

# Influence of technological processing on apple aroma analysed by high resolution gas chromatography–mass spectrometry and on-line gas chromatography-combustion/pyrolysis-isotope ratio mass spectrometry

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## Abstract

Extracts obtained by simultaneous distillation extraction (SDE) from industrial raw materials, namely single strength apple juices, and concentrates and aromas made thereof (each  $n = 31$ , from one production line; origin Poland, Germany, Turkey, Romania and China), as well as commercially available juices ( $n = 27$ ), were analysed by standard controlled capillary gas chromatography–mass spectrometry (HRGC–MS). During the technological processing from juice to the aroma, no qualitative changes in the apple aroma profile were observed. Major constituents of the juices and aromas under study were found to be 1-hexanol (juice, 0.06–5.9 mg/l; aroma, 47–685 mg/l), 1-butanol (juice, 0.1–4.7 mg/l; aroma, 17–370 mg/l); *E*-2-hexenol (juice, 0.01–3.4 mg/l; aroma, 12–300 mg/l); *E*-2-hexenal (juice, 0–3.0 mg/l; aroma 0–470 mg/l), and butyl acetate (juice, 0–1.7 mg/l; aroma, 0–165 mg/l). By far the major component of the apple juice concentrates under study was furfural (2.4–56 mg/kg). The observed occurrence of 3-methyl-1-butanol (juice, 0.01–2.1 mg/l; aroma, 1.5–134 mg/l) and, in part, its acetate (juice, 0–0.3 mg/l; aroma, 0–3.3 mg/l), both known not to be genuine apple constituents, was obviously caused by fermentative effects in the course of industrial juice production. In addition, on-line capillary gas chromatography–isotope ratio mass spectrometry was used in the combustion (C) and the pyrolysis (P) modes (HRGC–C/P–IRMS) for the determination of  $\delta^{13}\text{C}_{\text{V-PDB}}$  and  $\delta^2\text{H}_{\text{V-SMOW}}$  values of selected apple flavour constituents to check potential isotope discrimination during distillative aroma production. As shown by means of the representative examples of *E*-2-hexenal, 1-hexanol and *E*-2-hexenol, their  $\delta^2\text{H}_{\text{V-SMOW}}$  values were slightly depleted. However, authenticity assessment by stable IRMS will not be influenced by this effect.

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**Keywords:** Apple; Technological processing; Aroma profile; HRGC–MS; Isotope ratio mass spectrometry; HRGC–C/P–IRMS;  $^{13}\text{C}/^{12}\text{C}$  ratio,  $^2\text{H}/^1\text{H}$  ratio

## 1. Introduction

Apples are a highly favoured fruit, not only because of their good storage and manufacturing properties, but also owing to their unique flavour characteristics.

Apple juice is one of the most popular juices all over the world. In numerous studies, the aroma profiles of apples, apple juices and other apple products have been in the focus of research over the past 50 years (Dimick & Hoskin, 1983; Drawert, Heimann, Emberger, & Tressl, 1969; Dürr, 1981; Flath, Black, Guadagni, McFadden, & Schultz, 1967; Flath et al., 1969; Koch & Schiller, 1964; MacGregor, Sugisawa, & Matthews, 1964; Pailard, 1990). Besides the genuine aroma profile of several

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apple cultivars, the technological influences have been investigated, most of them on a laboratory scale (Dixon & Hewitt, 2001; Girard & Lau, 1995; Hern & Dorn, 2003; Lavilla, Puy, Lopez, Recasens, & Vendrell, 1999; Schreier, Drawert, & Mick, 1978; Schreier, Drawert, & Schmid, 1978; Schreier, Drawert, Steiger, & Mick, 1978). In the industrial juice production, however, several 100 kg of mash apples per hour are processed to apple juice. This juice is either pasteurized and bottled before being sold as commercial single strength juice or processed to apple juice concentrate and water phase/aroma by evaporators with aroma condensers (Schobinger, 2001). In spite of the basic knowledge, provided previously, on variations of the genuine volatiles during apple juice and aroma production (Kato et al., 2003; Su & Wiley, 1998), information about quantitative aroma profiles in the industrial production line from juice to aroma is rather scarce.

In addition, technology can influence the isotopic ratios of aroma compounds. Heating, vaporizing, distillation and other technologies are steps involving potential isotopic discrimination (Griffiths, 1998). Stable isotope analysis, in particular, isotope ratio mass spectrometry (IRMS) is the method of choice to assess the authenticity of aroma compounds (Richling, Heckel, & Schreier, 2003; Rossmann, 2001; Schmidt, Rossmann, & Werner, 1998); however, it is also important to verify that the natural isotopic ratios do not change significantly during technological processing in order to avoid misinterpretations.

