Effects of Aromatic Herb Flavouring on Carotenoids and Volatile Compounds in Edible Oil from Blue Sweet Lupin (*Lupinus angustifolius*)

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Running Title: **Volatile Compounds and Carotenoids of Lupin Oil Flavoured with Herbs**

Key words: *oil, lupin, aroma compounds, phytochemicals, herbs*, *relative odour activity value (ROAV*).

List of Abbreviations

Cumulative carotenoid content, CCC; neoxanthine equivalent, NEq.; odour activity value, OAV; retention index, RI; relative odour activity value, ROAV; solid-phase microextraction, SPME; supplementary information, SI; ultrasonic batch, US.

ABSTRACT

This study investigated the carotenoid and volatile compositions of one sample of lupin oil (*Lupinus angustifolius*), and five samples of lupin oil flavoured with aromatic herbs, namely, basil, chives, rosemary, sage, and thyme. Flavoured oils were obtained by macerating lupin oil with the herbs for 15 days, in the dark at 15 ± 1 °C. In all 11 carotenoids were identified by HPLC-DAD/APCI MS. (*all-E*)-Lutein and *β*-carotene were the most abundant. Thyme flavoured oil resulted the richest in carotenoids, with a cumulative carotenoid content of 195 ± 13.0 μg mL^{-1}. Volatile organic compounds were detected by headspace SPME-GC/MS analysis. Overall, 50 aroma compounds were determined, with alcohols, furans and terpenoids being the most abundant classes. Chives flavoured oil was the only sample to provide organosulphur compounds. Qualitatively, terpenoids were responsible for great differences amongst the samples, since unique terpenoid profiles were observed, e.g., isoterpinolene was only detected in sage flavoured oil, β-myrcene in rosemary flavoured oil, and thymol in thyme flavoured oil. The relative odour activity value (ROAV) was determined and employed to evaluate the contributions of the single compounds to the overall odour. The compounds with the greatest odour activity were 3-hexen-1-ol, hexanal, α-pinene, eucalyptol, and 2-pentylfuran.

Practical applications: Aromatic herbs have been traditionally used to enhance the flavour of food. The effects of herbs addition on lupin products has not been investigated yet, additionally, this is the first study that explored some quality characteristics of commercial lupin oil. Data indicated that the maceration of lupin oil with aromatic herbs had limited effects on the content of total carotenoids, nonetheless, it modified markedly the composition and relative proportions of the volatile organic compounds, and likely the overall aromas. Consumers are generally not familiar with the culinary use of lupin oil, nevertheless, the aromatization with herbs could increase its use. Lupin oil resulted rich in carotenoids. This is a useful information for the production of functional products with

47 healthy properties. Lupin oil could be recommended as a carotenoid-rich product and as an
48 alternative to more traditional table oils. Data from this study could contribute to the economic
49 valorisation of lupin oil.

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1. Introduction

Vegetable oils are primary components of the human diet, as they provide energy, essential fatty acids and vitamins. There are more than 40 different oil seeds and crops whose oil is intended to human consumption, however, only few are economically important, e.g., palm, soybean, canola, olive and sunflower. In order to meet the global demand for edible oils, it is of utmost importance to identify new oil seeds e/o crops that could contribute towards increasing the supply. Lupin (*Lupinus*) is a legume that is generally consumed in processed forms, and its protein and fibre fractions are found in several food products, e.g., beverages, bread and pasta [1]. In addition, lupin provides relatively high levels of lipids (about 10% w/w) [2], which have been investigated to a limited extent hitherto. Previous authors have focused on the fatty acid and phospholipid compositions [2,3]. Only few researchers have investigated the physicochemical properties, e.g., peroxide value, acidity, viscosity, etc. of lupin oil obtained on a lab-scale level [4,5].

Volatile organic compounds (VOCs) are responsible for oil aroma. They originate from the degradation of polyunsaturated fatty acids via enzymatic (lipoxygenase) or non-enzymatic pathways, i.e., in the presence of oxygen [6]. The aroma of lupin has been investigated previously, and a large spectrum of odour-active compounds was identified, e.g., alcohols, aldehydes, esters [6–8]. Nevertheless, authors focused mainly on raw beans (or beans processed to a limited extent) and lupin proteins, whereas information on the aroma compounds from lupin oil are still lacking.

Carotenoids are a wide group of lipophilic compounds with high colouring power, found abundantly in fruit and vegetables. Legumes provide different types of carotenoids with lutein being the prevalent , followed by zeaxanthin and β-carotene [9]. Up to now, limited research has been performed to investigate the carotenoid content of lupin. Fernández-Marín *et al* [10] investigated yellow lupins (*Lupinus luteus* L.) from Spain, and reported that wild lupins had higher carotenoid content (7.5 ± 2.6 μg g^{-1} DM) than the cultivated species (4.1 ± 0.3 μg g^{-1} DM).

Aromatic herbs have been traditionally employed to enrich the organoleptic and chemical characteristics of edible oils. Herbs, such as basil (*Ocimum basilicum*), chives (*Allium schoenoprasum*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), and thyme (*Thymus vulgaris*) have been utilised since early times as natural flavourings. In the present work, we hypothesised that the maceration of lupin oil with herbs would ameliorate the aroma and increase the carotenoid content of the oil. Lupin oil is a promising product for the food industry with limited market availability. The use of aromatic herbs to improve the organoleptic characteristics of lupin oil could be an inexpensive approach to increase its demand from consumers. At present, there are no other investigations performed on commercial lupin oil, and both the VOC and carotenoid compositions are unknown. This investigation aimed to establish the VOC and carotenoid profiles of commercially available plain lupin oil from blue sweet *Lupinus angustifulus*, and lupin oil flavoured with aromatic herbs, i.e., basil, chives, rosemary, sage, and thyme.

2 Materials and Methods

2.1 Chemicals and Plant Materials

Lupin oil from blue sweet lupin (*Lupinus angustifolius*) was purchased from Prolupin GmbH (Grimmen, Germany). The aromatic herbs were purchased from a local supermarket. General laboratory reagents were purchased from VWR International Oy (Helsinki, Finland). Diethyl ether, hexane, ammonium acetate, potassium hydroxide (pellet), butylated hydroxytoluene (BHT) (granular), and sodium chloride (large crystal) were of analytical grade. Methyl-*tert*-butyl ether (MTBE) was of HPLC grade. Methanol and acetonitrile were of LC-MS grade. The SPME fibre was purchased from Supelco (Bellefonte, PA, USA). Water was purified *in loco* with a Milli-Q water purification system (Merck KGaA, Darmstadt, Germany). Analytical standards of *(all-E)*-lutein, *(all-E)*-zeaxanthin, and β-carotene were purchased from Extrasynthese (Genay Cedex, France). The analytical standards of (9′Z)-neoxanthin and *(all-E)*-violaxanthin were bought from CaroteNature GmbH (Münsingen, Switzerland).

