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Enantioselective HS-SPME-GC-MS for Authentication of Natural and Synthetic Strawberry Flavour in Syrups

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Demand for a reliable distinction of the strawberry flavour naturalness stood in the beginning of this research. An efficient chiral column with CP-Chirasil-Dex CB stationary phase was involved in headspace solid-phase microextraction-gas chromatography-mass spectrometry analysis of strawberry flavour in 6 strawberry cultivars, 7 samples of strawberry flavourings and 14 samples of strawberry syrups to evaluate the authenticity of flavour naturalness and reveal adulteration. Enantiomeric ratios of 8 selected chiral volatiles (methyl 2-methylbutanoate, ethyl 2-methylbutanoate, 2-methylbutanoic acid, linalool, α-ionone, γ-decalactone, γ-undecalactone, δ-dodecalactone) were evaluated. Seven chiral volatiles contributed to the revelation of synthetic origin. γ-Decalactone (enantiomeric ratios: 100⁄0, 100⁄0, 99⁄1, 99⁄1, 100⁄0, and 99⁄1 in strawberries; 100⁄0 in 3 natural flavourings; 100⁄0 in syrup with natural flavour) was the most contributive indicator of synthetic and natural strawberry flavour, followed by ethyl 2-methylbutanoate (enantiomeric ratios: 0⁄100, 5⁄95, 5⁄95 in 3 natural flavourings, 0⁄100 in syrup with natural flavour). The best chiral resolution was observed for α-ionone (R_s>4). One syrup supposed to contain only natural strawberry flavouring was suspected to be adulterated regarding nearly racemic ratios of its four crucial chiral volatiles. Investigation of enantiomeric ratios of the unique selection of chiral volatiles and strawberry samples using the CP-Chirasil-Dex CB chiral column wider revealed possibilities for reliable recognition of strawberry flavour naturalness in complex matrices and in food samples in general.

INTRODUCTION

In recent years, findings on different sensory properties [Werkhoff *et al.*, 1993] or different biological activities [da Silva Rivas *et al*., 2012] of flavour compounds' enantiomers have appeared, as well as the studies demonstrating unlike enantiomeric composition of natural and synthetic flavours [Ravid *et al*., 2010]. Therefore, some consumers can prefer products with natural flavours and a credible flavour declaration on the label. The expression 'flavourings' is usually found without any additional description on food products. The probability of the synthetic origin in the case of 'flavourings' declaration is high, and even the declaration of natural flavouring can be misleading. Large assortment of strawberry products in local markets as well as the effort of producers to reduce expenses are among the factors that often lead to adulteration in the form of replacing the natural flavour declared on the product package with a synthetic one. The appropriate analytical testing to reveal flavour origin can be therefore very helpful.

Regarding limited possibilities for authentication of strawberry flavour (*e.g.* stable isotope ratio mass spectrometry which should be used as a set of values [Nitz *et al*., 1991; Schipilliti *et al.*, 2011] considering the overlapping isotopic ranges for γ-decalactone of synthetic and natural origin [Nitz *et al.*, 1991]), there is a crucial focus on the scope of possibilities in chiral gas chromatography (GC). To the best of our knowledge, there is paucity of literature dealing with the chiral GC for authentication of natural strawberry flavour and the available works focus mainly on fruit (Table 1), while even fewer studies have investigated synthetic strawberry flavour in commercial products [Cagliero *et al*., 2012; Kreck *et al*., 2001; Nitz *et al*., 1991; Ravid *et al*., 2010; Schipilliti *et al*., 2011]. In one study only [Kreck *et al*., 2001], chiral GC was used to authenticate strawberry flavour in syrup, a matrix that is different and can be more complicated, and moreover, just one syrup sample was researched therein. Besides that, to the best of found knowledge, there is a lack of studies investigating a rather wide range of chiral volatiles in commercial food products for the purposes of strawberry flavour authentication, despite its crucial importance. Kreck *et al*. [2001] investigated seven chiral volatiles, Cagliero *et al*. [2012] compared four chiral volatiles in strawberry yogurt, Schipilliti *et al.* [2011] two in yoghurt and candies, and Nitz *et al.* [1991] detected six γ-lactones in strawberry wines of which only two

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TABLE 1. Enantiomeric characterisation of selected volatiles of strawberries obtained by analysis on cyclodextrin stationary phases.

Elution order	Compound	t_{RI} (min)		t_{RII} (s)		LRI		Elution order of enantiomers		Enantiomeric comparison	Enantiomeric ratio	Chiral stationary phase[a]	Literature
		1.	2.	1.	2.	1.	2.	1.	2.				
1	Methyl 2-methylbutanoate							*R*	*S*	*S>>R*	97/3–99/1[e]	DIME-β-CD[i]	Kreck *et al.* [2001]
		9.7	10.2	1.11	1.13							EtTBS-β-CD[i] and CycloSil B[i]	Williams *et al.* [2005]
2	Ethyl 2-methylbutanoate							(-)-	(+)-	(+)>>(-)	95/5; 100/0; 99/1[bf]	Chirasil-β-Dex[i]	de la Peña Moreno *et al.* [2010]
								R	*S*	*S>>R*	97/3–99/1[e]	DIME-β-CD[i]	Kreck *et al.* [2001]
		11.6	12.1	1.30	1.30							EtTBS-CD[i] and CycloSil B[i]	Williams *et al.* [2005]
3	2-Methylbutanoic acid							*S*	*R*	*S>>R*	99/1[e]	DIME-β-CD[i]	Kreck *et al.* [2001]
		35.5 (*S*)		1.43 (*S*)								EtTBS-CD[i] and CycloSil B[i]	Williams *et al.*, [2005]
4	(β)-Linalool									*S>>R*	97/3–99/1[f]	Lipodex C[i]	Bernreuther&Schreier, [1991]
								(-)-	(+)-	*(+)>(-)*	57/43; 62/38; 60/40[bf]	Chirasil-β-Dex[i]	de la Peña Moreno *et al.* [2010]
						1226[d]	1233[d]	*R*(-)[d]	*S*(+)[d]			Rtβ-DEXsm[i]	Ravid *et al.* [2010]
						1296[d]	1298[d]	*S*(+)[d]	*R*(-)[d]			Rtγ-DEXsa	Ravid *et al.* [2010]
										S>>R	97/3–99/1[e]	Megadex DETTBS-β[i]	Schipilliti *et al.* [2011]
		37.8	38.5	2.05	2.00							EtTBS-CD[i] and CycloSil B[i]	Williams *et al.* [2005]
5	(*E*)-α-Ionon							*S*	*R*	*R>>S*	99/1[e]	DIME-β-CD[i]	Kreck *et al.* [2001]
						1502[d]	1512[d]	*S*(-)[d]	*R*(+)[d]			Rtβ-DEXsm[i]	Ravid *et al.* [2010]
6	γ-decalacton							*R*	*S*	*R>>S*	98/2[f]	Lipodex B	Bernreuther *et al.* [1989]
										R>>S	97/3–99/1[e]	DIME-β-CD[i]	Kreck *et al.* [2001]
								R	*S*	*R>>S*	>99% *R*[bf]	Lipodex D[i]	Mosandl *et al.* [1989]
										R>>S	99/1[f]	Lipodex B	Nitz *et al.* [1991]
						1680[d]	1688[d]	*R*(+)[d]	*S*(-)[d]	*R>>S*	90/10–100/0[eg]	Rtβ-DEXsm[i]	Ravid *et al.* [2010]
										R>>S	89/11–95/5[e]	Megadex DETTBS-β[i]	Schipilliti *et al.* [2011]
7	γ-undecalacton							*R*	S		traces[cf]	Lipodex B	Bernreuther *et al.* [1989]
						1745[d]	1748[cd]	*R*(+)[d]	*S*(-)[d]			Rtβ-DEXsm[i]	Ravid *et al.* [2010]
8	δ-dodecalacton							*S*(-)	*R*(+)	*R>S*	30.10/69.10[f]	Lipodex D[i]	Bernreuther *et al.* [1991]
								S(-)	*R*(+)	*R>S*	33/67–35.8/64.2[h]	Hydrodex β analogue[i]	Krammer *et al.* [2006]