It was therefore the aim of our studies: (i) to study the quantitative apple aroma profile in the industrial production line from juice to the separated aroma; (ii) to check potential isotope discriminations of typical apple aroma volatiles in the course of aroma production. For this purpose, authentic industrial raw materials and a number of commercial products were investigated. In this paper, we report the results obtained in these two research areas.

## 2. Materials and methods

### 2.1. Samples

Single strength apple juices, concentrates and aromas (each from one production line from Poland,  $n = 24$ , Germany,  $n = 3$ , Turkey,  $n = 2$ , Romania,  $n = 1$  and China  $n = 1$ ) were obtained by the Schutzgemeinschaft der Fruchtsaftindustrie e.V. (SGF), Nieder-Olm, Germany. Commercial German single strength apple juices ( $n = 19$ ) and juices made from concentrate ( $n = 8$ ) were purchased from local supermarkets. Reference aroma compounds were available from our laboratory collection; all other chemicals were purchased from Sigma–Aldrich (Steinheim, Germany). Solvents were redistilled before use.

### 2.2. Sample preparation

In each case, 500–1000 ml of juice, 250 g concentrate and 25 ml aroma were diluted with distilled water and, after addition of 2-methyl-1-pentanol as standard (500  $\mu\text{g}$ ), subjected to simultaneous distillation extraction (SDE, 2 h), using pentane–diethyl ether mixture (1:1, v/v). The extracts were dried over anhydrous sodium sulphate, filtered, and carefully concentrated to approximately 1 ml using a Vigreux column (45 °C).

References used in IRMS studies, namely *E*-2-hexenal (**9**), 1-hexanol (**11**), and *E*-2-hexenol (**13**), were dissolved (1 mg/ml) in diethyl ether and the solutions directly analysed by HRGC–MS and HRGC–C/P–IRMS. Model experiments comprised SDE of solutions of **9**, **11** and **13** (each 50 mg in 800 ml of water).

### 2.3. Gas chromatography–mass spectrometry (HRGC–MS)

An HP Agilent 6890 Series gas chromatograph with split injection (220 °C; 1:20) was directly coupled to an HP Agilent 5973 Network mass spectrometer (Agilent Technologies Inc., CA, USA). The flavour compounds were separated on a J&W DB-Wax fused silica capillary column (30 m  $\times$  0.25 mm, d.f. = 0.25  $\mu\text{m}$ ). The temperature programme was as follows: 3 min isothermal at 50 °C, then raised at 4 °C/min to 220 °C. Identification was achieved by comparison of linear retention indices and mass spectral data of sample constituents with those of authentic reference compounds.

For the separation of 2- and 3-methylbutyl acetate, as well as 2- and 3-methyl-1-butanol on a RH-1701 column (60 m  $\times$  0.25 mm, d.f. = 0.25  $\mu\text{m}$ ), a Fisons GC 8000 Series gas chromatograph with split injection (220 °C; 1:20) coupled to a Fisons Instrument MD 800 mass spectrometer was used. The temperature programme was as follows: 60 °C raised at 1 °C/min to 90 °C, then raised at 10 °C/min to 270 °C.

### 2.4. Enantioselective multidimensional gas chromatography–mass spectrometry (enantio-MDGC–MS)

A double-oven MDGC system (oven I, Fisons GC 8169; oven II, Fisons GC 8130) equipped with split injection (200 °C, 1:20) and a moving column switching system (MCSS) was coupled to a Fisons MD 800 mass spectrometer. Pre-separation was performed using a J&W DB-Wax fused silica capillary column (30 m  $\times$  0.25 mm i.d., d.f. = 0.25  $\mu\text{m}$ ) and temperature programme started at 50 °C and raised at 10 °C/min to 240 °C. Enantiomeric separation was performed using a modified cyclodextrin phase: 30% 2,3-diethyl-6-*t*-butyldimethylsilyl- $\beta$ -cyclodextrin in PS 086 (25 m  $\times$  0.25 mm i.d., d.f. = 0.15  $\mu\text{m}$ ), temperature programme

started at 50 °C, held for 20 min and raised at 2 °C/min to 200 °C.

### 2.5. Gas chromatography–isotope ratio mass spectrometry (HRGC–IRMS)

A Finnigan Delta plus XL isotope ratio mass spectrometer, coupled by an open-split via a combustion/pyrolysis (C/P) interface to an HP 6890 gas chromatograph (GC), was used. The GC was equipped with a J&W DB-Wax fused silica capillary column (60 m × 0.32 mm i.d.; d.f. = 0.25 µm). The following conditions were employed: 1 µl “splitless” injection (250 °C); temperature programme: 50–220 °C at 5 °C/min; helium flow, 2 ml/min. Pyrolysis interface temperature 1440 °C; hot ion source (Al<sub>2</sub>O<sub>3</sub>, *l* = 320 mm; 0.5 mm i.d.).