2.2 Preparation of Flavoured Lupin Oils

Flavoured oils were prepared by employing the method from Saoudi *et al* [11]. Briefly, flavoured oils were obtained by macerating lupin oil with the herbs (6% w/w) for 15 days in glass bottles, in the dark at 15 ± 1 °C.

2.3 Determination of Carotenoids

2.3.1 Extraction Process

The extraction of carotenoids was performed by adapting the method of Delpino-Rius *et al* [12]. The whole procedure was performed under dim light and butylated hydroxytoluene, 0.1% w/v, was added to the extraction solvents to minimize carotenoid degradation. Oil samples (0.5 mL) were

111 placed in glass tubes and suspended in 9.5 mL of hexane:diethyl ether (3:1, v/v). Then, 1 mL of this
112 solution was transferred in another test tube and dried under a stream of nitrogen. The dry residue
113 was saponified in a shaking incubator (Kottermann, GWB) for 60 min at 55 °C, using 1 mL of a
114 solution of 6% KOH in methanol (w/v). After the addition of 2 mL of sodium chloride solution (9%,
115 w/v), the mixture was placed on an orbital shaker for 15 min at 4 °C. Then, 4 mL of hexane:diethyl
116 ether (3:1, v/v) were added, and the mixture was vortexed and centrifuged (5 min; 1800g; 18 °C).
117 This step was repeated four times. The organic layers were combined and washed three times with 5
118 mL of chilled water, vortexed and centrifuged (5 min; 1800g; 18 °C). Subsequently, the organic
119 layers were dried under a stream of nitrogen and reconstituted in 1 mL of the injection solvent (A)
120 (acetonitrile/MBTE/methanol, 60/20/20, v/v/v, containing 0.1% of ammonium acetate w/v) (see
121 paragraph 2.3.2 HPLC-DAD Quantification), and sonicated in US bath for 5 min. Samples were
122 filtered through 0.22 µm PTFE membranes (VWR International, Finland). Four independent
123 samples of each oil were extracted.

124

125 **2.3.2 HPLC-DAD Quantification**

126 The liquid chromatography separation of carotenoids was performed adapting the method from
127 Mamatha *et al* [13]. Analyses were performed on a HPLC-DAD instrument (Shimadzu Corporation,
128 Kyoto, Japan), equipped with SIL-30AC autosampler, a sample cooler, two LC-30AD pumps, a
129 CTO-20AC column oven, an SPD-M20A diode array detector and a CBM-20A central unit. The
130 system was operated using LabSolutions Workstation software (Shimadzu). The chromatographic
131 separation was performed at 35 °C on a YMC C30 Carotenoid column (250 x 4.6 mm; 3 µm i.d.)
132 coupled to a C30 guard column (20 mm; 4 mm i.d.), both from Waters (Dublin, Ireland). The
133 mobile-phase solvents were (A) acetonitrile/MBTE/methanol, 60/20/20, v/v/v, containing 0.1% of
134 ammonium acetate w/v, and (B) water. The gradient used to separate the carotenoids was: 10% B
135 (0-25.0 min), 0% B (25.0-35.5 min), 10% B (35.5-42.0 min). The flow rate was 1 mL min^{-1}, the

injection volume was 10 µL, and the DAD was set at 350–500 nm. The quantification of carotenoids by DAD was performed using external standards. The list of the individual carotenoids identified by HPLC-PDA-MS, their chromatograms, the limits of detection (LOD) and quantification (LOQ) are included as Supporting Information (SI 1). The sum of the individual carotenoid compounds is referred here as cumulative carotenoid content (CCC).

2.3.3 HPLC-APCI-MS/MS Identification

The MS/MS analysis of carotenoids was performed adapting the methods from Delpino-Rius *et al* [12]. The analysis was carried out on a Waters Acquity UPLC in combination with a Waters 2996 PDA detector and a Waters Quattro Premier mass spectrometer. The instrument was operated using an atmospheric pressure chemical ionization source (APCI) in positive ion mode. The APCI parameters were as follows: corona voltage, 4.0 kV; extractor voltage, 3 V; source temperature, 120 °C; probe temperature, 350 °C; desolvation temperature, 150 °C; cone gas (nitrogen) flow, 10 L/h; and desolvation gas (nitrogen) flow, 150 L/h. Collision-induced dissociation was achieved using argon as the collision gas at a flow rate of 0.15 mL min^{-1} in the collision cell. Data were acquired using MassLynx 4.1 software (Waters, USA).

2.4 Analysis of Volatile Organic Compounds (VOCs)

2.4.1 Extraction by SPME

To establish the volatile profile of the oil samples, the main SPME extraction parameters, e.g., fibre coating, extraction time, extraction temperature, sample amount, and desorption time, were tested and optimised in a preliminary study (data not shown). Oil samples (2.0 g) were placed in a 20 mL headspace vial. The HS-SPME of the volatile fraction was performed with a 2 cm SPME fibre CAR/PDMS/DVB (Carboxen/Polydimethylsiloxane/Divinylbenzene; 50/30 µm) from Supelco

(Bellefonte, PA), at 45 °C for 30 min, applying agitation with a TriPlus RSH multipurpose autosampler (Thermo Scientific, Reinach, Switzerland).

2.4.2 GC-MS Conditions

GC-MS analyses were performed with a Trace 1310 (Thermo Scientific) gas chromatograph coupled to a TSQ 8000 EVO mass spectrometer (Thermo Scientific). The SPME fiber was desorbed into the injection port equipped with an 0.8 mm I.D. SPME liner (Restek, Bellefonte, PA) at 240 °C for 3 min. Compounds were separated with a HP-5MS column (30 m x 0.25 mm i.d. x 0.25 µm film thickness) from Agilent (Palo Alto, CA), using helium as carrier gas (1.2 mL min^{-1}). The oven temperature was programmed from 40 °C (held for 1 min) to 160 °C at 5 °C min^{-1}, then to 240 °C at 12 °C min^{-1} (held for 1 min). Mass spectra were recorded in electron impact (EI) mode at 70 eV within the mass range *m/z* 40–300. The transfer line and the ionization source were thermostated at 250 and 220 °C, respectively. The system was operated using Xcalibur 4.0 (Thermo Scientific).