Elution order – elution order of the compounds based on literature data; t_{RI} – retention time of enantiomer standards on EtTBS-CD column; t_{RII} – retention time of enanciomer standards on CycloSil B column; LRI – linear retention index; Enantiomeric comparison – comparison of enantiomers *R* and *S* according to their percentage proportion; Enantiomeric ratio – ratios between enantiomers *R* and *S*.
[a]For detailed composition see Table S3 in supplementary materials. [b]Data from literature were recalculated to enantiomeric ratios for the purposes of our study as: [(predominant enantiomer–minor t-enantiomer)/(predominant enantiomer+minor enantiomer)×100]. [c]Enantiomeric discrimination not possible. [d]Measured for standards. [e]Obtained from strawberries. [f]Obtained from strawberry extract. [g]Obtained from supernatan after lyophilization of strawberries. [h]Obtained from condensate strawberry water phase after lyophilization of strawberries. [i]β-cyclodextrin (β-CD) is the chiral selector of chiral stationary phase.

were functional. γ-Decalactone was a substance investigated wider than the others in strawberries (Table 1), but even this substance has been rarely studied in the literature in commercial products, just once in syrup and only in a single sample [Kreck *et al*., 2001]. The urgent need for a reliable authentication of the natural and synthetic origin of strawberry flavour by chiral gas chromatography-mass spectrometry (chiral GC-MS) based on the results of a wider range of substances as well as its application in strawberry syrups for revealing their adulteration stood in the beginning of our research.

The condition for such a determination was to find a chiral column allowing good separation of the enantiomers of the investigated substances. Modified forms of cyclodextrin are gaining in popularity as the bases of chiral stationary phases in GC enabling enantiomeric separation of almost all classes of volatile chiral compounds, highly effective in the flavour and fragrance field [Betzenbitcher *et al.*, 2022; Cagliero *et al.*, 2015, 2017; Engel, 2020]. For a specific case – a molecule of permethylated β-cyclodextrin separating enantiomers of selected flavours in GC – Lipkowitz's group described a lot of aspects of the separation mechanisms [Lipkowitz *et al*., 1997a,b; 1998]. Instead of different number of bondings ("three-point interaction model" – [Easson & Stedman, 1933; Fanali *et al.*, 2019]), the authors pointed out the same interactions of both enantiomers but their greater or lesser extent [Lipkowitz *et al*., 1997b]. Simultaneously, the authors concluded that permethylated β-cyclodextrin displayed its enhanced chiral discriminating capacity because of the spatial congruence of its domain of greatest enantiodifferentiation, found to be inside its macrocyclic cavity, with the analyte's preferred binding site [Lipkowitz *et al.,* 1998]. Later, the question arose, whether the formation of inclusion complexes was really a prerequisite for enantiorecognition. On the one hand, external complexes of selected enantiomers with β-cyclodextrine derivatives were observed in some other separation techniques, such as capillary electrophoresis [Servais *et al.*, 2012]; on the other hand, selected racemic compounds were enantioseparated also on the acyclic analogue ('acyclodextrin') of a specific β-cyclodextrin derivative in GC conditions [Sicoli *et al.*, 2005, 2007]. However, the enantioseparation factors α of compounds separated on the acyclic form were found mostly lower than those separated on their cyclic counterpart. Moreover, there was no study for GC conditions demonstrating the enantioseparations of similar compounds on the acyclic analogue of permethylated β-cyclodextrin studied by Lipkowitz's group. Current literature usually mentions the formation of inclusion complexes [Betzenbichler *et al*., 2022; Cagliero *et al.,* 2015; Scriba, 2016; Uccello-Barreta *et al*., 2010], but is more cautious about explaining the origins and mechanism of enantiorecognition, rather discussing factors enhancing the recognition efficiency or contributing to enantioselective process [Menestrina *et al.,* 2018; Uccello-Barreta *et al*., 2010; Wu *et al*., 2022]. Anyway, even nowadays there are studies considering inclusion interactions as a factor that can play an important role in enantioseparation [Betzenbichler *et al*., 2022]. The need for more explicit clarification of the enantioseparation mechanisms is often mentioned, as this would make it possible to prepare even a more universal enantioselector [Cagliero *et al.,* 2015; Engel, 2020]. Therefore, testing the functionality of chiral selectors by their direct application in the chromatographic analysis of real samples is a significant support in the field of enantioselectivity research. The CP-Chirasil-Dex CB column was chosen for our investigation due to its possible efficiency in separating enantiomers of the investigated enantiomeric pairs, due to the popularity of similar enantioseparating columns in relevant literature [Betzenbichler *et al*., 2022; Cagliero *et al.*, 2017; de la Peña Moreno *et al.*, 2010; Schmalzing *et al.*, 1992], as well as because of the results achieved on β-cyclodextrin phases in the analysis of strawberries (Table 1).

The knowledge about enantioselective recognition of synthetic and natural strawberry flavour has been gathered since the 20th century. Some volatiles provide specific distribution of enantiomers in their natural forms in different matrices, as it is obvious from the study by Nitz *et al.* [1991], providing several key findings. The authors assumed that chiral substances with one of the enantiomers significantly predominant in natural form, as they observed in the case of γ-decalactone and γ-dodecalactone, should be suitable for the evaluation of strawberry flavour naturalness, as they differ sufficiently from the racemic enantiomeric ratio (racemate) of their synthetic forms. At the same time, however, it depended on the lactones involved. The study showed almost pure *R*-enantiomer of γ-decalactone and γ-dodecalactone in strawberries and its absolute predominance in strawberry wines. In contrast, Nitz *et al.* [1991] found nearly racemic ratios of γ-hexa- and γ-heptalactone in strawberries and racemic γ-decalactone in deep frozen raspberries. Similarly, Bernreuther *et al*. [1991] noticed nearly racemic ratios for δ-octalactone in strawberries. Nitz *et al*. [1991] also discussed the potential effect of processing, microbial fermentation, and flavouring additives on the enantiomeric ratios, resulting in racemization. The authors [Nitz *et al*., 1991] noted that *R*-enantiomer of γ-decalactone observed in strawberry wines might be present due to an additive of microbiological origin, thus could not be reliably considered as a proof of natural flavour content. The following research extended these conclusions. In the study by Ravid *et al*. [2010], the authors focused closer on γ-decalactone in strawberries and strawberry products and similarly concluded that (*R*)-(+)-γ-decalactone was typical for the natural flavour (the enantiomeric purity ranged between 90 and 100% in 11 varieties of strawberries), for the synthetic flavour the enantiomeric ratio was equivalent to or close to the racemate. The authors highlighted the headspace-solid phase microextraction (HS-SPME) technique as the procedure without racemization. In turn, Kreck *et al*. [2001] explored various strawberry varieties and strawberry products to evaluate the influence of strawberry processing on enantiomeric composition of investigated volatiles. The results provided pure enantiomers for (*S*)-methyl 2-methylbutanoate, (*S*)-ethyl 2-methylbutanoate, (*R*)-γ-octalactone, (*R*)-α-ionone, (*R*)-γ-decalactone and (*R*)-γ-dodecalactone in strawberry flavour of natural origin, no racemization or creation of artefacts during sample processing was documented. Enantiomeric ratios close to the racemates were revealed in synthetic flavour. The authors explained that synthetic compounds are often racemic due to their nonselective chemical synthesis.

This present study focuses on eight chiral substances. Simultaneously, to the best of our knowledge, it is the first study focusing in detail on authentication of strawberry flavour in syrups and applying the CP-chirasil-Dex CB chiral column for strawberry flavour authentication. It is also the first study which compares enantiomeric ratios in 6 strawberry cultivars, 9 strawberry flavourings, and 14 strawberry syrups together in one research to evaluate functionality of the investigated chiral volatiles for authentication of strawberry flavour in complex matrices with variable composition. Analysis of a rather wide range of chiral volatiles, large number and unique selection of samples and the easily achievable technique of HS-SPME GC-MS providing no racemization [Ravid *et al*., 2010] in combination with the CP-chirasil-Dex CB chiral column are expected to enable more reliable authentication of strawberry flavour and extend the possibilities to recognize synthetic or natural strawberry flavour in strawberry syrups and strawberry products.

