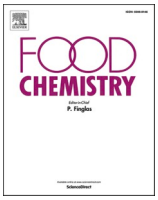
Food Chemistry 417 (2023) 135845

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



The potential of almonds, hazelnuts, and walnuts SFE-CO_2 extracts as sources of bread flavouring ingredients

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ARTICLE INFO

Keywords:
Food flavouring agents
Supercritical fluid extraction
Volatile compounds
Organoleptic profile

ABSTRACT

Nuts have been part of the human diet since our early ancestors, and their use goes beyond nutritional purposes, for example, as aromatic sources for dairy products. This work explores the potential of almond (*Prunus dulcis* (Mill.) DA Webb), hazelnut (*Corylus avellana* L.), and walnut (*Juglans regia* L.) extracts as sources of food flavouring agents, suggesting a new added-value application for lower quality or excess production fruits.

The extracts were obtained by supercritical fluid extraction with carbon dioxide and characterized by: quantification of the volatile fraction by HS-SPME GC–MS; sensory perception and description; and cytotoxicity against Vero cells. All extracts revealed potential as flavouring ingredients due to terpene abundance. No significant differences were observed for the minimal sensory perception, in which the odour threshold values ranged from 8.3×10^{-4} to 6.9×10^{-3} $\mu g \cdot mL^{-1}$ for walnuts and almonds extracts, respectively. In contrast, the cytotoxic potential differed significantly among the extracts, and *P. dulcis* extract presented lower cytotoxicity. Notes as woody, fresh, and green were identified in the volatile intensifiers obtained from the *P. dulcis* extract. Thus, almond extract was identified as the most promising ingredient to increase the sensory value of food products, namely bread. This potential was verified by an increase in the odour perception of bread after adding 4 μL of extract to each 100 g of bread dough. The quantified eucalyptol and D-limonene terpenes - found in the *P. dulcis* extract - have improved the release of the pleasant and natural volatile compounds from bread crust and crumb compared to the control bread chemical and sensory profiles.

1. Introduction

Nuts have been part of the human diet since ancient times. The health benefits associated with consuming these ingredients are documented in several scientific papers (Alasalvar et al., 2020; Beltrán et al., 2011). Almonds (*Prunus dulcis* (Mill.) DA Webb), hazelnuts (*Corylus avellana* L.), and walnuts (*Juglans regia* L.) are some of the most common edible nuts and are part of several industrial processes (Beltrán et al., 2011). These products are carefully selected, according to their size and weight, to offer the widely appreciated nutritional and bioactive composition (Beltrán et al., 2011). Since standard industrial processes and commercialization, in general, exclude lower-quality products, such as damaged nuts, these can potentially be reintegrated into the value chain as a potential source of aromatic molecules. Terpenes represent an attractive class of these molecules, as they are well-known volatile compounds that play a significant role in human perceptions and bring

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https://doi.org/10.1016/j.foodchem.2023.135845
Received 24 October 2022; Received in revised form 24 February 2023; Accepted 1 March 2023
Available online 11 March 2023

out sensations and feelings, influencing consumption choices (Franklin & Mitchell, 2019).

Aromatic molecules have successfully been incorporated into food products to increase customer acceptance (Tejedor-Calvo et al., 2021). However, there is limited knowledge about the volatile composition of nuts, and only a few works have focused on obtaining terpene-enriched fractions and their characterisation (Xiao et al., 2014). Furthermore, the quality of food products is strongly connected to their sensory, namely odour, properties (Franklin & Mitchell, 2019).