The volatile compound identification was based on authentic standards, when available, and comparison of the experimental spectra with those from Wiley 7 and Essential Oils mass spectral libraries (Wiley, New York, NY). Volatile compound identification was further confirmed by linear retention indices (RI) calculated using an *n*-alkane mixture (C7: C22), which were compared to those reported in Adams database [14] and Nist WebBook [15]. The software Xcalibur 4.0 was used to perform peak detection and integration. Semiquantitative data (percentage of total volatile composition) were directly calculated from the total ion current (TIC) peak areas, assuming no differences in the response factor for all the volatiles quantified.

2.4.3 Assignment of key odour compounds

The relative odour activity value (ROAV) is used to evaluate the contribution of individual compounds to the overall aroma. ROAV was employed by previous authors to identify the key odour compounds of foods [16,17]. In practise, the key odour compounds are determined based on their relative odour activity (ROAV). The formula of ROAV is defined as the Equation (1):

$$\mathrm{ROAVi} = 100 \times \frac{\mathrm{OAVi}}{\mathrm{OAVmax}}$$

Where OAVmax is the highest odour activity value (OAV) of all the volatile compounds and OAVi is the OAV of a specific volatile compound. OAV is calculated via the equation $OAV_i = C_i/OT_i$, where C_i is the concentration of the volatile compound in the sample, and OT_i is the odour threshold in water that is expressed in ppb. The odour thresholds in water of the compounds were taken from the literature [18–20]. ROAV ranges from 0 to 100. Volatile compound with ROAV $\geq$ 1 are considered as key odour compounds.

2.5 Statistical Analysis

Data reported are mean of four observations and values are expressed as mean ± SD. The statistical analysis was carried out using SPSS 23.0 for Windows (IBM, Armonk, NY, USA). Data were analysed using ANOVA to compare the groups, and the Tukey's HSD test was performed to allow for multiple comparisons. Differences among groups were considered significant at $p < 0.05$.

3 Results and Discussion

3.1 Carotenoid Analysis

The quantitative carotenoid profiles slightly differed amongst the oil samples (Figure 1). The CCC of the test oils ranged from 163 ± 7.42 to 195 ± 13.0 μg mL^{-1} in rosemary and thyme flavoured oils, respectively. Similar CCC were detected between the plain lupin oil (control) and the flavoured oils ($p < 0.05$), apart from thyme flavoured oil that resulted significantly richer in carotenoids than lupin oil ($p = 0.002$). Data agree with results from Karoui *et al* [21] who reported that the maceration with thyme increased markedly the carotenoid content of corn oil. The effect could be ascribed to carotenoids leaking from thyme leaves to the oil [22]. In general, regardless of the addition of aromatic herbs, the high content of carotenoids in plain lupin oil (171 ± 4.31 μg mL^{-1}) would make the oil an excellent source of the lipophilic pigments. On the contrary, results suggest that harsh processing methods would need to be employed to enrich edible oils with natural pigments, as the maceration with common culinary herbs might not be functional to this scope. Issaou *et al* [23] reported that the addition of herbs, such as oregano, rosemary and basil, to virgin olive oil produced slight differences in the carotenoid profile of the olive oil. To the authors' knowledge, this is the first study investigating the carotenoid content of lupin oil, and direct comparisons with data from the literature cannot be performed. Nevertheless, lupin oil resulted richer in carotenoids than other common edible oils, e.g., olive, canola, soy, corn and palm oils [23–25].

The HPLC-DAD-$APCI^+$-MS analysis of the experimental oils showed the presence of 11 carotenoids: (*all-E*)-luteoxanthin A, (*all-E*)-luteoxanthin B, (9'*Z*)-violaxanthin, (*all-E*)-lutein, (9'*Z*)-lutein, (7'*Z*)-lutein, (*all-E*)-zeaxanthin, 5,6-epoxy-β-carotene, β-carotene, (9'*Z*)-β-carotene, and an isomer of neoxanthine referred here as neoxanthin equivalent (NEq.). An exemplary chromatogram is reported in Figure 2. The qualitative carotenoid profiles of the experimental oils did not differ, as the 11 compounds were found in all the samples. In contrast, the quantitative analysis showed that statistically significant differences ($p < 0.05$) were found across the treatments in the concentrations

of NEq., (*all-E*)-luteoxanthin A, (*all-E*)-luteoxanthin B, (9'Z)-lutein, β-carotene, and (9'Z)-β-carotene. (*all-E*)-Lutein was the predominant carotenoid in all the oils, accounting for about 43% of the CCC, and ranging from 73.0 ± 6.21 to 80.0 ± 6.2 μg mL^{-1} in flavoured sage and thyme oils, respectively ($p < 0.05$). β-Carotene was the second most abundant carotenoid, making up about 33% of the CCC across the samples. Chives and thyme flavoured oils had significantly higher ($p < 0.05$) β-carotene levels (65.6 ± 5.33 and 64.5 ± 3.9 μg mL^{-1}, respectively) than plain lupin oil (53.9 ± 1.47 μg mL^{-1}). As previous investigations identified chives and thyme as carotenoid-rich matrices [22,26], it is likely that β-carotene have leached from the herbs into the oil. Although β-carotene and its isomers were identified as major compounds across the samples, carotenes were found in lower levels than xanthophylls. Earlier studies found that the photosynthetic tissues of lupin and aromatic herbs provide mainly xanthophylls, with lutein being the most predominant carotenoid [27,28]. Results from the present investigation support the hypothesis of Mellado-Ortega and Hornero-Mendez, who speculated extensively on the reason why lutein is the dominant carotenoid in many botanical species, e.g., legumes and cereals [29,30]. The authors postulated that the over-activation of the β, ε-branch of the carotenoid biosynthetic pathway develops lutein at the expenses of the β, β-branch, which controls the formation of zeaxanthin. In addition, the relatively low concertation of carotenes might be due to their hydroxylation to form lutein. It is noteworthy that (*all-E*)-violaxanthin was not detected as such in any of the samples, but its isomers were found ubiquitously across the test oils, and the peak tentatively identified as (9'Z)-violaxanthin was the predominant form. Violaxanthin is formed from epoxidation of zeaxanthin and is a precursor of neoxanthin [31]. It is plausible that a decline in zeaxanthin, averaging 9.58 ± 0.91 μg mL^{-1} in the experimental oils, might have led to the accumulation of (9'Z)-violaxanthin. As a general rule, carotenoids (particularly xanthophylls) are thought to degrade easily during seed ripening and processing due to the activity of epoxidase enzymes [32]. Remarkably, data from this investigation indicate that *Lupinus angustifolius* might retain carotenoids during ripening and processing, as great

concentrations of carotenoids were found in the commercial oil. In this regard, this study remarks on the need for future investigation.

