



ELSEVIER

Journal of Chromatography A, 684 (1994) 360–365

JOURNAL OF
CHROMATOGRAPHY A

Short communication

Enantiomeric and isotopic analysis of flavour compounds of some raspberry cultivars

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First received 7 March 1994; revised manuscript received 1 July 1994

Abstract

Analytical results from enantiomeric and isotopic analyses of four typical raspberry aroma compounds, α - and β -ionones, δ -decalactone and raspberry ketone, are reported. Different raspberry cultivars and commercial raspberry flavoured products were analysed, and a principal component analysis was carried out on the results. Using these methods, a cultivar discrimination was possible and significant information was obtained on the flavour of different cultivars. It was also possible to discriminate between natural and adulterated samples.

1. Introduction

Raspberry is a fruit mainly used in the food industry (baking, jam, syrup and juice). Therefore many authors have worked on its chemical characterization. Chromatographic methods of authentication were carried out [1] in 1965 and increasing numbers of components have been identified with the modernisation of analytical techniques since the study of Winter and Enggist [2]. Latrasse [3] proposed an aroma index in order to evaluate the flavour intensity and the "hedonic" part of several raspberries cultivars. In the 1980s particular attention was paid to a flavour impact molecule, 4-(*p*-hydroxyphenyl)-2-butanone, called raspberry ketone, whose olfactory impact was closely related to the fresh raspberry fruit. Several correlations between different cultivars were carried out [4,5].

New methods of detecting adulteration made

it possible for the analyst to discard sensory evaluation of raspberry ketone and to replace it by the examination of the chirality of certain raspberry aroma compounds such as α -ionone or theaspirane diastereoisomers [6].

In our study, we aimed to use modern methods to check the chiral and isotopic "quality" and to quantify flavour impact molecules. The present report shows that these two aspects of the analysis of the fruit can be described by principal component analysis (PCA) and can also determine the authenticity and sensory quality of the product.

2. Experimental

2.1. Samples

Natural samples of Mecker, Heritage and Williamette raspberry cultivars were provided by Sicoly and Flores. The origin of other cultivars

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was Ardeche (South of France). Raspberry-flavoured products (tea, syrup, juice) were bought on the local market.

2.2. Apparatus

Concentrated raspberry oils were analysed by GC-MS (Hewlett-Packard 5890 mass-selective detector) using the specific ion monitoring (SIM) method, which is specific and sensitive for particular molecules. Column: HP1, 25 m × 0.25 mm I.D., film thickness: 0.25 μm (Hewlett-Packard); carrier gas: He, 138 kPa; temperature program: 50°C for 5 min, 5°C min⁻¹ to 200°C, 25°C min⁻¹ to 295°C, 10 min at 295°C; temperature of injection: 280°C. Selected ions: *m/z* 192, 1 to 24.8 min (α- and β-ionones); *m/z* 99, 24.8 to 26 min (δ-decalactone); *m/z* 164, 26 min to end of run (raspberry ketone). Splitless injection; injection volume: 0.4 μl.

Chirality measurements were carried out on a Sichromat II (Siemens, Karlsruhe, Germany) with double ovens and pneumatic heart cut switching, allowing multidimensional gas chromatography (MDGC). Precolumn: 25 m × 0.32 mm I.D. coated with BP 20 from SGE; film thickness: 0.5 μm; carrier gas: He, 138 kPa; injection temperature: 250°C (flame ionization detection, FID); detector temperature: 250°C; program: 120°C for 5 min, 10°C min⁻¹ to 220°C. Main column: 25 m × 0.22 I.D. coated with 10% octakis-2,6-dimethyl-3-trifluoroacetyl-γ-cyclodextrin in OV-1701 (MEGA, Milan, Italy); carrier gas: He, 96 kPa; detector temperature: 250°C (FID); program: 110°C for 10 min, 1°C min⁻¹ to 180°C; injection volume: 1 μl.

Isotope ratios ¹³C/¹²C were measured on a MAT 252 (Finnigan, Bremen, Germany) with combined GC-combustion unit on CuO at 800°C—isotopic ratio mass spectrometry (IRMS) set on *m/z* 44, 45, 46. Column: HP1, 25 m × 0.25 mm I.D., film thickness: 0.25 μm (Hewlett-Packard); injection temperature: 285°C, splitless; carrier gas: He, 90 kPa; injection volume: 0.4 μl; temperature program: 50°C for 15 min, 5°C min⁻¹ to 140°C, 2°C min⁻¹ to 250°C, 15 min at 250°C.