The odour of edible products can be increased by adding essential oils or extracts, usually rich in terpenes. These extracts are usually obtained by conventional methods such as hydrodistillation, steam distillation or using organic solvents (*e.g.,* Soxhlet, maceration). However, these methodologies work over high temperatures and time, promoting chemical changes and negatively impacting thermosensitive compounds. Thus, there is an increasing interest in nonconventional methods for extracting natural ingredients, such as supercritical fluid extraction (SFE), which presents a high potential for industrial processes by extracting safe, stable, and selective target compounds from natural sources. These extracts can be applied in fine chemistry products such as foods, pharmaceuticals, and cosmetics (Manna et al., 2015). More precisely, SFE extraction using CO_2 as the solvent (SFE-CO_2) is a green and efficient methodology that allows the recovery of desirable and refined aromatic extracts (EX) and might avoid the formation of off-flavour compounds. SFE-CO_2 works from 73.8 bar and 32.1 °C, highly adjustable for the extraction of many thermolabile substances. Among the many advantages of this methodology, compared to conventional ones, are the absence of solvent in the final product and the use of CO_2 as a cheap, clean, and non-flammable solvent (Manna et al., 2015). The desired solubility and selectivity are achieved by applying low temperature and adjustable pressure (Mezzomo et al., 2010).

The natural aromas present in the nut's extracts can be effectively evaluated by headspace/solid phase microextraction gas chromatography/mass spectrometry (HS-SPME GC/MS) methods (Beltrán et al., 2011; Xiao et al., 2014). HS profile is an instrument to describe the odour attribute of volatile ingredients. On the other hand, thermodynamic properties such as vapour-liquid equilibrium play an important role in fragrance perception. The odours release is an effect of molecular interactions between the EX mixture and the environmental conditions, reflecting the balance of the gas-phase and the liquid-phase composition (Teixeira et al., 2011). The resulting perception is a combination of volatile compounds diffused in the air and detected by the human odour-active receptors, influenced by the stimuli and olfactory memories (Franklin & Mitchell, 2019; Teixeira et al., 2011).

Following previous studies on olfactory marketing strategies using natural extracts (Kessler et al., 2022a; Kessler et al., 2022b), this work aims to develop a sustainable approach to lower-quality nuts (*P. dulcis, C. avellana*, and *J. regia* species) by using them as a source of natural aromas for incorporation into a food matrix. To achieve this goal, SFE-CO_2 was used to recover enriched aromatic extracts, which were characterised by their chemical and sensory properties, and cytotoxic potential. Bread was used as a case model to address the outcomes regarding food sensorial perception by humans along with chemical characterisation of the products. The present study aims to contribute to a sustainable use of the nuts, namely lower-quality produce, through their reintegration into a chain of value as they are a rich source of natural aromatic ingredients with potential food applications.

2. Material and methods

2.1. Chemicals

D-Limonene (CAS 59889–27-5, 97%), thymol (CAS 89–83-8, 98.5%), and the series of alkanes C8-C40 (ref. 40147-U) were obtained from Sigma Aldrich (Madrid, Spain). *n*-Hexane (CAS 110–54-3, 99%) was purchased from Supelco (Madrid, Spain), while eucalyptol (CAS 470–82-6, 99%) was acquired from Alfa Aesar (Madrid, Spain). CO_2 food grade (CAS 124–38-9, 99.9%) was obtained from Linde (Lisbon, Portugal), and the SPME (Solid Phase Microextraction) fibres (DVB/CAR/PDMS) were supplied from Supelco (Bellefonte, USA).

2.2. Nuts

P. dulcis, C. avellana, and *J. regia* commercial nuts were kindly provided by Centro Nacional de Competências dos Frutos Secos - Associação CNCFS (Bragança, Portugal). The samples were gently ground (Hr7762/90 Mini Chopper, Philips Walita), and particles size with *mesh* 14 (<1.41 mm) were collected by a vibratory sieve shaker (D-42781, Retsch, Germany) and stored at 4 °C.

2.3. Extraction by supercritical CO_2

The nut samples (30 ± 0.05 g) were subjected to SFE-CO_2 extraction at 80 bar, 50 °C for two hours, according to a previously described procedure (Kessler et al., 2022b), using an existing equipment (Gomes et al., 2007). The equipment consists of a 1 L stainless steel extractor designed to work up to 200 bar. The depressurization valves were kept at 40 and 6 bar, respectively, to release the aromatic extracts, while the separator was set at −15 °C to avoid the compounds' volatilization. All extraction procedures were carried out in batch mode, with a single CO_2 feed, and the extracts were kept at 4 °C until further analysis.

