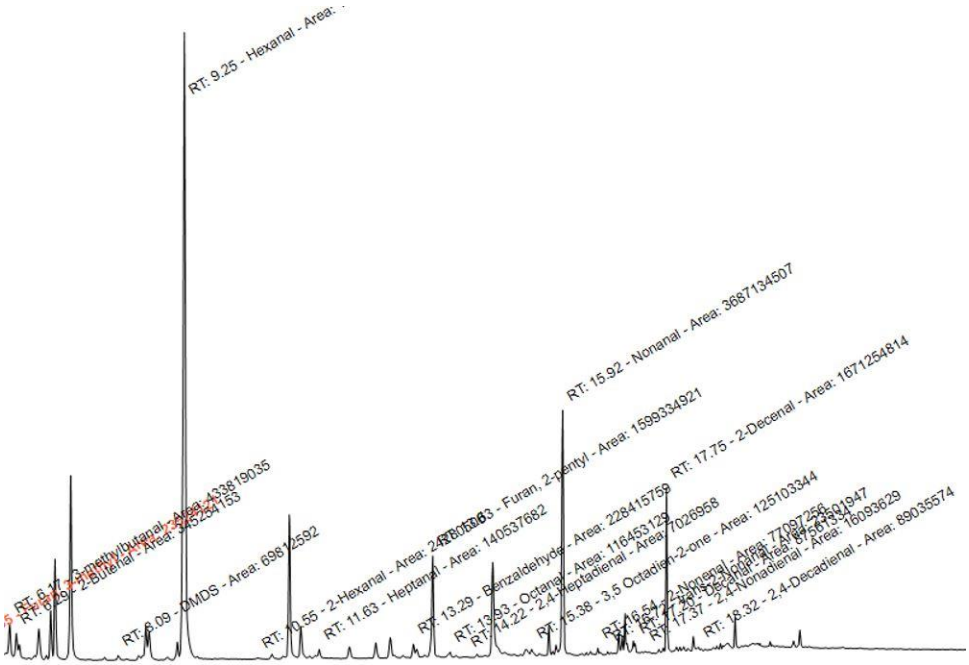

Figure 1*: HRGC-MS chromatogram obtained after a HS-Arrow extraction in the headspace above a 2.5% dispersion of PPC in water.*

A comparison of the headspace extraction techniques tested in both the dry product and in aqueous dispersion showed that HS-Arrow is the most efficient for effective analysis of the key oxidation related compounds. Also, HS-SPME and HS-Arrow extraction allow for lower (40°C) operational incubation temperature compared to HS-DHS (60°C) to detect the most relevant key aroma compounds. When higher incubation temperatures are used for the SPME and Arrow extractions, compounds as hexanal and 2-pentylfuran are overloaded for most samples.

At the other hand, DHS is more effective for very volatile compounds compared to HS and SPME. In Figure 2 the extraction efficiency is shown for some key oxidation related compounds in IF.

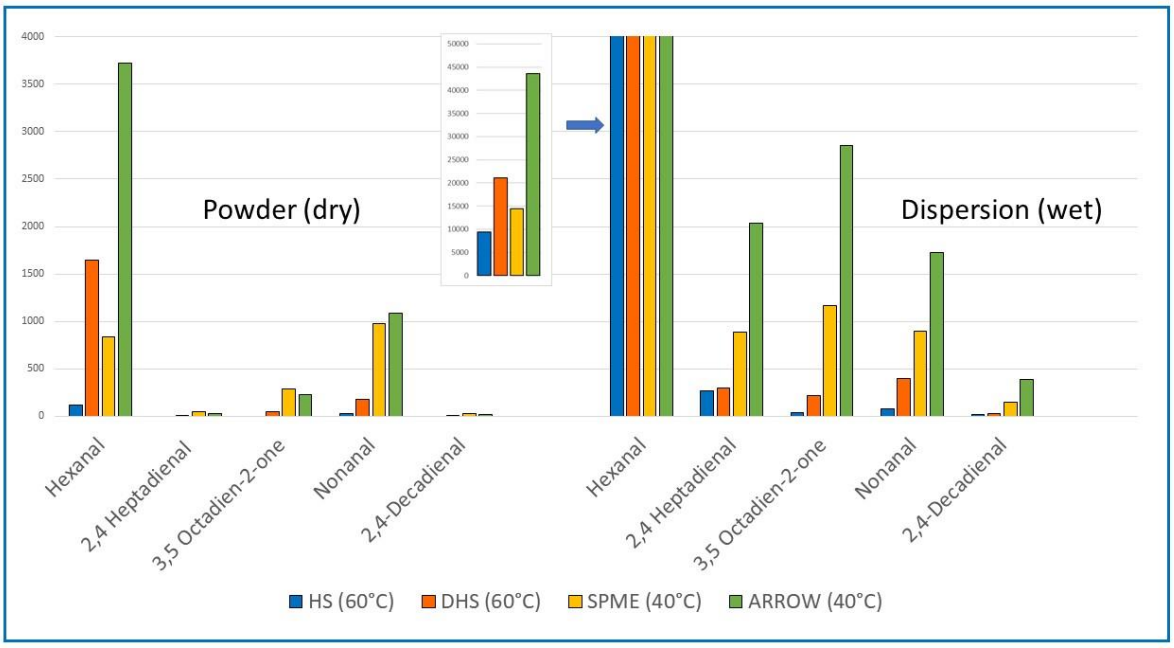

Figure 2*: Example of extraction efficiency comparison for an infant formula 1st age sample with added fish oil PUFA's like DHA and ARA.*

Besides the extraction efficiency of different techniques tested, Figure 2 also shows a difference in concentration in the headspace between the powder analyses and the analyses of the dispersions. The difference in flavour compound balance (e.g. 3,5-octadien-2-one, 2,4-heptadienal) will most probably lead to a different sensory perception. Similar observations were made for PPC and SPC. This means that the (off-) flavour quality of the protein powder as such and the (off-) flavour quality of the protein in application can be different, and it should be kept in mind that sensory evaluation of protein powder by smell only could lead to misinterpretation of the (off-flavour) perception when the intention is to use the protein powder in a moist product. Furthermore, some important and interesting compounds like lactones and 2,4,7-decatrienal were still difficult to detect using the headspace techniques studied. Stir Bar Sorptive Extraction (SBSE) could give possibilities here [4].

Conclusion

Headspace Solid Phase Micro Extraction using Arrow (HS-Arrow) in combination with true capillary cold trapping is the most sensitive and efficient extraction technique of the four tested techniques for a wide range of oxidation related HRGC-MS analyses. Although HS-Arrow in combination with HRGC-MS is a relative fast technique for the analyses of the key aroma compounds of plant protein related products and infant formula still some key aroma compounds could not be detected. Especially 2,4,7-decatrienal, an important key compound to detect "fishy" off-flavours in products with added DHA like infant formula, is missed by these four headspace techniques.

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