Results are expressed as $\delta = [(R_{\text{exp}} - R_{\text{ref}}) /$

$R_{\text{ref}}] \cdot 1000$, where R is the isotope ratio ¹³C/¹²C and where the reference is a calcium carbonate (the P.D. Belemnita of South Carolina). δ is expressed in ‰ [7].

PCA was carried out on Statgraphics US (STSC, Rockville, MD, USA) integral software.

2.3. Sample preparation

A 500-g amount of fresh raspberries was ground with 300 ml distilled water and 200 ml distilled acetone to improve the extraction from the pulp of certain aroma compounds such as raspberry ketone. Pulp and juice were separated by filtration through sand.

After acetone removal under vacuum at ambient temperature, the aqueous raspberry juice was extracted in a separatory funnel with 250 ml of a mixture of pentane-diethyl ether (50:50). The organic layer, containing most of the aroma compounds, was dried over anhydrous Na₂SO₄ and concentrated at 40°C to a volume of 25 ml. An injection volume of 1 μl is taken for the GC-MS analysis. The 25 ml were reduced further to 2 ml and 1 μl of the residual oil injected for MDGC and GC-IRMS analysis.

If the chromatogram is complex, the sample can be cleaned up by a 2-step process, viz. first washing the ether extract with sodium hydrogencarbonate (10%) and then with water, and then pouring the ether extract through a column of activated Florisil and eluting the flavour compounds with dichloromethane-acetone (90:10). After such a treatment, the quality of resolution and background are optimised.

3. Results and discussion

In the natural fruit one enantiomer predominates whereas synthetic molecules have a racemic distribution. In the same way, the ¹³C content of natural molecules can differ from the synthetic ones. The authentication methods are based on these two possibilities of discrimination [8].

Table 1
Chiral and isotope $^{13}\text{C}/^{12}\text{C}$ measurements with quantitative ratios of natural raspberry samples

Cultivar and characterization	δ -Decalactone enantiomeric excess (%)	δ -Decalactone isotope ratio $^{13}\text{C}/^{12}\text{C}$ (‰)	α -Ionone enantiomeric excess (%)	α -Ionone isotope ratio $^{13}\text{C}/^{12}\text{C}$ (‰)	Raspberry ketone isotope ratio $^{13}\text{C}/^{12}\text{C}$ (‰)	<i>cis</i> -3-Hexen-1-ol isotope ratio $^{13}\text{C}/^{12}\text{C}$ (‰)	Quantitative ratio $\text{R1} = \delta\text{C}_{10}/\alpha$ -ionone	Quantitative ratio $\text{R2} = \alpha$ -ionone/ β -ionone	β -Ionone isotope ratio $^{13}\text{C}/^{12}\text{C}$ (‰)
Natural Mecker (1993)	July	98 to 100 (S)	98 to 100 (R)	-34 to -39	-33 to -35	28.9 to 31.9	20 to 130	0.4 to 2.4	32.2 to -33.9
	August	98 to 99 (S)	98 (R)	36 to 39.4	33 to 36	30.6 to 32	20 to 31	0.9 to 1.0	32.3 to 31
Natural Heritage X (August 1993)	100 (S)	33.3 to 35.9	98 (R)	36.5 to 37.5	28.8 to 30.2	35.2	7 to 20	0.6 to 1.5	32.2 to -34.1
	99 (S)	-40.2	98 (R)	-36	30.1	n.d.	55	1.0	33.7
Natural wild (1993)	100 (S)	-37.2	99 (R)	-33.6	35.1	34.6	8	0.9	-33.0
Natural Williamette (1992)	99 (S)	35.0	99 (R)	-34.3	30.0	35.4	50	0.6	-34.4
Natural Malling Promise (1992)	99 (S)	34.0	100 (R)	-32.5	30.0	-33	16	0.7	n.d.

n.d. = Not detected; X is a Mecker's sample supplied under the name Heritage.