2.4. Chemical and sensory characterisation of terpene extracts

2.4.1. Extracts sampling by HS-SPME

In brief, 2 μL of each extract obtained under SFE-CO_2 conditions was transferred into a 20 mL headspace vial and sealed by an aluminium cap with PTFE septa. The samples were incubated at 50 °C for 5 min for temperature stabilisation, and then the SPME (50/30 μm of divinylbenzene-carboxen-polydimethylsiloxane - DVB/CAR/PDMS) fibre was exposed for 30 min to adsorb the aromas retained in the gas phase (headspace) (Almeida et al., 2021). The procedure was repeated at least three times for each sample.

2.4.2. Characterisation of nut's extracts by GC–MS

After the sampling time, the fibre was removed (by retracting it into the needle) and coupled into the GC–MS (TQ8040 NX Triple Quadrupole, Shimadzu, Japan) for thermal desorption of the volatiles by exposing the SPME fibre. The injector was kept at 270 °C, split ratio at 2 and high-pressure injection at 200 kPa for 0.50 min. After the high-pressure injection period, the fibre was retracted and removed from the injector. The chemical profile of the resulting extracts was performed by injecting 1 μL of the sample volume on the aforementioned GC–MS, equipped with a splitless injector and cross-bonded fused column (30 m × 0.25 mm, 0.25 μm film thickness) to low polarity phases (Rxi-5Sil MS, Restek, USA). The operational methodology has been previously described (Kessler et al., 2022b) where the temperature was programmed isothermally at 40 °C for 1 min, then increased from 40 to 200 °C for 2 min at 7 °C·min-^{1}, 200 to 250 °C for 2 min at 15 °C·min^{-1} and, finally, 250 to 280 °C for 1 min at 20 °C·min-^{1}. Ultrapure helium flow rate was set at 1 mL·min^{-1}. The analysis was carried out with ion and interface temperatures at 250 °C and 260 °C, respectively, with the mass scanning range maintained at *m/z* 40–500.

The identification of each molecule was accomplished by comparing the mass spectra with those obtained in the database software from the National Institute of Standards & Technology (NIST 21, 27, 107, 147) and the respective linear retention indices (LRI) calculated through Kovats retention index equation (Zellner et al., 2008). The same analysis conditions were applied to the used standards (alkanes C8-C40, D-limonene and thymol). To determine the LRI, the homologous series was used and compared with those reported in the literature. Finally, the selected molecules were quantified through calibration curves of each

correspondent analytical standard.

2.4.3. Sensory odour evaluation of terpene extracts

Each extract of *P. dulcis, C. avelana*, and *J. regia* nuts were evaluated by a panel of 12 individuals regarding their sensorial attributes following the protocols previously described (Kessler et al., 2022a). The Odour Detection Threshold (ODT) (ISO 13301:2018) (International Organization for Standardization 13301:2018, 2018) and (2) Quantitative Descriptive Analysis (QDA) (ISO 11035:1994) (International Organization for Standardization 11035:1994, 1994) were assessed. All panellists received a brief training under International Standard ISO 8586:2012 instructions (International Organization for Standardization 8586:2012, 2012). The tests were carried out in a clear room with good ventilation and lighting, at a controlled temperature of 20 °C and with no disturbing noises. Additionally, social and hygiene rules were adopted due to SARS-CoV-2 concerns.

2.4.4. Odour detection threshold (ODT)

For each extracted sample, a series of sequential water dilutions were prepared in a concentration range of 5.0×10^{-8} to 1.0×10^{-2} μg·mL^{-1} and presented for individual assessment. The range of concentrations was defined by preliminary tests (data not shown). After sniffing the samples, in ascending order, one by one, the panellists marked the one where they had a minimum perception of the odour (ODT). The overall ODT values were calculated by linear interpolation, obtained by the concentration in which at least 50% of the evaluators were stimulated.