3.2 VOCs and Relative Contents

The volatile profiles of one plain lupin oil and five lupin oils flavoured with aromatic herbs were established by HS-SPME followed by GC-qMS. Exemplary chromatograms are included in the supplementary section (SI 2). A total of 50 VOCs were identified, including one organic acid, two esters, eight alcohols, four aldehydes, three organosulfur compounds, two furans, nine monoterpene hydrocarbons, ten cyclic terpenes, ten oxygenated monoterpenes, and one sesquiterpene. Their relative contents, i.e., relative percentage of the total GC peak areas, are shown in Table 2. Apart from hexanal, all VOCs identified in lupin oil were also found across the flavoured oils. Chives flavoured oil was the only to provide organosulfur compounds (1.08 ± 0.43%). According to Kremr *et al* [33], methyl propyl disulphide, dipropyl disulphide, and propyl-*trans*-1-propenyl disulphide are main sulphur compounds in fresh plants of chives. Sulphur volatile compounds derive from decomposition of the amino acids cysteine and methionine, and have a strong aroma activity even at trace levels. Indeed, our preliminary (unpublished) data indicated that the maceration with chives altered the aroma of lupin oil to a large extent, conferring the oil with a marked pungent odour. With regard to the group of aldehydes, these compounds are common products of autoxidation reactions, occurring during processing and storage of oils. Sage flavoured oil was the only sample lacking of aldehydes. On the contrary, rosemary flavoured oil showed the highest relative concentrations of total aldehydes (6.87 ± 0.54%) with hexanal being the main contributor (5.56 ± 0.52%). Related to this, Perestrelo *et al* [34] found several aldehydes in the volatile profile of thyme flavoured olive oil, whereas Upadhyay *et al* [35] found that sage extracts enhanced the oxidative stability of sunflower oil. When herbs are added to food, the quality of the food has a great influence on the behaviour of the phytochemicals from herbs. Further, phytochemicals are reported to act as pro-oxidants at very

high concentrations [35], which can explain the different VOC profiles of sage and thyme flavoured oils. Previous studies performed on edible oils correlated the presence of C6 aldehydes such as hexanal with green, apple or grass aroma [36]. Within the group of alcohols, sage was the only flavoured oil to lack of hexanol, which was a major compounds of the other oils. As a result, the relative content of alcohols in sage flavoured oil (15.4 ± 0.83%) was approximately half that of plain lupin oil (33.4 ± 1.76%). Hexanol and 3-hexen-1-ol derive from the lipoxygenase pathway, and for this reason, they can be found in a wide range of edible oils [34,36]. Organic acids confer sour and pungent notes to oils, causing sensory defects. Acetic acid is commonly identified in commercial oils. It is noteworthy that the relative content of acetic acid in the flavoured oils was several times lower than in plain lupin oil (37.8 ± 1.12%), with thyme flavoured oil showing the lowest relative content of acetic acid (0.76 ± 0.18%). Apart from chives flavoured oil, terpenoids were responsible for major qualitative differences between the plain lupin and the flavoured oils. Plain lupin oil provided only four terpenoids: α-thujene (2.81 ± 0.15%), *p*-cimene (0.62 ± 0.12%), α-phellandrene (3.32 ± 0.11%) and limonene (0.30 ± 0.07%). On the contrary, about 30 terpenoids were identified in the flavoured oils. These compounds are considered important volatile compounds in the commercialization of oils, as their presence is highly appreciated by consumers. Data showed that the maceration with aromatic herbs influenced greatly the terpenoid profile of the oils. Rosemary flavoured oil resulted the richest in terpenoids and was the only one to provide β-pinene (2.59 ± 0.06%), β-myrcene (1.74 ± 0.21%), pinocarvone (0.08 ± 0.03%), 2-carene (0.73 ± 0.08%) α-terpineol (0.16 ± 0.05%), sabinene hydrate (0.17 ± 0.10%), verbenone (2.43 ± 0.32%), and *trans*-caryophyllene (0.07 ± 0.01%). Thyme flavoured lupin oil lacked oxygenated monoterpens, nevertheless, terpenoids accounted for about 60% of its total volatile profile. Thymol and carvacrol are volatile markers used to determine the identity, origin and quality of thyme [37]. Indeed, the maceration of thyme provided the lupin oil with these two cyclic terpenes (1.90 ± 0.28% and 0.04 ± 0.01%, respectively). Previous studies showed that camphor and borneol are major

compounds of sage essential oil [38]. Both terpenoids were identified in sage flavoured oil (9.16 ± 0.49% and 0.30 ± 0.04%, respectively). Oxygenated terpenes from sage, e.g., α-thujone, β-thujone, bornyl acetate, have strong odour activity [38] and might have provided the lupin oil with sage flavour.

To the authors' knowledge, there are no previous studies investigating the aroma profile of lupin oil, nevertheless, processed lupin fractions, e.g., fibre and protein isolates, have been investigated. Bader *et al* [39] reported that acetic acid conferred sour attributes to lupin flour. Stephany *et al* [6] investigated lupin fibre by HRGC-O and found hexanal to be a major odour compound. Schindler *et al* [40] found that the lactic fermentation improved the aroma of lupin proteins by decreasing the content of hexanol and hexanal. To sum up, different qualitative profiles where obtained by macerating lupin oil with aromatic herbs. The greatest differences were due to the presence of terpenoids in basil, rosemary, sage and thyme flavoured oils, and organosulfur compounds in chives flavoured oil. It is apparent that the maceration with culinary herbs improved the sensory characteristics of the lupin oil, since it enriched the plain oil with several aromatic molecules. The process of maceration resulted alternative to the conventional processes of essential oil addition and solvent extraction for oil flavouring, which are time-consuming and require large amounts of solvents.