^a Measurements made on a mixture of different samples.Table 2
Chiral and isotope $^{13}\text{C}/^{12}\text{C}$ measurements with quantitative ratios of raspberry-flavoured products

Commercial Samples	δ -Decalactone enantiomeric excess (%)	δ -Decalactone isotope ratio $^{13}\text{C}/^{12}\text{C}$ (‰)	α -Ionone enantiomeric excess (%)	α -Ionone isotope ratio $^{13}\text{C}/^{12}\text{C}$ (‰)	Raspberry ketone isotope ratio $^{13}\text{C}/^{12}\text{C}$ (‰)	<i>cis</i> -3-Hexen-1-ol isotope ratio $^{13}\text{C}/^{12}\text{C}$ (‰)	Quantitative ratio $\text{R1} = \delta\text{C}_{10}/\alpha$ -ionone	Quantitative ratio $\text{R2} = \alpha$ -ionone/ β -ionone	β -Ionone isotope ratio $^{13}\text{C}/^{12}\text{C}$ (‰)
Syrups	SA	-34	100 (R)	-35.8	-31.9	n.d.	11	50	-29.5
	SB	-26.3	4 (R)	-27.8	-24.0	n.d.	0.5	9	-26.3
Heavy Juice	BA	-28.3	100 (R)	-34.3	-29.1	n.d.	23	3	-33.1
	BB	-29.5	4 (R)	-26.0	-29.3	n.d.	4	2.1	-26.0
Tea	0	-27.6	8 (R)	n.m.	-25.2	n.d.	0.2	100 ^a	n.d.

n.m. = Detected but not measurable; n.d. = not detected.

^a R2 theoretically equal to +∞.

Four flavour impact molecules of raspberry were selected: α - (CAS No. 127-41-3) and β -ionones (CAS No. 14901-07-6), giving for the first a woody violet-like odor, whereas the second makes a more floral impact; δ -decalactone (CAS No. 705-86-2) which provides a peachy/apricot-like olfactory impression and the raspberry ketone or 4-(*p*-hydroxyphenyl)-2-butanone (CAS No. 5471-51-2), whose impact is clearly reminiscent of fresh raspberry.

From previous chiral studies, the enantiomeric distribution was expected to be strongly in favour of the *R* form for the *trans*- α -ionone [9] whereas the δ -decalactone occurs only in its *S* form [10] in the fruit.

The observed strong enantiomeric excesses of α -ionone and δ -decalactone in the natural products (Table 1) agreed with the literature [6], whereas in some cases commercial raspberry-flavoured products (Table 2) gave nearly racemic distributions.

The $^{13}\text{C}/^{12}\text{C}$ isotope ratio of the raspberry ketone (Table 1) and δ -decalactone revealed significant differences between the nature of identical molecules of synthetic origin and the natural molecules, particularly for δ -decalactone which showed a strong negative isotopic deviation (-34% to -40%). *cis*-3-Hexen-1-ol showed a relatively low ^{13}C content. Table 1 gives the isotope ratios found in the different cultivars. Wild fruits showed lower δ values for raspberry ketone than cultivated species and this can help to distinguish the two groups.

Concentrations of the four chosen molecules are useful parameters. There was an increase in the level of aroma compounds within the Mecker cultivars from July to August; these measurements explain the fact that July raspberries did not have as pronounced a taste as those from August. The results we obtained are quite different from those given in the literature [5]. However, in the determination of such concentrations, the initial quantity of raspberries extracted needs to be known accurately. Such accurate information is rarely available in the case of commercial products. In order to avoid having to depend on the initial quantity, we chose to compare internal ratios such as those shown in

Table 1. This made it possible to compare the results from our natural samples and from samples where the quantity extracted and the method of extraction were not known.

When PCA was applied to the data a two-dimensional representation explained 77% of the variations. The six parameters chosen were δ -decalactone enantiomeric excess, α -ionone enantiomeric excess, raspberry ketone $^{13}\text{C}/^{12}\text{C}$ isotope ratio, δ -decalactone $^{13}\text{C}/^{12}\text{C}$ isotope ratio, quantitative ratio of raspberry ketone to δ -decalactone and quantitative ratio of δ -decalactone to α -ionone.

The first steps of PCA analysis extracted α -ionone enantiomeric excess and δ -decalactone $^{13}\text{C}/^{12}\text{C}$ isotope ratio as the physicochemical properties which distinguish between the synthetic group on the left of Fig. 1 and the natural group on the right. A third discriminant axis is obtained with the isotope ratio of raspberry ketone but that parameter is not sufficient to authenticate a sample because the values for natural and synthetic products are too close.

Further conclusions can be drawn in a second step of PCA (Fig. 1) when ratios of flavour compounds are examined. It is interesting to note that the quantity ratio of δ -decalactone/ α -ionone appears as a discriminant axis between different natural samples. From July to August, the Mecker cultivar zone has dropped due to the increase in α -ionone which is responsible for the development of a woody odour like in the wild species. The Heritage cultivar is close to the August Meckers, but forms a distinct group, the quantity ratios α/β -ionones being somewhat higher than those of the Meckers. The cultivars, Malling Promise and Williamette, can be grouped by PCA with the Meckers from August and July respectively. The wild raspberries form a group with low δ -decalactone/ α -ionone quantity ratios, which correlates with the fact that these fruits have a strong woody wild odour. A second factor which separates the wild variety from the cultivated ones is its very low $^{13}\text{C}/^{12}\text{C}$ isotope ratio for raspberry ketone.