Combustion was performed by oxidative reactor (Al<sub>2</sub>O<sub>3</sub>, 0.5 mm i.d., 1.5 mm o.d., 320 mm) with Cu, Ni, Pt (each 240 mm × 0.125 mm) to CO<sub>2</sub> at 960 °C; water separation was by Nafion membrane.

For pyrolysis to H<sub>2</sub> the effluent from the GC passed through a ceramic tube (Al<sub>2</sub>O<sub>3</sub>; 0.5 mm i.d., 320 mm) at 1440 °C.

In addition, coupling of elemental analysers (EA) (<sup>13</sup>C/<sup>12</sup>C, Euro Vector EA 3000, Milano, Italy; temperature, 1000 °C; <sup>2</sup>H/<sup>1</sup>H, HT Sauerstoff-Analysator, HEKA-Tech, Wegberg, Germany; temperature, 1460 °C) to the IRMS was realized for off-line control and determination of reference samples.

Daily system stability checks were carried out by measuring reference samples with known <sup>13</sup>C/<sup>12</sup>C and <sup>2</sup>H/<sup>1</sup>H ratios. Stability check of the used reference gases was continuously performed by measuring International Atomic Energy Agency (IAEA, Vienna, Austria) standards with defined <sup>13</sup>C/<sup>12</sup>C and <sup>2</sup>H/<sup>1</sup>H ratios (for <sup>13</sup>C/<sup>12</sup>C IAEA-CH-7 and for <sup>2</sup>H/<sup>1</sup>H IAEA-CH-7, NBS22 oil and V-SMOW).

The isotope ratios were expressed per mil (‰) deviation relative to the V-PDB and V-SMOW international standards. Results were expressed in δ values as follows (for δ<sup>2</sup>H<sub>V-SMOW</sub>, corresponding formula is valid)

$$\delta^{13}\text{C}_{\text{V-PDB}} (\text{‰}) = \left( \frac{R_{\text{sample}} - R_{\text{V-PDB}}}{R_{\text{V-PDB}}} \right) \times 1000,$$

where *R* is the isotope ratio <sup>13</sup>C/<sup>12</sup>C.

For <sup>13</sup>C/<sup>12</sup>C and <sup>2</sup>H/<sup>1</sup>H. measurements, calibration of the IRMS was done with certified CO<sub>2</sub> (declared = δ<sup>13</sup>C<sub>V-PDB</sub> = −24.9‰) and H<sub>2</sub> (δ<sup>2</sup>H<sub>V-SMOW</sub> declared = −270 ± 10‰, Messer Griesheim, Frankfurt, Germany).

In general, sixfold determinations were carried out, and standard deviations calculated. The latter were ±0.1‰ and ±5‰ for <sup>13</sup>C<sub>V-PDB</sub> and δ<sup>2</sup>H<sub>V-SMOW</sub> determinations, respectively. Additional peak recognition was performed by reference compounds and HRGC–MS registered under separation identical to samples.

In order to determine the δ<sup>2</sup>H<sub>V-SMOW</sub> values of the apple volatiles, the system reliability had to be proven by measuring commercial references ‘off-line’ via the equipped elemental analyser (EA) (Hör, Ruff, Weckerle, König, & Schreier, 2001). Comparison of the data recorded by EA–C/P–IRMS revealed good agreement with that determined by HRGC–C/P–IRMS analysis. The areas of linearity for the δ<sup>2</sup>H<sub>V-SMOW</sub> determinations were from 1 to 4.5 µg (on column) for compounds **9**, **11** and **13**, respectively, as already found previously (Hör et al., 2001). The influence of sample preparation on the <sup>2</sup>H/<sup>1</sup>H isotope ratio, checked by model SDE extractions, was found to be within the range of standard deviation and thus negligible (data not shown).

## 3. Results and discussion

### 3.1. Apple aroma profiles

About 80 volatile apple aroma compounds were routinely detected by HRGC–MS in the samples under study. The recorded qualitative data agreed with those of previous studies (Paillard, 1990); representative aroma profiles of authentic apple juice, concentrate and aroma are shown in Fig. 1. The amounts of main aroma compounds determined in single strength juices and aromas are listed in Table 1. Major constituents of the samples under study were found to be 1-hexanol (**11**; juice, 0.06–5.9 mg/l; aroma, 47–685 mg/l), 1-butanol (**7**; juice, 0.1–4.7 mg/l; aroma, 17–370 mg/l); *E*-2-hexenal (**9**; juice, 0–3.0 mg/l; aroma 0–470 mg/l); *E*-2-hexenol (**13**; juice, 0.01–3.4 mg/l; aroma, 12–300 mg/l) and butyl acetate (**5**; juice, 0–1.7 mg/l; aroma, 0–165 mg/l).