MATERIALS AND METHODS

Samples

Strawberry cultivars of Viva (S1), Dart Select (S2), Korona (S3), Evie 2 (S4), Malvina (S5), and Elsanta (S6) were purchased in the Czech Republic in local Prague markets; 7 strawberry flavourings (A to F, where B, C, F were natural, see Table S1 – supplementary materials) were obtained from Darmstadt, Germany; and 14 syrups with strawberry flavour (1–14, syrup 1 and 3 with natural strawberry flavour, see Table S2 – supplementary materials) were bought in the Czech Republic in Prague shops. Strawberries were homogenized by T18 basic ULTRA-TURAX (IKA® group, Staufen, Germany) and stored for 6 months in a freezer at –24°C. Flavouring samples were kept in originally closed packages and stored at 4°C in a fridge, while syrups were kept at room temperature of 20°C.

Chemicals

Butyl acetate (≥98%), ethyl butyrate (≥98%), hexanal (98%), (*E*)-hex-2-enal (≥95%), hexyl acetate (99%), 3-methylbutyl acetate (≥97%), (–)-linalool (95%) and (+)-γ-decalactone (≥97%) were provided in analytical quality; the mixture of alkane standards C8-C20 (40 mg/L, in *n*-hexane) was of analytical standard grade. The substances were purchased from Sigma Aldrich (St. Louis, Missouri, USA), subsidiary company of Merck KGaA (Darmstadt, Germany). Sodium chloride (p.a.) was obtained from Penta (Prague, Czech Republic). Helium (purity 99.998%) was supplied by Linde Gas (Prague, Czech Republic).

Sample preparation and isolation of volatiles

Preparation procedure and the main isolation steps (as well as chromatographic conditions described later) were inspired by Schipilliti *et al*. [2011] and adjusted to our conditions of measurements. Approximately 2 g of each homogenized defrosted strawberry sample as well as 2 g of each syrup sample were separately placed into vials (10 mL glass vial, Supelco, Bellefonte, Pensylvania, USA) with 2 mL of saturated NaCl solution. Two microlitres of each flavouring were mixed with 100 mL of NaCl solution (mixture of 20 g of NaCl and 100 mL of distilled water), and 4 mL were placed into a vial. The mixture of butyl acetate, ethyl butyrate, hexanal, (*E*)-hex-2-enal, hexyl acetate, 3-methylbutyl acetate, linalool, γ-decalactone served as a quality control sample. One microliter of each substance was transferred by 1 μL microsyringe (Hamilton, Giarmata, Romania) into the same vessel containing 1 L of distilled water. The mixture was ultrasonicated for 30 s, 10 times diluted with distilled water, the final concentrations of substances were approximately in the range of 0.08–0.1 mg/L. Four millilitres were placed into a vial with 1.5 g of NaCl. The mixture was always measured no later than 2–3 days after its preparation. In the case of alkane standards, 5 μL of the mixture were analysed immediately after being placed into vials, blanks were prepared as 4 mL of distilled water. All vials with the analysed solutions were sealed with 1.3 mm thick PTFE/silicone septum and 18 mm thread magnetic stainless steel screw cap (Supelco). HS-SPME extraction of volatiles was performed with 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane fibre (Supelco) and Autosampler Combi Pall (Agilent Technologies, Santa Clara, CA, USA). Samples were incubated for extraction for 15 min at 50°C. Afterwards, the fibre was held for 15 min at the temperature of 50°C in the head space. Samples were exposed to agitation (8 Hz) during both procedures, incubation as well as extraction.

Analysis of volatiles

The gas chromatograph System 7890A coupled to an inert mass selective detector with a triple-axis detector 5975C (Agilent Technologies) was used. Enantiomeric separation was enabled by the CP-Chirasil-Dex CB chiral column (β-cyclodextrin directly bonded to dimethylpolysiloxane) of 25 m × 250 μm × 0.25 μm (Agilent Technologies) using helium as the carrier gas at a flow rate of 0.82 mL/min. Volatile compounds were thermally desorbed for 2 min at the temperature of 220°C in the GC injector port. The sample was injected in a split mode at the split ratio of 1:1. The GC oven initial temperature of 50°C was held for 2 min and subsequently increased up to 200°C with a ramp rate of 2°C/min. The complete analysis took 79 min. The mass spectrometer was operating in the scan range (*m*/*z*) between 40 and 550 amu, temperature of the ion source of 230°C and temperature of quadrupole of 150°C. Identification of volatile compounds was performed by comparing their mass spectra with Mass Spectral Library NIST 11 (National Institute of Standards and Technology, Gaithersburg, MD, USA) included in GC-MS data analysis software MSD ChemStation (version G1701EA E.02.02.1431, Agilent Technologies). The order of identified volatiles, their retention time (t_R) and linear retention indices (LRIs, calculation based on H. van den Dool and D. J. Kratz equation [Zellner *et al*., 2008] using retention time of selected alkane standards and eluted substances) were compared with literature data (Table 1). Calculation of the chiral resolution (R_S) was based on the equation (1).

$$R_S = [(t_{R2} - t_{R1})]/[(W_1 + W_2)/2] \quad (1)$$

where: t_{R1} and t_{R2} are retention times of enantiomers 1 and 2 and W_1 and W_2 baseline widths of chromatographic peaks of enantiomers 1 and 2.

The (enantio)separation factor α has been calculated according to the equation (2).

$$\alpha = k_2/k_1 \quad (2)$$

where: $k_1 = (t_{R1} - t_{R0})/t_{R0}$, $k_2 = (t_{R2} - t_{R0})/t_{R0}$ and t_{R0} is the dead time.

Enantiomeric ratios (E ratio) were expressed based on peak areas of enantiomers (*R* and *S*) as:

$$E_{ratio} = [R/(R + S) \times 100] / \{100 - [R/(R + S) \times 100]\} \quad (3)$$

or

$$E_{ratio} = [S/(S + R) \times 100] / \{100 - [S/(S + R) \times 100]\} \quad (4)$$

Two equivalent aliquots of each sample of strawberry cultivar, flavouring and syrup were analysed. Reliability was evaluated as part of the verification of the method as relative standard deviation (RSD) from at least 2 determinations of the total area of the quality control sample during 1 series of measurements (1.5–12.5% within all monitored series). Repeatability was calculated for the γ-decalactone enantiomers as RSD from two equivalent aliquots for all samples, in 64% of cases it was 0–6% (in strawberries in 89% of cases), in 28% it was 7–11%, and in 8% of cases (in flavours A, D, G) it was up to 15%.

RESULTS AND DISCUSSION

The condition for authentication of strawberry flavour was based on two main suppositions. The first one, the efficiency of the chiral column, was met. As can be seen in Figure 1, the enantiomers were well distinguishable thanks to the CP-Chirasil-Dex CB stationary phase. 2-Methylbutanoic acid, linalool, α-ionone, γ-decalactone and γ-undecalactone achieved chiral resolution in the baseline level (except for γ-decalactone, all of them in all samples with their paired enantiomers). The best resolution of enantiomers was obtained for α-ionone (R_S>4), their least differentiation for methyl 2-methylbutanoate. Elution order of these volatiles was in agreement with their elution order on chiral columns in studies by Williams *et al*. [2005] and Ravid *et al*. [2010] with LRIs values very close to those reported in literature (see t_R and LRIs in Table 1 and Table 2). The second supposition – selection of volatiles having enantiomeric composition in natural strawberry flavour different from the composition in its synthetic form – achieved gratifying results. An absolute predominance of one enantiomeric form was characteristic for all positively detected investigated volatiles in our strawberries (Table 2; the results were comparable to those found for strawberries, reported for each substance mostly in only one or two studies so far, as can be seen in Table 1). Moreover, such predominance was observed even on our flavourings of natural origin subjected to processing during production, while the near-racemates in flavourings with the expected synthetic origin were obtained (Table 3). It was an important finding supporting reliable application of these volatiles for authentication of strawberry flavour in syrups. Thanks to the fulfilled suppositions, it was possible to focus on the authentication of strawberry flavour in syrups. The exception for linalool in strawberries reported in one study [de la Peña Moreno *et al*., 2010] and shown in Table 1 was supposed to be due to processing to strawberry extract, as the racemization during specific processing conditions was noticed for this substance [Bernreuther & Schreier, 1991; Marchelli *et al*., 1996] and created one more incentive to test this substance in our samples and conditions.