3.3 Determination of Key Volatile Components

The contribution of the detected VOCs to the odour of the experimental oils was assessed by determining the individual proportions of VOCs and their sensory thresholds [16]. VOCs with ROAV from 1 to 100 were recognised as key odour compounds, whereas VOCs providing ROAV between 0.1 and 1 contributed to the overall aroma to a lesser extent [17]. The orthonasal odour thresholds in water and the odour attributes of the VOCs are shown in Table 3. The compound 2-pentylfuran had odour threshold of 6 ppb, and was found at relatively high content in plain lupin oil

and in basil, chives, and thyme flavoured oils. It is likely that in these samples, 2-pentylfuran gave the highest contribution to the general odour, as its relative odour activity value (ROAVmax) was 100. Furans are major odour compounds, providing a flavour of grass and cooked caramel [41]. It is acknowledge that 2-pentylfuran arises from oxidation of linoleic acid [42]. Previous authors identified 2-pentylfuran in a variety of foods, including fermented meat, fish, and baked products [43]. In chives flavoured oil, methyl propyl disulphide resulted a key odorant with ROAV > 5.. Methyl propyl disulphide was found at low relative concentrations in the oil, nevertheless, it exhibited strong odour activity. Methyl propyl disulphide derives from the Strecker degradation of sulphur containing amino acids [17], and as having an odour threshold as low as 1 ppb, can influence markedly the aroma of the products in which is present, even at minor concentrations. In sage flavoured oil, eucalyptol gave the highest contribution to the odour, obtaining the ROAVmax of 100. Eucalyptol has a floral, woody and grassy fragrance and was identified by previous authors as a major VOC in leaves of sage [44]. In rosemary flavoured oil α-pinene constituted an abundant compound and obtained the ROAVmax of 100, giving the highest contribution to the overall aroma. In the aroma study performed by Sonmezdag *et al* [45], α-pinene was recognised as a critical odorant in the headspace of pistachio oil.

In general, data showed that the maceration with aromatic herbs provided the oils with unique odour profiles, since few compounds resulted ubiquitous key odorants across the samples, namely hexanal and limonene. The VOC profiles of the flavoured oils were complex, although they could be reduced down to the few compounds having the ROAV between 1 and 100, e.g., eugenol, eucalyptol, linalool, in basil flavoured oil (2.18, 56, and 25.9, ROAVs, respectively), propyl-*trans*-1-propenyl disulphide in chives flavoured oil (ROAV = 2.27), isoterpinolene in sage flavoured oil (ROAV = 20.8), β-miricene and eucalyptol and in rosemary flavoured oil (4.52 and 32.4 ROAVs, respectively), α-pinene, *p*-cyminene, and γ-terpinene in thyme flavoured oil (97.5, 7.12, and 3.39 ROAVs, respectively). Most of these key odour compounds were terpenoids with low odour

350 threshold. In this regard, data are in agreement with previous research in which aromatic herbs, due
351 to their content in terpenes, have augmented the odour characteristics of a wide-range of edible oils
352 [34,46].

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4. CONCLUSIONS

This study investigated the volatile compounds and carotenoid profile of plain lupin oil and lupin oil flavoured with aromatic herbs, i.e., basil, chives, rosemary, sage, and thyme. A total of 11 carotenoids were identified. (*all-E*)-Lutein was the main carotenoid across the samples. Thyme flavoured oil was the only to provide significantly higher CCC than plain lupin oil. Most of the carotenoids identified belonged to the group of xanthophylls, nevertheless, β-carotene was found at relatively high concentrations. The analysis performed by HS-SPME/GC-qMS on the experimental oils revealed the presence of 50 VOCs, belonging to different groups, namely organic acids, esters, alcohols, aldehydes, organosulphur compounds, furans, and terpenoids. The greatest qualitative differences were imparted by organosulphur compounds in chives flavoured oil, and by terpenoids, since every sample had a unique terpenoid profile, e.g., isoterpinolene was only detected in sage flavoured oil, β-myrcene in rosemary flavoured oil, thymol in thyme flavoured oil. These differences were due to the maceration of lupin oil with the aromatic herbs, which are recognised as great sources of terpenoids and other volatile aroma compounds. About 20 key odorants were determined by calculating the ROAV index. The most active odour compounds, i.e. ROAV $\geq$ 30, were 3-(*E*)-hexen-1-ol, hexanal, α-pinene, eucalyptol, and 2-pentylfuran.

Lupin oil is a food product largely unknown to consumers. The use of aromatic herbs could represent an inexpensive method to ameliorate its aroma and increase its presence on the market. In addition, the potential health properties of lupin oil, due to the high concentration of bioactive carotenoids, might be used by food manufacturers to provide health-conscious consumers with new food products of added value. To the authors' knowledge, this is the first investigation performed on commercially available lupin oil. This study improves the scientific knowledge on lupin oil and provides information that could foster the economic valorisation of the product. Further research is needed to accomplish the full characterization of lupin oil and explore its utilization in new food applications.

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TABLE AND FIGURES

Table 1. Individual carotenoid profiles of the experimental oils.

	neo-xanthin equival.	**(*all-E*)-luteo-xanthin A**	**(*all-E*)-luteo-xanthin B**	**(9Z)-viola-xanthin**	**(*all-E*)-lutein**	**9-*cis*-lutein**	**7-*cis*-lutein**	**zea-xanthin**	**5,6-epoxy-β-carotene**	**β-carotene**	**9-*cis*-β-carotene**
lupin	5.54 ± 0.69 **a**	1.09 ± 0.02 **c,d**	2.89 ± 0.07 **d**	1.47 ± 0.06 **a**	74.2 ± 1.6 **a**	3.59 ± 0.05 **a,b**	2.77 ± 0.08 **a**	9.73 ± 0.21 **a,b**	5.20 ± 0.26 **a**	53.9 ± 1.8 **b**	10.2 ± 0.6 **b**
basil	2.52 ± 0.13 **b,c**	2.23 ± 0.21 **a**	4.73 ± 0.28 **b**	1.61 ± 0.15 **a**	75.7 ± 1.0 **a**	3.74 ± 0.04 **a,b**	2.58 ± 0.18 **a**	9.84 ± 0.17 **a,b**	5.02 ± 0.17 **a**	58.4 ± 4.4**a,b**	10.8 ± 0.8 **b**
chives	1.44 ± 0.25 **c**	2.34 ± 0.23 **a**	5.28 ± 0.50 **a**	1.60 ± 0.21 **a**	74.1 ± 5.5 **a**	3.77 ± 0.38 **a,b**	2.77 ± 0.30 **a**	8.72 ± 0.82 **b**	5.55 ± 0.09 **a**	65.6 ± 5.3 **a**	13.1 ± 0.8 **a**
rosemary	2.99 ± 0.38 **b**	0.99 ± 0.23 **d**	2.88 ± 0.19 **d**	1.41 ± 0.15 **a**	73.5 ± 4.0 **a**	3.22 ± 0.24 **b**	2.52 ± 0.25 **a**	8.89 ± 0.61 **b**	5.02 ± 0.34 **a**	51.6 ± 2.2 **b**	10.5 ± 0.6 **b**
sage	5.28 ± 0.78 **a**	0.87 ± 0.06 **d**	2.31 ± 0.16 **e**	1.53 ± 0.16 **a**	73.0 ± 6.2 **a**	3.32 ± 0.27 **b**	2.62 ± 0.27 **a**	9.41 ± 0.66 **b**	5.60 ± 0.33 **a**	54.8 ± 3.1 **b**	9.75 ± 0.55 **b**
thyme	6.41 ± 1.03 **a**	1.37 ± 0.24 **b,c**	3.94 ± 0.37 **c**	1.62 ± 0.25 **a**	80.0 ± 6.2 **a**	3.89 ± 0.30 **a**	2.61 ± 0.17 **a**	10.9 ± 0.9 **a**	5.06 ± 0.37 **a**	64.5 ± 3.9 **a**	14.4 ± 1.3 **a**