In apple juice concentrate (Table 2), apart from 1-hexanol (**11**; 0–0.2 mg/kg) and 1-butanol (**8**; 0–0.5 mg/kg), most of the typical apple volatiles, such as the esters, ethyl butanoate (**3**) or butyl acetate (**5**), were only found in traces. The presence of small amounts of the alcohols **11** and **8** can be explained by their known occurrence as glycosidically bound forms in apple fruit, from which they are liberated by thermal processing under acidic conditions (Schwab & Schreier, 1990). By far the major component of the apple juice concentrates under study was, as expected, furfural (**15**; 2.4–56 mg/kg).

In addition, the aroma profile of several commercial single strength apple juices and juices made from concentrate were analysed. Compared to the products obtained as raw materials from the industry, the commercial single strength juices contained much lower amounts of aroma compounds (Table 3). The reasons for the differences observed in the aroma yields could be related to influences of cultivars or losses during pasteurisation, bottling, packaging and storage. Commercial apple juices made from concentrate contained the characteristic apple aroma components in similar

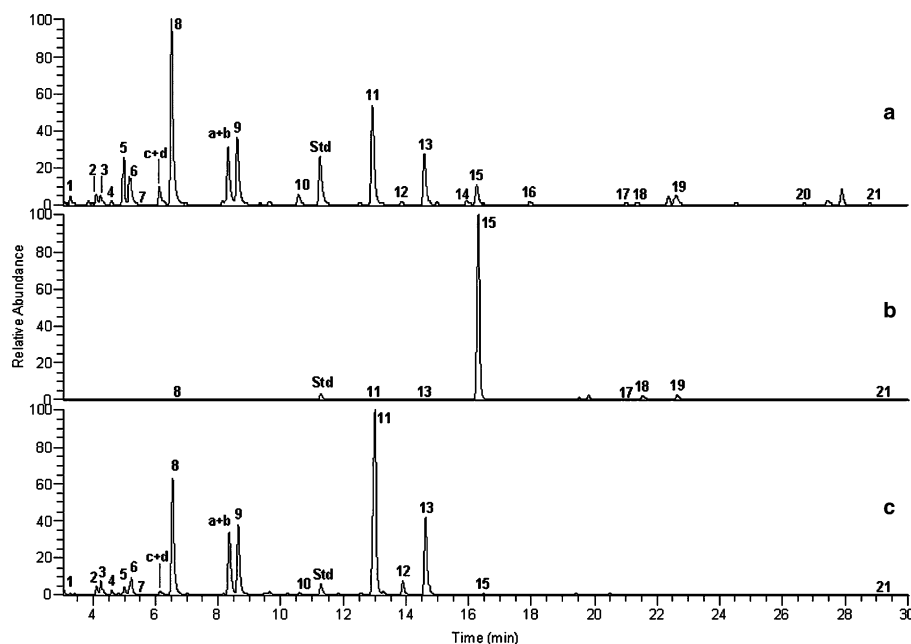


Fig. 1. Representative total ion chromatograms (TIC) of apple volatiles in the production line of (a) single strength juice, (b) apple concentrate and (c) apple juice aroma. The peak numbers correspond to those in Tables 1–3.

Table 1

Aroma compounds determined by HRGC–MS in industrial single strength apple juices (a) and apple juice aromas (b) (each  $n = 31$ )

Peak no.	Aroma compound	(a)		(b)	
		Range (mg/l)	Mean (mg/l)	Range (mg/l)	Mean (mg/l)
1	Propyl acetate	0–0.1	0.03	0–27	4.1
2	1-Propanol	0–0.3	0.1	0–43	10.7
3	Ethyl butanoate	0–0.3	0.1	0–37	13.0
4	Ethyl 2-methylbutanoate	0–0.1	0.05	0–25	4.1
5	Butyl acetate	0–1.7	0.4	0–165	29
6	Hexanal	0–0.6	0.2	0–95	20
7	2-Methylpropanol	0–0.5	0.2	0–175	21
c + d	2/3-Methylbutyl acetate	n.s.	n.s.	n.s.	n.s.
8	1-Butanol	0.1–4.7	2.5	17–370	154
a + b	2/3-Methyl-1-butanol	n.s.	n.s.	n.s.	n.s.
9	<i>E</i> -2-hexenal	0–3.0	0.9	0–470	107
10	Hexyl acetate	0–0.7	0.1	0–28	9.5
11	1-Hexanol	0.06–5.9	3.0	47–685	270
12	<i>Z</i> -3-hexenol	0–0.9	0.2	0–79	15
13	<i>E</i> -2-hexenol	0.01–3.4	1.2	12–300	100
14	Acetic acid	0–5.3	0.4	0–2.0	0.4
15	Furfural	0–25	1.6	0–21	1.1
16	Benzaldehyde	0–0.3	0.06	0.02–45	4.6
17	Butanoic acid	0–1.2	0.2	0–0.2	0.01
18	Phenylacetaldehyde	0–1.1	0.05	0–4.2	0.5
19	2-Methylbutanoic acid	0–5.9	0.7	0–5.2	0.4
20	$\beta$ -Damascenone	0–0.1	0.03	0–5.1	0.8
21	2-Phenylethanol	0–2.3	0.2	0–2.6	0.4
	1,3-Octandiol	0–5.4	0.2	n.d.	n.d.
	4-Vinylguaiacol	0–0.8	0.04	n.d.	n.d.