As can be seen in Table 2, Table 3, and Table 4, the tested samples of strawberries and flavourings were clearly poorer in volatile contents than syrup samples. Ethyl 2-methylbutanoate was missing in strawberries, methyl 2-methylbutanoate and 2-methylbutanoic acid in strawberry flavourings, α-ionone, γ-undecalactone, δ-dodecalactone in both. Single peak predominance of ethyl-2-methylbutanoate and α-ionone in strawberries was considered based on the study by Kreck *et al*. [2001], which is – to the best of our knowledge – the only source providing it in pure strawberries (Table 1). The deficit of the substances could lie in the selection of cultivars. The absolute predominance of one of the paired enantiomers required for natural flavour as a condition enabling distinguishing the synthetic flavour [Nitz *et al*., 1991], was found in the case of methyl 2-methylbutanoate, 2-methylbutanoic acid and linalool (also called β-linalool) in strawberries in which they were detected, and in the case of γ-decalactone even in all strawberries. Moreover, the condition was fulfilled also for linalool, ethyl 2-methylbutanoate and γ-decalactone in the samples of strawberry flavourings declared as natural. Pure *R*-peak of γ-decalactone was again present in all of them (Table 3, samples B, C, F), the effect of processing steps on its enantiomeric ratio in the natural form was not noticeable at all. It already highlighted the great ability of γ-decalactone to indicate the natural strawberry flavour. Slight differences from 100% one-peak predominance observed for linalool in strawberries S4, S5, and S6; and for γ-decalactone in S3, S4, and S6 could be caused by cultivar selection, for ethyl 2-methylbutanoate in naturally declared strawberry flavourings C and F also by processing, the complication described for several other chiral substances in literature [Nitz *et al*., 1991; Ravid *et al*., 2010]. Unfortunately, there is a lack of comparable findings in literature to suppose other reasons for negligible racemization (up to 5%) of ethyl 2-methylbutanoate in flavourings or to consider the presence of its *R*-enantiomer due to the material of different natural origin (up to 5% allowed by legislation [Guidance Document, 2019]). The reason for linalool's enantiomeric reverse order (*S*>>*R* with *S* eluted first) in strawberry cultivars S1 and S2 (Table 2) and in naturally declared strawberry flavourings B and F (Table 3), contrary to the order reported in literature (*S*>>*R*, *R* eluted first, Table 1), could be similar to the one discussed in studies by Cagliero *et al*. [2017] and Mosandl *et al*. [1990]. Cagliero *et al*. [2017] mentioned that changes of elution order of enantiomers from members of homologous series on the same column could be related to the strength of interactions between enantiomer and cyclodextrin stationary phase; therefore, it was unreliable to correlate the absolute configuration (*R* or *S*) with the elution order. Moreover, Mosandl *et al*. [1990] demonstrated changes in the elution order

TABLE 2. Enantiomeric ratios and characterization of selected chiral volatiles in strawberries (S1–S6)

Elution order	Compound	E order		t_R (min)	LRI (calc.)	α	Peak area (×10³, Abundance×second)**					
							S1	S2	S3	S4	S5	S6
1	Methyl 2-methylbutanoate	1.	*R*	7.0	821	1.03	nd	nd	nd	nd	nd	nd
		2.	*S*	7.1–7.5	823–833		1542	nd	nd	580	163	nd
				E ratio			0/100	na	na	0/100	0/100	na
2	Ethyl 2-methybutanoate	1.		8.7–9.4	860–877	1.03	nd	nd	nd	nd	nd	nd
		2.		8.9–9.6	865–881		nd	nd	nd	nd	nd	nd
				E ratio			na	na	na	na	na	na
3	2-Methylbutanoic acid	1.	*S*	28.6–29	1198–1205	1.02	nd	nd	nd	255	42	1122
		2.	*R*	29.2–29.4	1208–1212		nd	nd	nd	nd	nd	nd
				E ratio			na	na	na	100/0	100/0	100/0
4	(β)-Linalool	1.	*S, R*	31.1–31.2	1241–1242	1.00	1419	2815	nd	459	710	540
		2.	*S*	31.3–31.4	1244–1246		nd	nd	nd	10542	12060	5720
				E ratio			100/0	100/0	na	4/96	6/94	9/91
5	(*E*)-α-Ionone	1.		42.8–42.9	1450–1452	1.02	nd	nd	nd	nd	nd	nd
		2.		43.8	1469		nd	nd	nd	nd	nd	nd
				E ratio			na	na	na	na	na	na
6	γ-Decalactone	1.	*R*	49.9–50.6	1589–1604	1.01	438	26741	16806	5571	626	8013
		2.	*S*	50.3–50.7	1597–1606		nd	nd	188	43	nd	78
				E ratio			100/0	100/0	99/1	99/1	100/0	99/1
7	γ-Undecalactone	1.		55.0–55.1	1696–1698	1.00	nd	nd	nd	nd	nd	nd
		2.		55.3	1702		nd	nd	nd	nd	nd	nd
				E ratio			na	na	na	na	na	na
8	δ-Dodecalactone	1.		60.7–60.8	1822–1824	1.00	nd	nd	nd	nd	nd	nd
		2.		60.9	1826		nd	nd	nd	nd	nd	nd
				E ratio			na	na	na	na	na	na

nd – not detected; na – not available; Elution order – elution order of the substances monitored in our study; E order – enantiomeric order – elution order of enantiomers *R* and *S* obtained by comparison of their percentage proportions in literature (column E comparison, Table 1) with their peak areas in tested samples; t_R – retention time of the enantiomers in strawberries, flavourins, syrups; LRI (calc.) – calculated linear retention index, calculation is based on H. van den Dool and D. J. Kratz equation [Zellner *et al*., 2008]; α – calculated separation factor; E ratio – enantiomeric ratio – percentage ratio of the peak areas of the substance's enantiomers *R* and *S* (for the calculation equation see the section Materials and Methods); **Results of enantiomers (source data from GS-MS chromatogram) represent the measured peak areas integrated by RTE integrator of the software used.

TABLE 3. Enantiomeric ratios and characterization of selected chiral volatiles in strawberry flavourings (A–G)

Elution order	Compound	E order		Peak area (×10³, Abundance×second)**						
				A	B	C	D	E	F	G
1	Methyl 2-methylbutanoate	1		nd	nd	nd	nd	nd	nd	nd
		2		nd	nd	nd	nd	nd	nd	nd
		E ratio		na	na	na	na	na	na	na
2	Ethyl 2-methylbutanoate	1	*R*	13923	0	185	14638	5067	11686	9089
		2	*S*	21405	4572	3194	28128	9612	215517	20390
		E ratio		39/61	0/100	5/95	*34/66*	*35/65*	5/95	*31/69*
3	2-Methylbutanoic acid	1		nd	nd	nd	nd	nd	nd	nd
		2		nd	nd	nd	nd	nd	nd	nd
		E ratio		na	na	na	na	na	na	na
4	(β)-Linalool	1	*S*	nd.	155	nd	nd	nd	569	nd
		2	*R*	nd	nd	nd	nd	nd	nd	nd
		E ratio		na	100/0	na	na	na	100/0	na
5	(E)-α-Ionone	1		nd	nd	nd	nd	nd	nd	nd
		2		nd	nd	nd	nd	nd	nd	nd
		E ratio		na	na	na	na	na	na	na
6	γ-Decalactone	1	*R*	1573	548	236	3363	493	761	1319
		2	*S*	888	nd	nd	3182	483	nd	549
		E ratio		64/36	100/0	100/0	51/49	51/49	100/0	71/29
7	γ-Undecalactone	1		nd	nd	nd	nd	nd	nd	nd
		2		nd	nd	nd	nd	nd	nd	nd
		E ratio		na	na	na	na	na	na	na
8	δ-Dodecalactone	1		nd	nd	nd	nd	nd	nd	nd
		2		nd	nd	nd	nd	nd	nd	nd
		E ratio		na	na	na	na	na	na	na