Data (μg mL^{-1}) is presented as mean ± SD and represents mean of four independent measurements. Values with unlike letters (a-d) within the same column differ significantly ($p < 0.05$).

Table 2. Relative content (%) of VOCs in the oil samples.

No.	name of VOCs	RI	lupin oil	basil flavoured oil	chives flavoured oil	sage flavoured oil	rosemary flavoured oil	thyme flavoured oil
organic acids								
1	acetic acid	n/a	37.8 ± 1.1	7.63 ± 1.51	14.9 ±0.4	11.9 ± 3.5	4.25 ± 0.04	0.76 ±0.18
	total		37.8 ± 1.1	7.63 ± 1.51	14.9 ±0.4	11.9 ± 3.5	4.25 ± 0.04	0.76 ±0.18
esters								
2	isopentyl formate	792						2.06 ± 0.51
3	hexyl acetate	1099					0.36 ± 0.03	
	total						0.36 ± 0.03	2.06 ± 0.51
alcohols								
4	1-penten-3-ol	n/a					0.39 ± 0.07	
5	pentanol	804	3.47 ± 0.20	4.05 ± 0.14	4.61 ± 0.77	1.45 ± 0.21	3.70 ± 0.35	4.54 ± 0.32
6	3-(*E)*-hexen-1-ol	855	0.64 ± 0.03	1.18 ± 0.16	0.50 ± 0.11	13.9 ± 0.8	0.17 ± 0.04	
7	hexanol	871	28.1 ± 1.8	32.0 ± 2.2	33.4 ± 1.7		27.4 ± 0.6	20.7 ± 1.3
8	benzyl alcohol	1043	1.17 ± 0.06	0.62 ± 0.04	1.55 ± 0.18			0.56 ± 0.04
9	phenethyl alcohol	1218					0.06 ± 0.01	
10	eugenol	1360		1.59 ± 0.20				
11	methyl eugenol	1407		1.09 ± 0.20				
	total		33.4 ± 1.8	40.5 ± 2.2	40.1 ± 1.9	15.4 ± 0.8	31.8 ± 0.7	25.8 ± 1.3

No.	name of VOCs	RI	lupin oil	basil flavoured oil	chives flavoured oil	sage flavoured oil	rosemary flavoured oil	thyme flavoured oil
aldehydes								
12	3-methyl butanal	654					0.60 ± 0.09	
13	hexanal	804	3.10 ± 0.12	2.86 ± 0.31	5.90 ± 0.12		5.56 ± 0.52	6.65 ± 0.20
14	phenylacetalal-dehyde	1109					0.55 ± 0.10	
15	2-octenal	1149					0.16 ± 0.02	
	total		3.10 ± 0.12	2.86 ± 0.31	5.90 ± 0.12		6.87 ± 0.54	6.65 ± 0.20
organosulfur compounds								
16	methyl propyl disulphide	946			0.16 ± 0.03			
17	dipropyl disul-phide	1104			0.78 ± 0.43			
18	propyl-*trans*-1-propenyl disul-phide	1113			0.14 ± 0.03			
	total				1.08 ± 0.43			
	furans							
19	γ-butalactone	920	4.66 ± 0.26	3.08 ± 0.07	4.33 ± 0.05	0.25 ± 0.05	0.07 ± 0.01	1.53 ± 0.20
20	2-pentylfuran	990	14.0 ± 0.1	14.6 ± 0.4	18.8 ± 0.8	7.17 ± 0.32	4.46 ± 0.88	8.92 ± 0.15
	total		18.6 ± 0.2	17.7 ± 0.4	23.1 ± 0.8	7.42 ± 0.32	4.53 ± 0.88	10.4 ± 0.2

No.	name of VOCs	RI	lupin oil	basil flavoured oil	chives flavoured oil	sage flavoured oil	rosemary flavoured oil	thyme flavoured oil
monoterpene hydrocarbons								
21	α-thujene	929	2.81 ± 0.15	3.28 ± 0.28	5.76 ± 0.49	5.77 ± 0.33	0.16 ± 0.02	3.01 ± 0.20
22	α-pinene	939					16.5 ± 1.5	8.70 ± 0.03
23	camphene	954					3.35 ± 0.30	0.83 ± 0.12
24	β-pinene	964					2.59 ± 0.06	
25	sabinene	975						0.79± 0.03
26	β-myrcene	993					1.74 ± 0.21	
27	α-terpinene	1008				2.43 ± 0.19		3.30 ± 0.03
28	*p*-cymene	1022	0.62 ± 0.12	0.46 ± 0.08	0.83 ± 0.11	0.60 ± 0.12	0.38 ± 0.05	10.6 ± 0.3
29	γ-terpinene	1050		2.26 ± 0.10			0.27 ± 0.03	17.7 ± 0.4
	total		3.43 ± 0.19	6.00 ± 0.31	6.59 ± 0.51	8.80 ± 0.40	25.0 ± 1.5	44.9 ± 0.6
cyclic terpenes								
30	2-carene	995					0.73 ± 0.08	
31	δ-3-carene	1001					2.79 ± 0.10	
32	α-phellandrene	1007	3.32 ± 0.11	3.35 ± 0.15	5.29 ± 0.23	1.19 ± 0.13	0.41 ± 0.02	3.71 ± 0.04
33	limonene	1025	0.30 ± 0.07	0.95 ± 0.05	1.48 ± 0.12	1.99 ± 0.22	2.70 ± 0.26	0.59 ± 0.05
34	eucalyptol	1030		16.3 ± 0.4		16.1 ± 0.8	10.7 ± 0.3	
35	isoterpinolene	1068				4.20 ± 0.45		
36	terpinolene	1085		0.49 ± 0.04		0.43 ± 0.09		
37	*o*-methylthymol	1229						0.12 ± 0.00