For each component, ranges of amounts and mean values are given.

n.s. = not separated on DB-Wax.

n.d = not detected.

ranges, the very volatile substances in lower concentrations, and the less volatile substances in higher amounts than detected in the industrial samples under study

(Table 3). Thus, in contrast to the results recently obtained in the course of studies on commercial pineapple juice (Elss et al., 2005), and in the commercialisation of

Table 2  
Aroma compounds determined by HRGC–MS in apple juice concentrates ( $n = 31$ )

Peak no.	Aroma compound	Range (mg/kg)	Mean (mg/kg)
2	1-Propanol	0–0.2	tr
3	Ethyl butanoate	0–0.3	tr
5	Butyl acetate	0–0.3	tr
6	Hexanal	0–0.1	0.02
7	2-Methylpropanol	0–0.4	0.03
<b>c + d</b>	2/3-Methylbutyl acetate	n.s.	n.s.
8	1-Butanol	0–0.5	0.08
<b>a + b</b>	2/3-Methyl-1-butanol	n.s.	n.s.
9	<i>E</i> -2-hexenal	0–0.1	0.02
11	1-Hexanol	0–0.2	0.04
12	<i>Z</i> -3-hexenol	tr	tr
13	<i>E</i> -2-hexenol	0–0.6	tr
14	Acetic acid	0–0.8	0.1
15	Furfural	2.4–56	12
16	Benzaldehyde	0–0.3	0.04
17	Butanoic acid	0–0.08	0.01
18	Phenylacetaldehyde	0–1.5	0.4
19	2-Methylbutanoic acid	0–2.8	1.0
20	$\beta$ -Damascenone	0–0.6	0.2
21	2-Phenylethanol	0–0.7	0.05
	1,3-Octandiol	0–1.8	0.6
	4-Vinylguaiacol	0–1.4	0.4

For each component, ranges of amounts and mean values are given.

tr = < 0.01 mg/l.

n.s. = not separated on DB-Wax.

apple juices made from concentrate, the legislative requirements concerning the use of recovery aroma seem to be fulfilled.

The HRGC–MS analysis of the apple volatiles in juice and water phase samples was not only carried out with a DB-Wax column but also with a RH-1701 column. This phase allows the separation of 2- and 3-methyl-1-butanol (**a/b**) as well as their corresponding acetates (**c/d**). The 2-methyl-branched volatiles are considered to be genuine apple constituents but the 3-methyl-branched are not; they are thought to arise from fermentation (Schumacher et al., 1998). As shown in Fig. 2, alcohol **a** dominated, quantitatively, in all samples. According to the literature, **a** and **c** are found to occur exclusively in nearly enantiomerically pure (*S*)-form (>98 (*S*)) in the samples (König & Schreier, 1999; Schumacher et al., 1998).

The amounts of 3-methyl-1-butanol (**b**), already more than 20 years ago classified as ‘undesirable’ for apple (Dürr & Schobinger, 1981), ranged from 0.01 to 2.1 and 1.5 to 134 mg/l in industrial juices and aromas, respectively. None of the samples was free of 3-methyl-1-butanol (**b**). Its acetate – with ranges of 0–0.3 and 0–3.3 mg/l in juices and aromas, respectively, was found in 45% of the samples under study. Obviously,

Table 3  
Aroma compounds determined in commercial single strength apple juices (**a**,  $n = 19$ ) and commercial apple juices made out of concentrates (**b**,  $n = 8$ )