** See the legend below Table 2 for details. Coelutions were noticed for the results in italics.

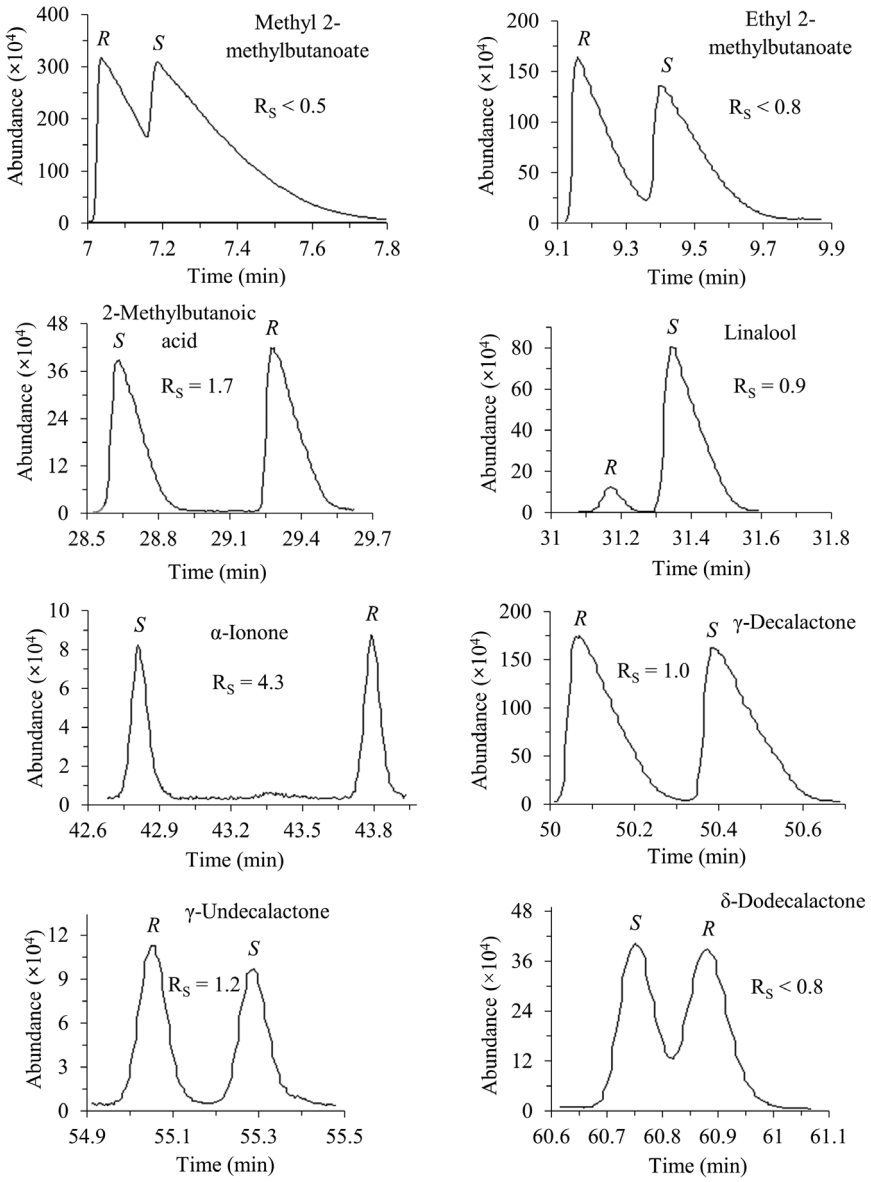

FIGURE 1. The enantiomeric separations on CP-Chirasil-Dex CB in selected syrups (methyl 2-methylbutanoate, ethyl 2-methylbutanoate, 2-methylbutanoic acid, α-ionone, γ-decalactone, γ-undecalactone, δ-dodecalactone) and in strawberry sample (linalool). R_S – chiral resolution.

of enantiomers in homologous series of selected volatiles directly on permethylated β-cyclodextrin phase. Possible influence of processing or addition of acidic additives on the enantiomeric ratios of linalool in strawberry products resulting in enantiomeric size reverse order (*R*>>*S*, *R* eluted first) was also conceivable. Anyhow, regarding literature discussing such influence [Bernreuther & Schreier, 1991; Cagliero *et al*., 2012; Marchelli *et al*., 1996; Nitz *et al*., 1991], including mentions of possible impact of acidic conditions on linalool [Bernreuther & Schreier, 1991; Marchelli *et al*., 1996], or alternatively observing nearly racemic ratios of linalool in strawberry extracts [de la Peña Moreno *et al*., 2010], the triggered ratio changes did not lead to absolute predominance of opposite enantiomer there; instead racemization or partial racemization is rather discussed. A shift in retention time seemed as an unlikely reason. Although slight shifts in retention times were observed for some substances, in the case of linalool the retention time values of each enantiomer were in a stable range (Table 2). Therefore, both alternatives of *R* and *S* enantiomeric order are considered in Table 2. Nearly racemic ratios of dominant γ-decalactone and ethyl 2-methylbutanoate in the rest of flavourings indicated the presence of synthetic flavours in accordance with our supposition. The coelution of ethyl 3-methylbutanoate with ethyl 2-methylbutanoate in strawberry flavourings of D, E, and G finally did not have any significant impact on the possibility to distinguish between natural and synthetic forms, as the ratios would be indicative of synthetic flavour in both cases, with coverage by ethyl 3-methylbutanoate or without it. Based on visual estimation, exclusion of ethyl 3-methylbutanoate from the second eluted peak could lead to ratios close to the racemates.

By closer focusing on syrups (Table 4), it is evident that the possibility to distinguish between synthetic and natural flavour for investigated chiral volatiles in syrups is more

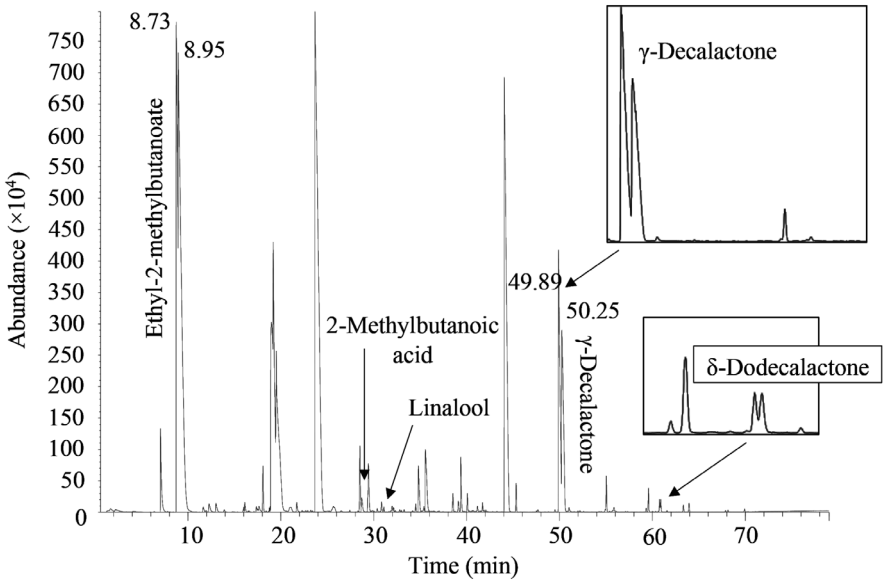

FIGURE 2. Chiral GC-MS spectrum of syrup 1 suspected of adulteration.

evaluable due to their higher presence and overall larger spectrum of results than in the samples of strawberry flavourings and strawberries. Exceptions were observed for methyl 2-methylbutanoate in syrup 9 and 12 and for linalool in syrups 2, 5, 6, 9, and 10. For these samples with strawberry flavour not declared as 100% natural, only their dominant single peaks were recorded. Accordingly, in addition to the declared flavourings or strawberry flavouring indicating possible content of synthetic flavourings, the samples contained strawberry fruit juice, a supposed source of natural strawberry flavour. Therefore, the single peaks of linalool and methyl-2-methylbutanoate could be of natural origin, similar situation as in the peach flavour mixture of synthetic and natural origin in the study by Ravid *et al*. [2010]. For the named samples, these two substances could not be included in the authentication of synthetic flavour. Thus, linalool did not contribute to such authentication in any of the cases. Despite the susceptibility of linalool to racemization under specific processing conditions [Bernreuther & Schreier, 1991; Marchelli *et al*., 1996], no racemates were observed in our conditions.

More contributive results were obtained for other crucial substances in syrups. Ethyl 2-methylbutanoate was found in syrup 3 as a single enantiomeric peak consistent with the supposition for syrup's natural flavour. No racemization due to processing was observed. According to its label, syrup 1 should contain only strawberry flavouring of natural origin, similarly to syrup 3. However, the enantiomeric ratio (31/69) of ethyl 2-methylbutanoate indicated a very high probability of synthetic flavour addition, even by considering the nearly racemic ratios of other 3 chiral volatiles in this syrup including γ-decalactone (57/43) – see Table 4 and Figure 2, and a range of the enantiomeric ratios of ethyl 2-methylbutanoate in other syrups without natural flavour declaration (Table 4). It could be assumed that ethyl 2-methylbutanoate might be a useful indicator for distinguishing between natural and synthetic flavour. Coelution of *S*-enantiomer in five syrups (Table 4) was similar to those observed in the flavourings with the same insignificant impact as it was described above.