2.4.5. Quantitative Descriptive analysis (QDA)

The extracts were diluted at 1.0×10^{3} μg·mL^{-1} to allow their odour perception by the panellists and for the respective characterisation according to the sensory descriptors: woody, green, fresh, citrus, floral, sweet, spicy, fruity, or oily (Miyazaki et al., 2012). Panellists scored the sensory descriptors by following an unstructured scale, where 0 represented "low intensity" and 9 corresponded to "high intensity" according to a preliminary explanation about the odour properties of *P. dulcis*, *C. avellana*, and *J. regia* extracts.

2.4.6. Cytotoxicity analysis

The extracts were dissolved in aqueous DMSO (50%, v/v) at 8 mg•mL^{-1} concentration and further diluted in the range of 400 to 6.25 μg•mL^{-1}. The cytotoxic properties were assessed against the monkey non-tumour cell line Vero (kidney cells, ATCC® CCL81.4), using the sulforhodamine B assay (Barros et al., 2013). Ellipticine was used as a positive control, while the negative control was represented by a suspension of cells. The results were expressed in GI_{50} values (concentration that inhibited 50% of the cell proliferation). Three independent assays were performed in duplicate.

2.5. Chemical and organoleptic properties of bread enriched with P. dulcis

2.5.1. Bread making and samples preparation

Fresh wheat bread dough was provided by the company M. Ferreira & Filhas Lda. (also known as "Pão de Gimonde") located in Bragança, Portugal, under refrigerated conditions. After the reception, the dough was frozen and stored at −20 °C until further usage. For each assay, the dough was defrosted overnight at 4 °C, sliced into 100 g pieces and backed at 240 °C for 15 min in a convection oven model (2000 W, O30-B Moulinex series). The incorporation of *P. dulcis* extracts obtained by SFE-CO_2 was performed according to a previously described protocol (Kessler et al., 2022a). In summary, extracts were directly added to a known amount of dough to achieve the desired final concentration on each batch of bread and mixed through a food processor for 20 s (Thermomix® TM5). *P. dulcis* extract was selected as it was considered the most promising for final application presenting organoleptic properties of interest for the bakery industry. The range of extract concentrations to be incorporated into the bread is delimited by the results obtained in the Odour Threshold (ODT) and Vero cells proliferation inhibition (GI_{50}) analyses. The aroma of bread crumb and crust samples was analysed immediately after 30 min of cooling (*t*0) and after 4 h had passed (*t*4). In this regard, bread crust and crumb samples were carefully separated, frozen with liquid nitrogen and grinded (Hr7762/90 Mini Chopper, Philips Walita) to obtain a particle size of > 1 mm at *t*0 *and t*4. Each sample was prepared in triplicate.

2.5.2. Characterisation of bread odour by HS-SPME GC–MS

About 1000 ± 5 mg of each sample prepared as described in section 2.5.1 was transferred into a 20 mL headspace vial and sealed by an aluminium cap with PTFE septa. The samples were incubated at 50 °C for 5 min for temperature stabilisation, previously to the SPME (50/30 μm, DVB/CAR/PDMS) fibre exposure for 30 min. The same procedure was applied to the stored bread (*t*4). GC–MS settings for sampling and analysis are described elsewhere (Kessler et al., 2022a). The volatile composition was presented according to the molecule's percentage values, and their identification performed as described in Section 2.4.2. The most abundant volatiles (*D*-limonene and eucalyptol) present in the incorporated samples were quantified. Calibration curves for each correspondent analytical standard were obtained by spiking bread crust samples with the respective concentration range (*D*-limonene: $2.59 \times 10^{-4} - 6.66 \times 10^{-2}$ μg•g^{-1}; eucalyptol: $1.14 \times 10^{-3} - 1.37 \times 10^{-1}$ μg•g^{-1}). The limits of detection (LODs) were calculated as 3 times the signal-to-noise ratio (S/N), while the limits of quantification (LOQs) were calculated as 10 times the signal-to-noise ratio (S/N).