Peak no.	Aroma compound	(a)		(b)	
		Range (mg/l)	Mean (mg/l)	Range (mg/l)	Mean
1	Propyl acetate	0–0.05	0.02	0–0.04	0.01
2	1-Propanol	0–0.6	0.1	0–0.2	0.05
3	Ethyl butanoate	0–0.2	0.1	0–0.1	0.04
4	Ethyl 2-methylbutanoate	0–0.07	0.03	0.04	0.01
5	Butyl acetate	tr–0.4	0.1	0–0.2	0.09
6	Hexanal	0–0.3	0.09	0–0.3	0.08
7	2-Methylpropanol	0–0.2	0.08	0–0.4	0.1
<b>c + d</b>	2/3-Methylbutyl acetate	n.s.	n.s.	n.s.	n.s.
8	1-Butanol	1.0–3.2	1.8	0.03–3.6	0.9
<b>a + b</b>	2/3-Methyl-1-butanol	n.s.	n.s.	n.s.	n.s.
9	<i>E</i> -2-hexenal	0–0.8	0.3	0–1.0	0.3
10	Hexyl acetate	0–0.1	0.05	0–0.1	0.04
11	1-Hexanol	0.8–3.2	1.8	0.2–3.4	1.4
12	<i>Z</i> -3-hexenol	0.01–0.1	0.04	0–0.1	0.04
13	<i>E</i> -2-hexenol	tr–1.0	0.4	0–0.9	0.3
14	Acetic acid	0–0.3	0.03	0–0.2	0.05
15	Furfural	0.1–7.7	1.2	0.8–5.6	2.0
16	Benzaldehyde	tr–0.08	0.02	0–0.1	0.03
17	Butanoic acid	0–0.2	0.02	0–0.09	0.02
18	Phenylacetaldehyde	0–0.02	0.01	0–0.1	0.04
19	2-Methylbutanoic acid	0–0.6	0.1	0.03–0.9	0.3
20	$\beta$ -Damascenone	0–0.03	0.02	tr–0.1	0.04
21	2-Phenylethanol	0–0.2	0.09	0–0.2	0.06
	1,3-Octandiol	0–0.3	0.09	0–0.8	0.2
	4-Vinylguaiacol	tr–0.08	0.03	0–0.2	0.07

For each component, ranges of amounts and mean values are given.

n.s. = not separated on DB-Wax.

tr = < 0.01 mg/l.



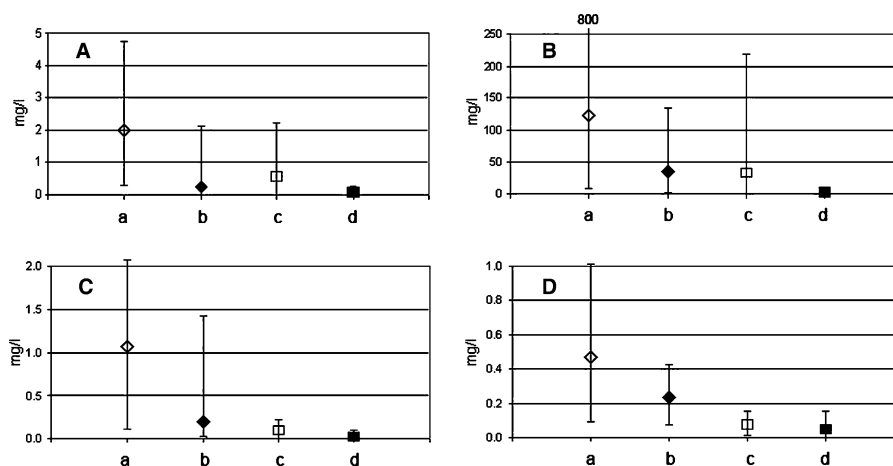


Fig. 2. Average amounts (mg/l) of 2-methyl-1-butanol (a), 3-methyl-1-butanol (b), 2-methyl-butyl acetate (c), and 3-methylbutyl acetate (d) in industrial single strength apple juices (A) and apple juice aromas (B) (each  $n = 31$ ), as well as commercial single strength apple juices (C;  $n = 19$ ) and commercial apple juices made from concentrate (D;  $n = 8$ ) separated on a RH-1701 column. Lowest and highest values are indicated.

fermentative effects cannot be completely excluded under industrial apple fruit juice processing conditions. At present, industrial and government organisations are discussing regulations to determine limits of the presence of **b** and **d** in fruit juice production.

### 3.2. Technology and isotopic discrimination

In order to check if apple juice aroma processing can modify the isotopic data of apple juice volatiles,  $\delta^{13}\text{C}_{\text{V-PDB}}$  and  $\delta^2\text{H}_{\text{V-SMOW}}$  values of three selected apple aroma compounds were measured in single strength apple juices and their corresponding aromas by HRGC–C/P–IRMS. As the two samples were each derived from one production line, it is possible to draw conclusions from the obtained data about potential thermodynamic isotopic effects during technological treatment. In Fig. 3, the results for  $^{13}\text{C}/^{12}\text{C}$  and  $^2\text{H}/^1\text{H}$  ratio determinations