No.	name of VOCs	RI	lupin oil	basil flavoured oil	chives flavoured oil	sage flavoured oil	rosemary flavoured oil	thyme flavoured oil
38	thymol	1291						1.90 ± 0.28
39	carvacrol	1300						0.04 ± 0.01
	total		3.62 ± 0.13	21.9 ± 0.4	6.77 ± 0.26	23.9 ± 1.0	17.3 ± 0.4	6.36 ± 0.29
oxygenated monoterpenes								
40	linalool	1102		3.78 ± 0.16			0.72 ± 0.10	
41	α-thujone	1105				19.1 ± 1.0		
42	β-thujone	1117				3.70 ± 0.45		
43	camphor	1145		0.37 ± 0.02		9.16 ± 0.49	4.17 ± 0.32	
44	borneol	1166				0.30 ± 0.04	1.73 ± 0.28	
45	pinocarvone	1169					0.08 ± 0.03	
46	sabinene hydrate	1176					0.17 ± 0.03	
47	α-terpineol	1198					0.16 ± 0.05	
48	verbenone	1206					2.43 ± 0.32	
49	bornyl acetate	1289				0.55 ± 0.15	0.42 ± 0.05	
	total			4.15 ± 0.15		32.8 ± 1.2	9.88 ± 0.55	
sesquiterpenes								
50	*trans*-caryophyllene	1415					0.07 ± 0.01	
	total						0.07 ± 0.01	

Data represent the mean values of four independent replicates, and are expressed as relative percentage of the total GC peak areas. The retention index (RI) was relative to C7-C22 *n*-alkanes, and calculated on HP-5MS column. n/a = not available.

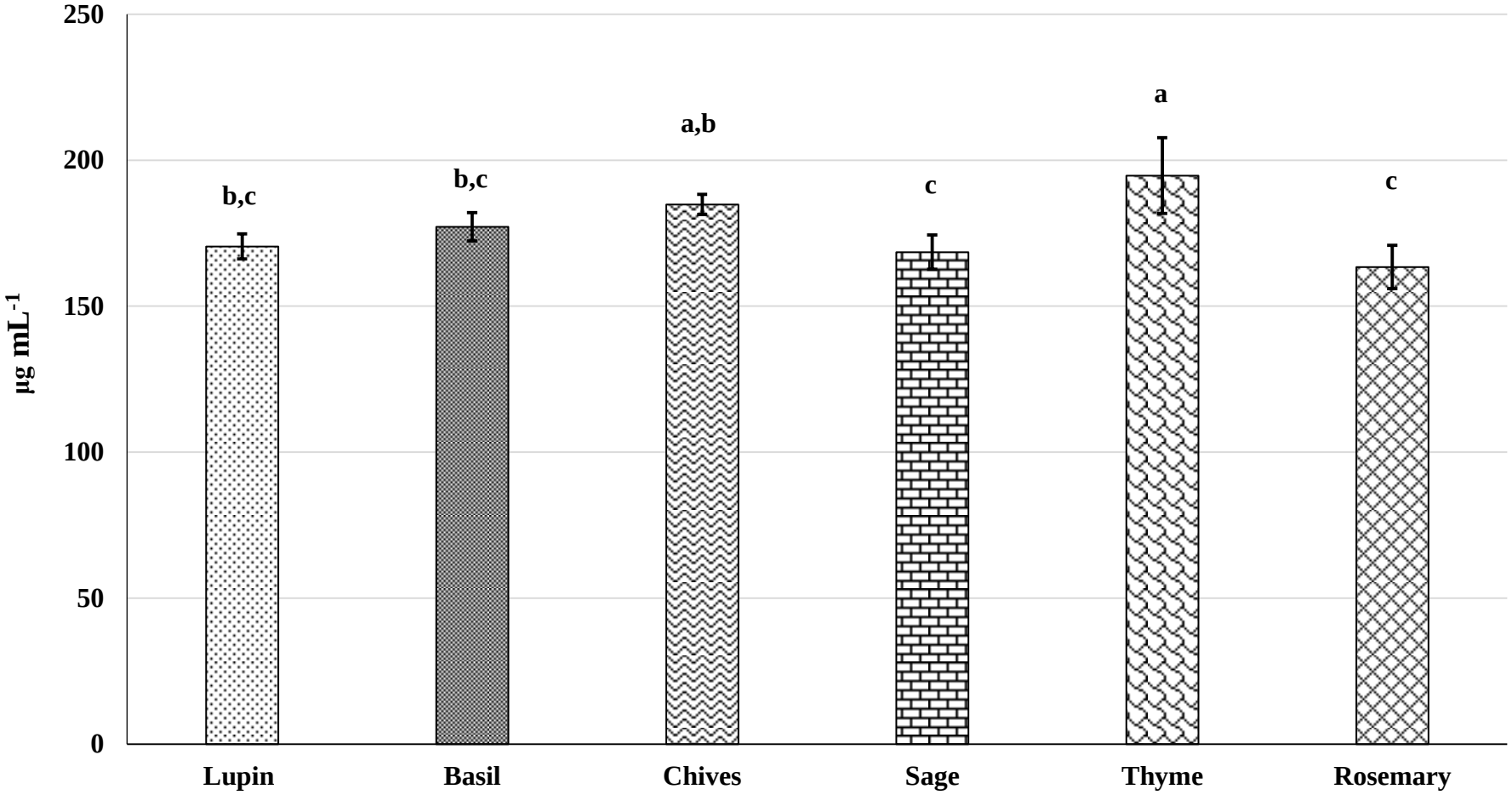
Table 3. ROAV and odour characteristics of the experimental oils.