The problematic coelution was noticed for another monitored volatile, namely the 2-methylbutanoic acid. Its second eluted enantiomer coeluting with methyl salicylate (calculated LRI 1213) in syrup samples 1, 2, and 8, with phenylethyl acetate (calculated LRI 1212) in samples 9 and 12 or its first eluted enantiomer with cyclohexasiloxane derivative (calculated LRI 1197) in syrup 4, were responsible for the impossibility to accurately determine its enantiomeric ratio in the named syrups. The ability of the substance to distinguish between natural and synthetic flavour was not judged in the case of flavour deficit (syrup 3, syrup 6 with only one detected enantiomer instead of two expected). In the case of coelution in syrups 1 and 8, substances' near racemates could be estimated after visual recognition of the substances' overlapped areas or from extracted ion chromatograms, whereas only the latter was possible in the case of syrups 2 and 12. For syrups 4 and 9, the estimation was problematic due to the extensive overlap with the enantiomer. In five syrups without observed coelutions, ratios were similar to those of γ-decalactone or ethyl 2-methylbutanoate, confirming potential for synthetic flavour recognition. Enantiomeric reverse order observed among them and among the extracted ion chromatograms of 2-methylbutanoic acid in coeluting samples might have the same reason as the one described for linalool in strawberry flavourings.

In the case of α-ionone, almost exact racemic ratios were observed in syrups 5, 9, 11 and 12 (Table 4). Moreover, results of γ-decalactone and ethyl 2-methylbutanoate in the same syrups as well as the result of δ-dodecalactone in syrup 5 supported the supposition of synthetic flavour origin due to the racemic ratios found. Despite the low content, the enantiomeric separation of α-ionone was perfect (Figure 1).

By closer focus on γ-decalactone in syrups, benefits of this substance for recognition of flavour origin are evident even in this matrix. The substance was again present in all

TABLE 4. Enantiomeric ratios and characterization of selected chiral volatiles in strawberry syrups (1–14)

Elution order	Compound	E order		Peak area (×10³, Abundance×second)**													
				1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Methyl 2-methyl butanoate	1	*R*	nd	nd	nd	25779	nd	nd	nd	nd	nd	nd	20492	nd	nd	nd
		2	*S*	nd	nd	nd	51606	nd	nd	nd	nd	3529	nd	43164	2732	nd	nd
		E ratio		na	na	na	33/67	na	na	na	na	0/100	na	32/68	0/100	na	na
2	Ethyl 2-methylbutanoate	1	*R*	76682	17069	nd	30346	17775	7477	18405	11723	21799	10296	28476	19635	25895	12877
		2	*S*	168455	25417	122	122636	22863	8555	46036	20885	47454	11725	48487	42222	80916	15237
		E ratio		31/69	40/60	0/100	20/80	44/56	47/53	*29/71*	*36/64*	*31/69*	47/53	37/63	*32/68*	*24/76*	46/54
3	2-Methylbutanoic acid	1	*S, R*	1935	585	nd	14366	1316	59	850	1376	1147	2884	4145	1281	1097	206
		2	*R, S*	5434	988	nd	8404	1765	nd	1149	3356	2597	3351	5318	2558	898	594
		E ratio		*26/74*	*37/63*	na	*63/37*	43/57	100/0	43/57	*29/71*	*31/69*	46/54	44/56	*33/67*	55/45	26/74
4	(β)-Linalool	1	*S*	806	106	nd	nd	277	128	nd	nd	474	165	nd	nd	nd	nd
		2	*R*	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
		E ratio		100/0	100/0	na	na	100/0	100/0	na	na	100/0	100/0	na	na	na	na
5	(*E*)-α-Ionone	1	*S, R*	nd	nd	nd	nd	381	nd	nd	nd	96	nd	176	119	nd	nd
		2	*R, S*	nd	nd	nd	nd	415	nd	nd	nd	104	nd	175	126	nd	nd
		E ratio		na	na	na	na	48/52	na	na	na	48/52	na	50/50	49/51	na	na
6	γ-Decalactone	1	*R*	46393	24578	1003	40184	21575	10944	16493	15991	29372	12693	6096	25569	14431	14344
		2	*S*	35524	21053	nd	17137	15568	7722	15514	15226	22950	9951	5406	25200	14383	11858
		E ratio		57/43	54/46	100/0	70/30	58/42	59/41	52/48	51/49	56/44	56/44	53/47	50/50	50/50	55/45
7	γ-Undecalactone	1	*R*	nd	nj	nd	nd	nd	nd	nd	859	1700	nj	nd	1794	559	nd
		2	*S*	nd	387	nd	nd	nd	nd	nd	650	1506	367	nd	1676	507	nd
		E ratio		na	100/0	na	na	na	na	na	57/43	53/47	100/0	na	52/48	52/48	na
8	δ-Dodecalactone	1	*S*	871	46	nd	nd	1664	296	126	226	nd	nd	nd	nd	nd	nd
		2	*R*	944	57	nd	nd	1883	323	134	259	nd	nd	nd	nd	nd	nd
		E ratio		48/52	45/55	na	na	47/53	48/52	48/52	47/53	na	na	na	na	na	na

** See the legend below Table 2 for other details. Coelutions were noticed for the results in italics; nj – not judgeable due to coelution.

the samples tested (Table 4). Regarding pure *R*-enantiomer found in syrup 3 declared with only natural strawberry flavouring, the absolute predominance of *R*-enantiomer in our (Table 2) and previously analysed strawberries (Table 1), as well as in strawberry flavourings of natural origin (Table 3), processing steps did not seem to affect the enantiomeric ratio of γ-decalactone in its natural form, not even in the case of syrups. However, other factors, as *R*-enantiomer of unwanted microbial origin [Hidalgo *et al*., 2013; Nitz *et al*., 1991; Rong *et al*., 2017; Silva *et al*., 2021; Vandamme & Soetaert, 2002], might be considered as a possibility of uncertainty. Nearly racemic enantiomeric ratios in the samples were comparable with the ratios of other investigated substances, namely the ethyl 2-methylbutanoate, α-ionone, γ-undecalactone, δ-dodecalactone, evaluable ratios of 2-methylbutanoic acid and two ratios of methyl 2-methylbutanoate (Table 4). Findings of nearly racemic ratios of γ-decalactone in syrups containing flavour of supposedly synthetic origin were similar to those of flavourings of synthetic origin (Table 3) and they are comparable with results of γ-decalactone in synthetic strawberry flavour of other previously analysed commercial products [Kreck *et al*., 2001; Ravid *et al*., 2010; Schipilliti *et al.,* 2011]. These findings document its unified character in strawberry flavour. With regards to all results and conclusions, γ-decalactone seemed to be the most reliable indicator of synthetic and natural strawberry flavour. Nevertheless, the uncertainty had to be taken into consideration.

Nearly racemic ratios strengthening the supposition of synthetic flavourings in syrups 8, 9, 12, and 13 were observed also for γ-undecalactone (Table 4). The single enantiomer in syrups 2 and 10 was supposed to be found due to full coverage of the first enantiomer by larger peak of coeluting nonadecane. Absence of γ-undecalactone in syrup 3 assumed to contain only natural flavour was in this case in accordance with our expectations. With the exception of Bernreuther *et al*. [1989], who demonstrated traces of γ-undecalactone in strawberries, the presence of this volatile in natural strawberry flavour was not discussed in other literature works dealing with strawberry flavour authentication (probably due to the awareness of the volatile's absence), while in the same works the substance's racemates were related to synthetic flavours of other fruits [Cagliero *et al*., 2012; Ravid *et al*., 2010]. Our observations could be explained as a potential of this volatile to distinguish between synthetic and natural strawberry flavour. Nonetheless, more literature sources dedicated to γ-undecalactone enantiomers in natural strawberry flavour exposed to various processing techniques in different matrices could help clarify this issue more.