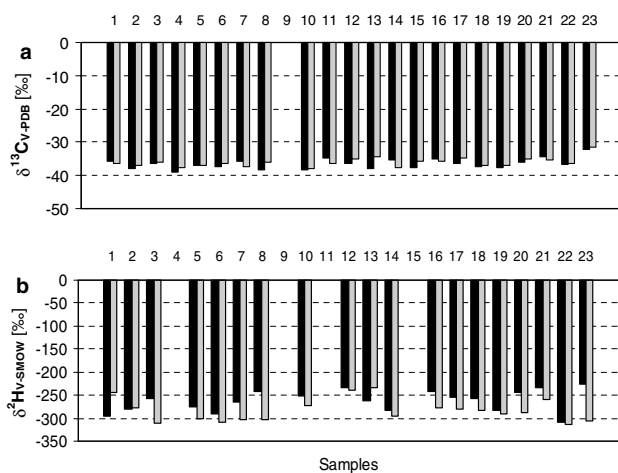


Fig. 3.  $\delta^{13}\text{C}_{\text{V-PDB}}$  (a) and  $\delta^2\text{H}_{\text{V-SMOW}}$  values (‰) (b) measured by HRGC–C/P–IRMS of *E*-2-hexenal (9), in apple juices and corresponding apple juice aromas.

of *E*-2-hexenal (9) in the samples are presented. For  $\delta^{13}\text{C}_{\text{V-PDB}}$  values of 9 (juice,  $-38.9\text{‰}$  to  $-32.2\text{‰}$ ; aroma,  $-39.1\text{‰}$  to  $-31.5\text{‰}$ ), no significant alterations during processing were observed. The  $\delta^2\text{H}_{\text{V-SMOW}}$  values of 9, however, showed slight depletion in the aroma samples (juice,  $-308\text{‰}$  to  $-227\text{‰}$ ; aroma,  $-318\text{‰}$  to  $-233\text{‰}$ ), as also indicated by data correlation outlined in Fig. 4.

Comparable results were found by measuring 1-hexanol (11, Fig. 5). While no definite changes in the  $\delta^{13}\text{C}_{\text{V-PDB}}$  values of 11 influenced by apple juice aroma production took place (juice,  $-42.4\text{‰}$  to  $-39.6\text{‰}$ ; aroma,  $-42.5\text{‰}$  to  $-38.4\text{‰}$ ), an increase of the lighter hydrogen isotopes in the aroma after distillation was detected. With the exception of one sample ( $-237\text{‰}$ ), the  $\delta^2\text{H}_{\text{V-SMOW}}$  values of the analysed apple juices ranged between  $-176\text{‰}$  and  $-74\text{‰}$ , whereas that of 11 in the aromas varied from  $-215\text{‰}$  to  $-99\text{‰}$ . This depletion of  $\delta^2\text{H}_{\text{V-SMOW}}$  values is also apparent after data correlation, as outlined in Fig. 6.

In contrast to 11, no significant isotopic effect caused by technological treatment could be observed in the case of *E*-2-hexenal (13), either in the  $\delta^{13}\text{C}_{\text{V-PDB}}$  or in the

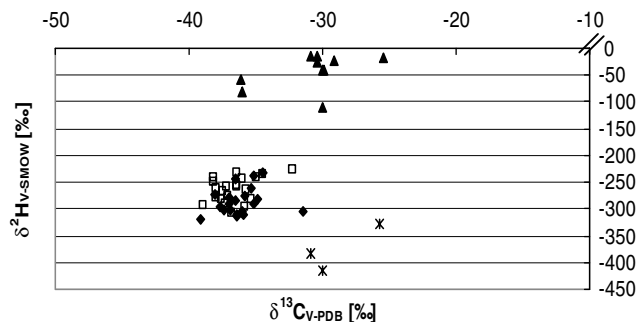


Fig. 4. Correlation of  $\delta^{13}\text{C}_{\text{V-PDB}}$  and  $\delta^2\text{H}_{\text{V-SMOW}}$  values (‰) of *E*-2-hexenal (9), in apple juices (□), corresponding apple juice aromas (◆), natural references (\*) and synthetic references (▲).

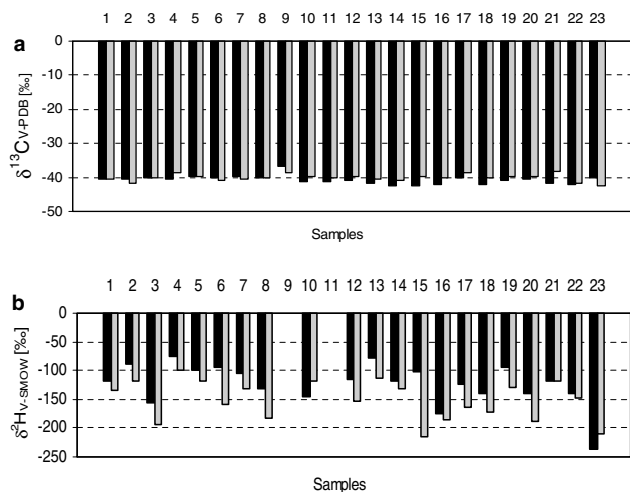


Fig. 5.  $\delta^{13}\text{C}_{\text{V-PDB}}$  (a) and  $\delta^2\text{H}_{\text{V-SMOW}}$  values (‰) (b) measured by HRGC-C/P-IRMS of 1-hexanol (11), in apple juices and corresponding apple juice aromas.