name of VOCs	odour description	odour threshold (ppb)	relative odour activity value (ROAV)					
			lupin oil	basil flavoured oil	chives flavoured oil	sage flavoured oil	rosemary flavoured oil	thyme flavoured oil
alcohols								
3-(*E*)-hexen-1-ol	grassy, green	70	0.39	0.70	42.4		0.09	
eugenol	cloves	30		2.18				
aldehydes								
hexanal	grass, tallow, fat	4.5	29.8	26.1	42.4		44.9	99.5
2-octenal	fatty	3					1.9	
organosulfur compounds								
methyl propyl disulphide	garlic	1			5.10			
propyl-*trans*-1-propenyl disulphide	vegetable sulphide	2			2.27			
furans								
2-pentylfuran	fruity	6	100	100	100	89.0	27.0	100
monoterpene hydrocarbons								
α-pinene	citrus, pine	6					100	97.5
β-pinene	green, pine	140					0.67	

name of VOCs	odour description	odour threshold (ppb)	relative odour activity value (ROAV)					
			lupin oil	basil flavoured oil	chives flavoured oil	sage flavoured oil	rosemary flavoured oil	thyme flavoured oil
sabinene	woody	75						0.70
β-myrcene	spicy	14					4.52	
p-cymene	weak citrus	100	0.27	0.19	0.27	0.44	0.14	7.12
γ-terpinene	lemon	350		0.15			0.03	3.39
cyclic and oxygenated terpenes								
2-carene	sweet, pungent	15					1.76	
δ-3-carene	citrus	100					1.01	
α-phellandrene	weed	200	0.71	0.69	0.81	0.44	0.08	1.25
limonene	citrus, orange	10	1.28	3.92	4.72	14.5	9.84	3.94
eucalyptol	eucalyptus, balsam woody	12		56		100	32.4	
isoterpinolene	herbal, woody, pine	15				20.8		
thymol	medicinal, woody and spicy	86						1.49
linalool	woody, lavender	6		25.9			4.38	

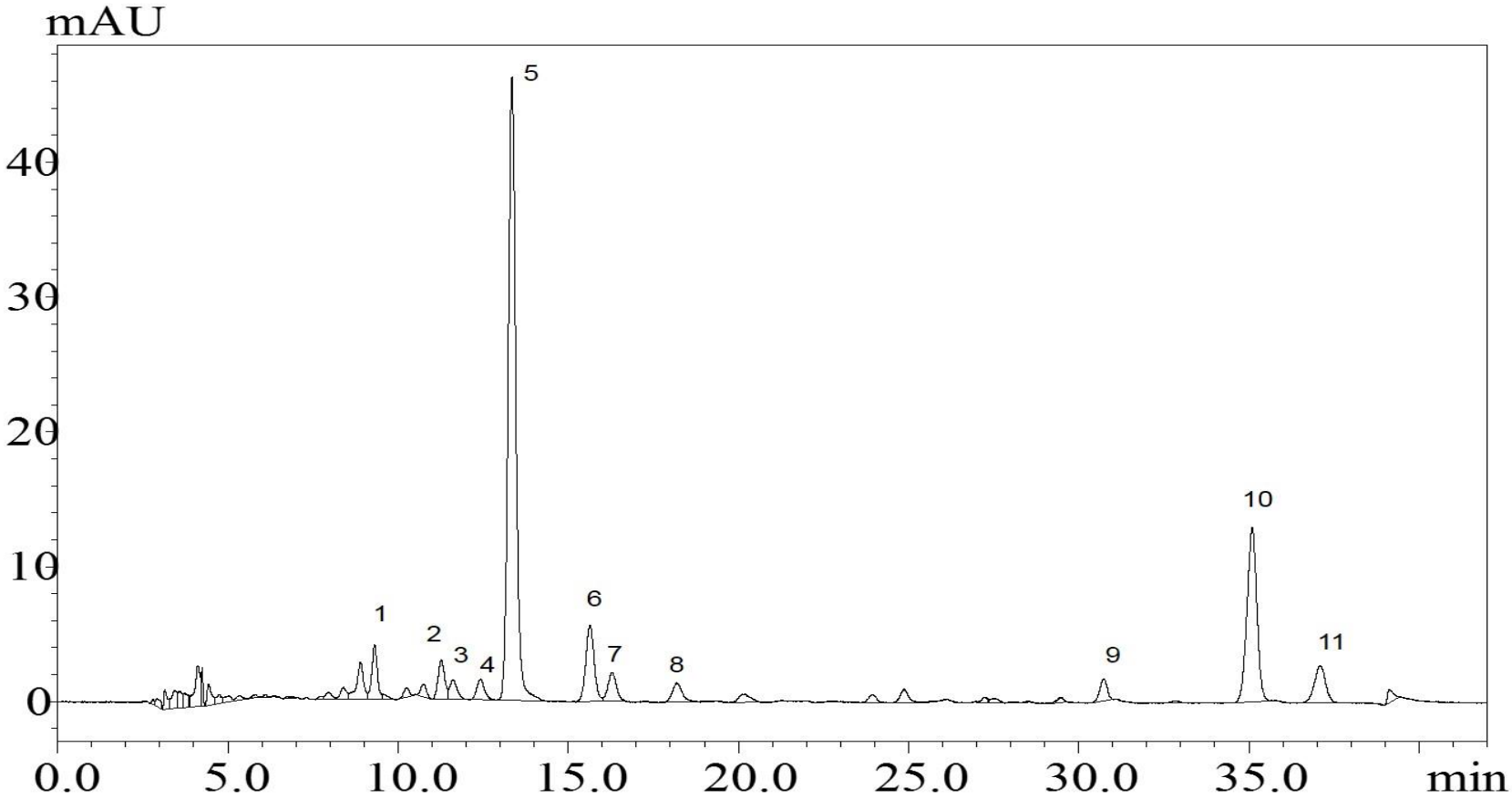
The odour descriptors and the orthonasal odour thresholds in water of VOCs were obtained from the literature.[18,47]

Figure 1. Cumulative carotenoid content.



Data is presented as mean ± SD and represents mean of four independent measurements. Values with unlike letters (a-c) differ significantly ($p < 0.05$). Results are expressed as µg mL^{-1}.

Figure 2. Exemplary chromatogram obtained at 450 nm. Selected data from chives flavoured oil.



Refer to Material and Methods for chromatographic details. Description: (1) neoxanthin equivalent, (2) (*all-E*)-luteoxanthin A, (3) (*all-E*)-luteoxanthin B, (4) (*9'Z*)-violaxanthin, (5) (*all-E*)-lutein, (6) (*all-E*)-zeaxanthin, (7) 9-*cis*-lutein, (8) 7-*cis*-lutein, (9) 5,6-epoxy-β-carotene, (10) (*all-E*)-β-carotene, and (11) 9-*cis*-β-carotene.