The last substance of interest was represented by δ-dodecalactone. Enantiomeric pairs with ratios close to the racemate were found in 6 samples including syrup 1 that was declared as natural (Table 4). Regarding enantiomeric ratio of δ-dodecalactone in the processed strawberry extract (*S*(-)30.1%/*R*(+)69.1%) [Bernreuther *et al*., 1991] and concentrated strawberry water phase (*S*(-)33%/*R*(+)67% – *S*(-)36%/*R*(+)64%) [Krammer *et al.*, 2006] known from literature as well as any information recorded for the tested syrup 3, expected to contain natural flavouring in accordance with information on the label, there was too little information to consider the enantiomeric ratio in the natural form. Anyhow, the near-racemates of the substance in our other syrup samples and consistency with observations for dominant γ-decalactone and ethyl-2-methyl butanoate, including syrup 1 declared with strawberry flavour of natural origin, were obvious (Table 4). Considering the whole complex of our results on the investigated volatiles, the accusation of adulteration was made in case of syrup 1.

Regarding the issue of coelutions, it is recommended to implement a second GC column, if possible [Gomes da Silva *et al*., 2008]. Anyway, the contained strawberry flavour is often a complicated mixture of compounds with variable composition, therefore the coelution in individual cases can be observed in any conditions. Also in this regard, authentication based on the evaluation of a large number of functional chiral volatiles is crucial.

CONCLUSIONS

The implemented CP-Chirasil-Dex CB chiral column simultaneously with adjusted measurement conditions enabled determining enantiomeric ratios of the monitored chiral volatiles in the studied strawberries, strawberry flavourings, and syrups; and evaluating the strawberry flavour authenticity with contribution of seven chiral volatiles. Especially due to γ-decalactone and ethyl 2-methylbutanoate, supported by observations on 2-methylbutanoic acid and γ-dodecalactone, it was possible to make accusation of adulteration concerning one syrup sample. In the case of γ-decalactone, results were available for all tested samples and in all cases indicated natural or synthetic form in accordance with the corresponding enantiomeric ratios. Ethyl 2-methylbutanoate was similarly contributive, detected in all syrups and flavourings. Racemates of α-ionone and γ-undecalactone from positively detected samples supported the observed results as well as 2-methylbutanoic acid in the case of evaluable values. Methyl-2-methylbutanoate contributed to the evaluation in two cases. The results found for strawberries, natural flavourings, and one natural syrup gave evidence that there was no or negligible racemization during processing in strawberry flavour, which is an important condition for reliable authentication. However, for the practical purposes, it is recommended to evaluate the strawberry flavour authenticity in syrups on the basis of the enantiomeric ratios obtained for a rather wide range of chiral volatiles considered for each syrup individually. The CP-Chirasil-Dex CB column was efficient in enantiomeric separations (five of the eight volatiles investigated achieved baseline resolution, while the resolution value was even greater than 4 for α-ionone) and represents an easily accessible technique together with the method described above.

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CONFLICT OF INTEREST

Authors declare no conflicts of interest.

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SUPPLEMENTARY MATERIAL

Supplementary data related to this article can be found at http://journal.pan.olsztyn.pl/Enantioselective-HS-SPME-GC-MS-for-Authentication-of-Natural-and-Synthetic-Strawberry,152237,0,2.html. Strawberry flavourings and syrups used in the study. Composition of chiral stationary phases.

REFERENCES

1. Bernreuther, A., Bank, J., Krammer, G., Schreier, P. (1991). Multidimensional gas chromatography/mass spectrometry: A powerful tool for the direct chiral evaluation of aroma compounds in plant tissues I. 5-alkanolides in fruits. *Phytochemical Analysis, 2*(1), 43–47. https://doi.org/10.1002/pca.2800020109
2. Bernreuther, A., Christoph, N., Schreier, P. (1989). Determination of the enantiomeric composition of γ-lactones in complex natural matrices using multidimensional capillary gas chromatography. *Journal of Chromatography A, 481*, 363–367. https://doi.org/10.1016/S0021-9673(01)96780-9
3. Bernreuther, A., Schreier, P. (1991). Multidimensional gas chromatography/mass spectrometry: A powerful tool for the direct chiral evaluation of aroma compounds in plant tissues. II. Linalool in essential oils and fruits. *Phytochemical Analysis, 2*(4), 167–170. https://doi.org/10.1002/pca.2800020405
4. Betzenbichler, G., Huber L., Kräh, S., Morkos, M.-L.K., Siegle, A.F., Trapp, O. (2022). Chiral stationary phases and applications in gas chromatography. *Chirality*, *34*(5), 732–759. https://doi.org/10.1002/chir.23427
5. Cagliero, C., Bicchi, C., Cordero, C., Rubiolo, P., Sgorbini, B., Liberto, E. (2012). Fast headspace-enantioselective GC–mass spectrometric-multivariate statistical method for routine authentication of flavoured fruit foods. *Food Chemistry*, *132*(2), 1071–1079. https://doi.org/10.1016/j.foodchem.2011.10.106
6. Cagliero, C., Sgorbini, B., Cordero, C., Liberto, E., Rubiolo, P., Bicchi, C. (2015). Cyclodextrin derivatives as stationary phases for the GC separation of enantiomers in the flavor and fragrance field. In: K.H. Engel, G. Takeoka (Eds.), *Importance of Chirality to Flavor Compounds, American Chemical Society*, Chapter 2, *ACS Symposium Series, 1212*, 15–34. https://doi.org/10.1021/bk-2015-1212.ch002
7. Cagliero, C., Sgorbini, B., Cordero, C., Liberto, E., Rubiolo, P., Bicchi, C. (2017). Enantioselective gas chromatography with cyclodextrin in odorant analysis. In: A. Buettner (Ed.), *Springer Handbook of Odor*, Springer, Cham, Switzerland, pp. 409–438. https://doi.org/10.1007/978-3-319-26932-0_19
8. da Silva Rivas, A.C., Lopes, P.M., de Azevedo Barros, M.M., Costa Machado, D.C., Alviano, C.S., Alviano, D.S. (2012). Biological activities of α-pinene and β-pinene enantiomers. *Molecules*, *17*(6), 6305–6316. https://doi.org/10.3390/molecules17066305
9. de la Peña Moreno, F., Blanch, G.P., Flores, G., del Castillo, M.L.R. (2010). Development of a method based on on-line reversed phase liquid chromatography and gas chromatography coupled by means of an adsorption–desorption interface for the analysis of selected chiral volatile compounds in methyl jasmonate treated strawberries. *Journal of Chromatography A, 1217*(7), 1083–1088. https://doi.org/10.1016/j.chroma.2009.10.037
10. Easson, L.H., Stedman, E. (1933). Studies on the relationship between chemical constitution and physiological action: Molecular dissymmetry and physiological activity. *Biochemical Journal*, *27*(4), 1257–1266. https://doi.org/10.1042/bj0271257
11. Engel, K.H. (2020). Chirality: An important phenomenon regarding biosynthesis, perception, and authenticity of flavor compounds. *Journal of Agricultural and Food Chemistry*, *68*(38), 10265–10274. https://doi.org/10.1021/acs.jafc.0c01512
12. Fanali, Ch., D'Orazio, G., Gentili, A., Fanali, S. (2019). Analysis of enantiomers in products of food interest. *Molecules*, *24*(6), art. no. 1119. https://doi.org/10.3390/molecules24061119
13. Gomes da Silva, M.D.R., Cardeal, Z., Marriott, P.J. (2008). Comprehensive two-dimensional gas chromatography: Application to aroma and essential oil analysis. In: H. Tamura, S.E. Ebeler, K.K. Gary, R. Takeoka (Eds.). *Food Flavour*, American Chemical Society, Washington, DC, pp. 3–24. https://doi.org/10.1021/bk-2008-0988.ch001
14. Guidance Document on EC Regulation on Flavourings (2019). *European Flavour Association.* Brussels, Belgium [accessed: 14 February 2022]. https://cdn2.assets-servd.host/aggressive-shelduck/production/Documents/Guidance/effa-guidance-document-on-the-ec-regulation-on-flavourings-updated-july-2019.pdf
15. Hidalgo, C., Torija, M., Mas, A., Mateo, E. (2013). Effect of inoculation on strawberry fermentation and acetification processes using native strains of yeast and acetic acid bacteria. *Food Microbiology*, *34*(1), 88–94. https://doi.org/10.1016/j.fm.2012.11.019
16. Krammer, G.E., Weckerle, B., Brennecke, S., Weber, B., Kindel, G., Ley, J., Hilmer, J.M., Reinders, G., Stöckigt, D., Hammerschmidt, F.J., Ott, F., Gatfield, I., Schmidt, C.O., Bertram, H.J. (2006). Product-oriented flavor research: Learnings from the past, visions for the future. *Molecular Nutrition & Food Research*, *50*(4–5), 345–350. https://doi.org/10.1002/mnfr.200500204
17. Kreck, M., Scharrer, A., Bilke, S., Mosandl, A. (2001). Stir bar sorptive extraction (SBSE)-enantio-MDGC-MS–a rapid method for the enantioselective analysis of chiral flavour compounds in strawberries. *European Food Research and Technology*, *213*(4), 389–394. https://doi.org/10.1007/s002170100397
18. Lipkowitz, K.B., Coner, B., Peterson, M.A., Morreale, A. (1997a). Enantioselective binding in gas chromatography: A computational study of chiral selection bypermethyl-β-cyclodextrin. *Journal of Physical Organic Chemistry, 10*(5), 311–322. https://doi.org/10.1002/(SICI)1099-1395(199705)10:5<311::AID-POC871>3.0.CO;2-P
19. Lipkowitz, K.B., Coner, R., Peterson, M., Morreale, A., Shackelford, J. (1998). The principle of maximum chiral discrimination:

chiral recognition in permethyl-β-cyclodextrin. *Journal of Organic Chemistry, 63*(3), 732–745. https://doi.org/10.1021/jo9717090

20. Lipkowitz, K.B., Pearl, G., Coner, B., Peterson, M.A. (1997b). Explanation of where and how enantioselective binding takes place on permethylated β-cyclodextrin, a chiral stationary phase used in gas chromatography. *Journal of the American Chemical Society*, *119*(3), 600–610. https://doi.org/10.1021/ja963076x
21. Marchelli, R., Dossena, A., Palla, G. (1996). The potential of enantioselective analysis as a quality control tool. *Trends in Food Science & Technology, 7*(4), 113–119. https://doi.org/10.1016/0924-2244(96)10011-X
22. Menestrina, F., Ronco, N.R., Romero L.M., Castells, C.B. (2018). Enantioseparation of polar pesticides on chiral capillary columns based on permethyl-β-cyclodextrin in matrices of different polarities. *Microchemical Journal*, *140*, 52–59. https://doi.org/10.1016/j.microc.2018.03.037
23. Mosandl, A., Hener, U., Hagenauer-Hener, U., Kustermann, A. (1989). Direct enantiomer separation of chiral γ-lactones from food and beverages by multidimensional gas chromatography. *Journal of High Resolution Chromatography, 12*(8), 532–536. https://doi.org/10.1002/jhrc.1240120807
24. Mosandl, A., Rettinger, K., Fischer, K., Schubert, V., Schmarr, H.G., Maas, B. (1990). Stereoisometric flavor compounds XLI: New applications of permathylated β-cyclodextrin phasse in chiral CGC analysis. *Journal of High Resolution Chromatography*, *13*(5), 382–385. https://doi.org/10.1002/jhrc.1240130522
25. Nitz, S., Kollmannsberger, H., Weinreich, B., Drawert, F. (1991). Enantiomeric distribution and 13C/12C isotope ratio determination of γ-lactones: appropriate methods for the difterentiation between natural and non-natural flavours? *Journal of Chromatography A*, *557*, 187–197. https://doi.org/10.1016/S0021-9673(01)87132-6
26. Ravid, U., Elkabetz, M., Zamir, C., Cohen, K., Larkov, O., Aly, R. (2010). Authenticity assessment of natural fruit flavour compounds in foods and beverages by auto-HS–SPME stereoselective GC–MS. *Flavour and Fragrance Journal, 25*(1), 20–27. https://doi.org/10.1002/ffj.1953
27. Rong, S., Yang, S., Li, Q., Cai, B., Guan, S., Wang, J., Zhou, Y., Chen, Y. (2017). Improvement of γ-decalactone production by stimulating the import of ricinoleic acid and suppressing the degradation of γ-decalactone in *Saccharomyces cerevisiae*. *Biocatalysis and Biotransformation, 35*(2), 96–102. https://doi.org/10.1080/10242422.2017.1289182
28. Schipilliti, L., Dugo, P., Bonaccorsi, I., Mondello, L. (2011). Headspace-solid phase microextraction coupled to gas chromatography–combustion-isotope ratio mass spectrometer and to enantioselective gas chromatography for strawberry flavoured food quality control. *Journal of Chromatography A, 1218*(42), 7481–7486. https://doi.org/10.1016/j.chroma.2011.07.072
29. Schmalzing, D., Jung, M., Mayer, S., Rickert, J., Schurig, V. (1992). Extending the scope of enantiomer separations on Chirasil-Dex by GLC: Comparison with permethylated β-cyclodextrin dissolved in OV-1701. *Journal of High Resolution Chromatography, 15*(11), 723–729. https://doi.org/10.1002/jhrc.1240151106
30. Scriba, G.K. (2016). Chiral recognition in separation science – an update. *Journal of Chromatography A*, *1467*, 56–78. https://doi.org/10.1016/j.chroma.2016.05.061
31. Servais, A.C., Rousseau, A., Dive, G., Frederich, M., Crommen, J., Fillet, M. (2012). Combination of capillary electrophoresis, molecular modelling and nuclear magnetic resonance to study the interaction mechanisms between single-isomer anionic cyclodextrin derivatives and basic drug enantiomers in a methanolic background electrolyte. *Journal of Chromatography A*, *1232*, 59–64. https://doi.org/10.1016/j.chroma.2011.10.010
32. Sicoli, G., Jiang, Z., Jicsinsky, L., Schurig, V. (2005). Modified linear dextrins ("acyclodextrins") as new chiral selectors for the gas-chromatographic separation of enantiomers. *Angewandte Chemie International Edition*, *44*(26), 4092–4095. https://doi.org/10.1002/anie.200500509
33. Sicoli, G., Pertici, F., Jiang, Z., Jicsinszky, L., Schurig, V. (2007). Gas-chromatographic approach to probe the absence of molecular inclusion in enantioseparations by carbohydrates. Investigation of linear dextrins ("acyclodextrins") as novel chiral stationary phases. *Chirality: The Pharmacological, Biological, and Chemical Consequences of Molecular Asymmetry*, *19*(5), 391–400. https://doi.org/10.1002/chir.20383
34. Silva, R., Coelho, E., Aguiar, T.Q., Domingues, L. (2021). Microbial biosynthesis of lactones: Gaps and opportunities towards sustainable production. *Applied Sciences, 11*(18), art. no. 8500. https://doi.org/10.3390/app11188500
35. Uccello-Barretta, G., Vanni, L., Balzano, F. (2010). Nuclear magnetic resonance approaches to the rationalization of chromatographic enantiorecognition processes. *Journal of Chromatography A*, *1217*(7), 928–940. https://doi.org/10.1016/j.chroma.2009.10.070
36. Vandamme, E.J., Soetaert, W. (2002). Bioflavours and fragrances via fermentation and biocatalysis. *Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology*, *77*(12), 1323–1332. https://doi.org/10.1002/jctb.722
37. Werkhoff, P., Brennecke, S., Bretschneider, W., Güntert, M., Hopp, R., Surburg, H. (1993). Chirospecific analysis in essential oil, fragrance and flavor research. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, *196*(4), 307–328. https://doi.org/10.1007/BF01197930
38. Williams, A., Ryan, D., Guasca, A.O., Marriott, P., Pang, E. (2005). Analysis of strawberry volatiles using comprehensive two-dimensional gas chromatography with headspace solid-phase microextraction. *Journal of Chromatography B, 817*(1), 97–107. https://doi.org/10.1016/j.jchromb.2004.05.021
39. Wu, D., Ma, C., Fan, G.C., Pan, F., Tao, Y., Kong, Y. (2022). Recent advances of the ionic chiral selectors for chiral resolution by chromatography, spectroscopy and electrochemistry. *Journal of Separation Science*, *45*(1), 325–337. https://doi.org/10.1002/jssc.202100334
40. Zellner, B., Bicchi, C., Dugo, P., Rubiolo, P., Dugo, G., Mondello, L. (2008). Linear retention indices in gas chromatographic analysis: a review. *Flavour and Fragrance Journal, 23*(5), 297–314. https://doi.org/10.1002/ffj.1887