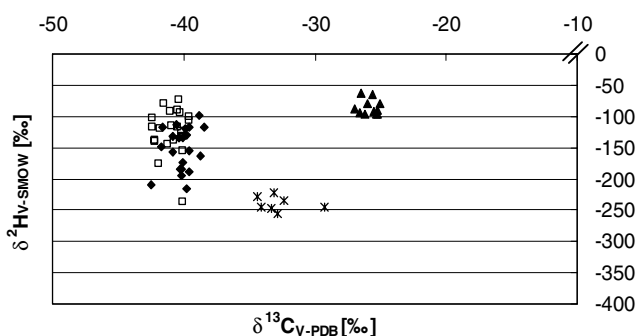


Fig. 6. Correlation of  $\delta^{13}\text{C}_{\text{V-PDB}}$  and  $\delta^2\text{H}_{\text{V-SMOW}}$  values (‰) of 1-hexanol (11), in apple juices ( $\square$ ), corresponding apple juice aromas ( $\blacklozenge$ ), natural references ( $*$ ) and synthetic references ( $\blacktriangle$ ).

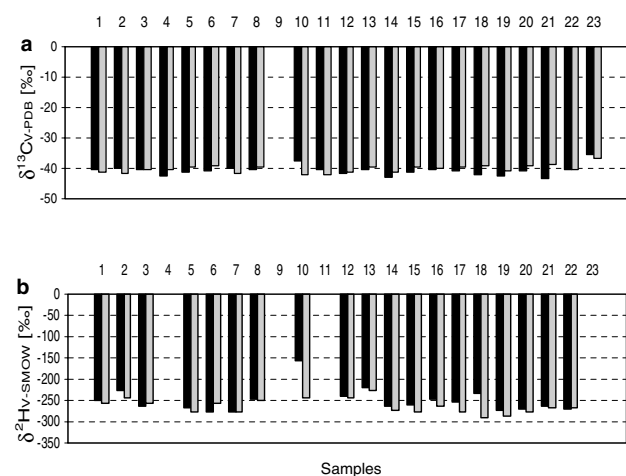


Fig. 7.  $\delta^{13}\text{C}_{\text{V-PDB}}$  (a) and  $\delta^2\text{H}_{\text{V-SMOW}}$  values (‰) (b) measured by HRGC-C/P-IRMS of *E*-2-hexenol (13), in apple juices and corresponding apple juice aromas.

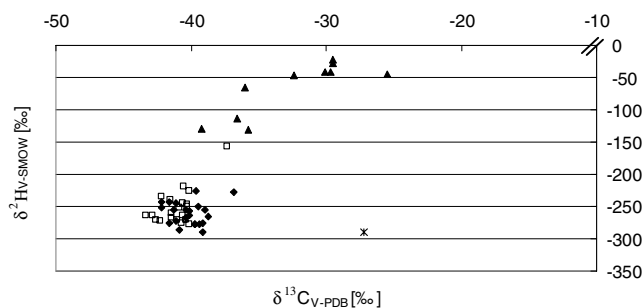


Fig. 8. Correlation of  $\delta^{13}\text{C}_{\text{V-PDB}}$  and  $\delta^2\text{H}_{\text{V-SMOW}}$  values (‰) of *E*-2-hexenol (13), in apple juices ( $\square$ ), corresponding apple juice aromas ( $\blacklozenge$ ), natural references ( $*$ ) and synthetic references ( $\blacktriangle$ ).

$\delta^2\text{H}_{\text{V-SMOW}}$  data (Fig. 7).  $\delta^{13}\text{C}_{\text{V-PDB}}$  values (juice,  $-43.3\text{‰}$  to  $-37.3\text{‰}$ , aroma,  $-42.2\text{‰}$  to  $-36.8\text{‰}$ ) and  $\delta^2\text{H}_{\text{V-SMOW}}$  data of 13 (juice,  $-278\text{‰}$  to  $-219\text{‰}$  – exception  $157\text{‰}$  – aroma,  $-289\text{‰}$  to  $-225\text{‰}$ ) showed no consistent tendency for enrichment or depletion during apple juice aroma processing (Fig. 8).

#### 4. Conclusions

In the course of industrial apple aroma production, the qualitative aroma profile is preserved. For the first time, the ranges of amounts of main aroma components were determined, providing additional knowledge for quality assessment of apple juice. Using HRGC-P/C-IRMS analysis, a slight trend to depletion of  $\delta^2\text{H}_{\text{V-SMOW}}$  values of  $\text{C}_6$ -aroma compounds was observed. However, it is so small that authenticity assessment by stable IRMS is not influenced at all by this effect.

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