To test the power of PCA, sample X was provided under the name "Heritage". An analysis of the parameters and comparison with the

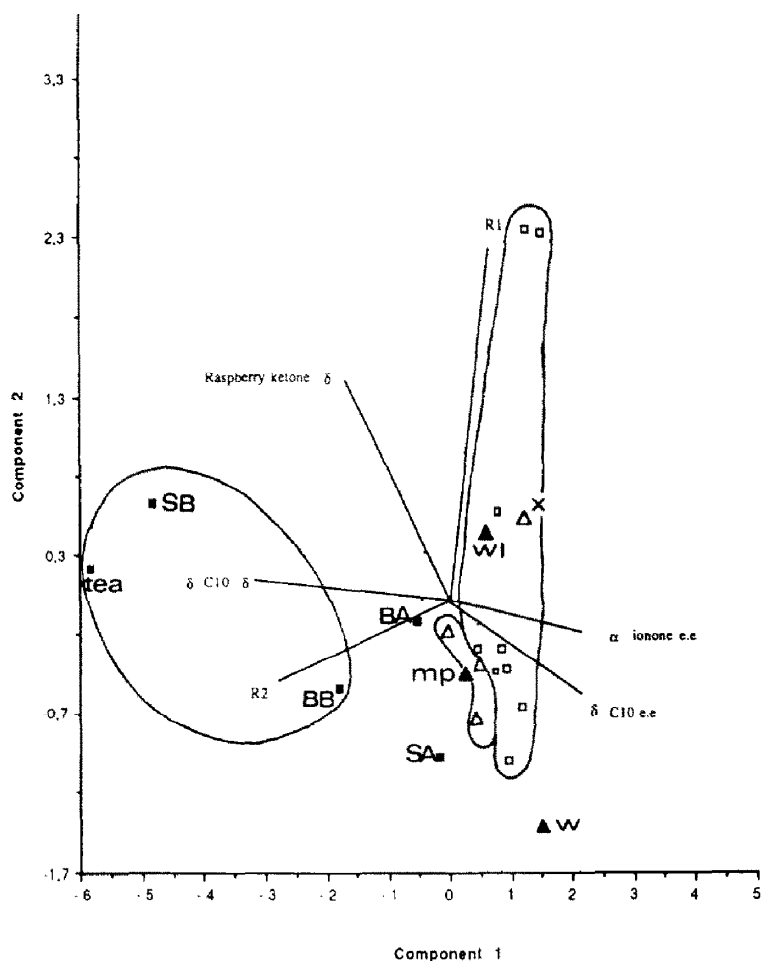


Fig. 1. Principal component analysis of raspberries samples. ■ = Raspberry-flavoured products (tea, syrup, heavy juice); □ = Mecker cultivar; △ = Heritage cultivar; ▲ = other raspberry cultivars; W = wild, mp = Malling Promise, WL = Williamette; e.e. = enantiomeric excess; δ = isotope ratio $^{13}\text{C}/^{12}\text{C}$; X = sample supplied to test discriminant power of the method; R1 = quantitative ratio raspberry ketone/ δ -decalactone; R2 = quantitative ratio δ -decalactone/ α -ionone.

PCA showed that sample X belonged to the Mecker group, a fact which was confirmed by the supplier.

Commercial samples like syrup SB, heavy juice BB and the tea were found to belong to the synthetic area on the left.

PCA provides a great deal of information about the nature of the fruit both with respect to authenticity and flavour. However, there is no correlation between naturalness and olfactory impressions.

4. Conclusions

The simultaneous chiral and isotopic measurement of aroma compounds together with their quantification produces results that can be used to perform PCA. The introduction of quantification parameters to an analysis brings out important points:

(1) A cultivar discrimination is possible and significant information about the taste of the different cultivars can be obtained.

(2) Although climatic variations influence the level of aroma compounds in natural samples it is still possible to discriminate between natural and adulterated samples.

Chiral chromatography and IRMS are powerful complementary methods for screening raspberry flavour in authentication problems.

Acknowledgements

We are very grateful to Mr. Massardier (Sicoily, St. Laurent d'Agny, France) and Mr. Meurisse (Flores, Grenoble, France) for providing samples and for profitable collaboration.

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