2.5.3. Sensory odour evaluation of bread

The sensory odour evaluation of bread was performed based on a previously presented methodology (Kessler et al., 2022a). In this regard, the International Standard ISO 8586:2012 instructions (International Organization for Standardization 8586:2012, 2012) were followed, and the sensory evaluation of the bread odour was addressed by 19 panellists. A Multiple Comparison Test (MCT) was applied to characterise the odour of bread crumb and crust. In this sense, panellists were questioned about the odour deviation of unknown samples compared to a reference one (relative-to-reference rating), as suggested by ISO 13299:2016 (International Organization for Standardization 13299:2016, 2016). Four concentrations of the *P. dulcis* extract were used: 0, 2, 4 and 8 μL•100 g^{-1} of bread, which was presented to the panellists with random codes to be compared with a reference (bread without any additional ingredient). Each panel member pointed out a description based on a non-structured scale ranging from 1 -"much more intense odour of bread than control" to 5 - "much less intense odour of bread than control". Similarly, the presence of a distinct/unusual odour besides the bread odour was expressed through a second non-structured scale: 1 - "distinct odour much more intense than control" to 5 - "distinct odour much less intense than control" for both crust and crumb. The result was given by the average value indicated by the panel evaluators.

2.6. Statistical analysis

The significant differences between the extraction yield, the main compounds, and the sensory odour analysis were evaluated using the ANOVA and Tukey tests, with $\alpha = 0.05$ (significance level). To explain the relationship between chemical composition and sensory descriptions of the extracted products, an "odour map" was performed by the Principal Component Analysis (PCA) procedure based on the generalised inverse (Statistica StatSoft, version 14, USA) after data range standardising.

The extraction yield is obtained by equation (1):

$$Yield(\%) = \left[\frac{m_{\text{SFE}-CO_2\ \text{extract}}(\text{g})}{m_{\text{Sample weight (d.b.)}}(\text{g})}\right] \times 100 \quad (1)$$

Table 1
HS volatile composition of extract and extraction yields of *P. dulcis, C. avellana*, and *J. regia* species, obtained by supercritical CO_2 fluid extraction.

N	Compounds	MS/MS	LRI[1]	RT (min)	*P. dulcis* (%)	*C. avellana* (%)	*J. regia* (%)
1	α-Thujene	93, 77, 91	925	8.416	–	–	0.15 ± 0.02
2	α-Pinene	93, 41, 53	935	8.718	0.6 ± 0.1	2.6 ± 0.1	2.0 ± 0.2
3	Camphene	93, 121, 107	950	9.191	0.12 ± 0.02	–	0.273 ± 0.003
4	Sabinene	93, 77, 41	971	9.812	–	–	0.059 ± 0.001
5	β-Pinene	93, 41, 69	978	10.007	0.18 ± 0.04	0.689 ± 0.005	0.27 ± 0.02
6	β-Myrcene	41, 93, 69	994	10.508	0.9 ± 0.1	0.74 ± 0.04	0.81 ± 0.03
7	3-Carene	93, 121, 41	1018	11.255	0.11 ± 0.01	–	0.326 ± 0.003
8	*p*-Cymol	119, 91, 134	1025	11.509	3.0 ± 0.2	8.44 ± 0.2	4.66 ± 0.15
9	D-Limonene	68, 93, 136	1031	11.682	15 ± 1	48.1 ± 0.7	6.88 ± 0.09
10	Eucalyptol	43, 81, 93	1033	11.773	2.7 ± 0.4	12.2 ± 0.9	2.5 ± 0.1
11	γ-Terpinene	93, 136, 43	1063	12.736	0.9 ± 0.2	3.18 ± 0.02	3.0 ± 0.1
12	Sabinene hydrate	93, 43, 71	1071	13.018	–	–	0.40 ± 0.03
13	Dihydromyrcenol	59, 43, 41	1081	13.332	0.7 ± 0.1	0.86 ± 0.04	–
14	Terpinolene	93, 59, 43	1088	13.563	0.10 ± 0.02	–	–
15	β-Linalool	71, 41, 43	1104	14.050	2.6 ± 0.4	2.21 ± 0.03	0.92 ± 0.08
16	Nonanal	57, 41, 82	1109	14.165	0.49 ± 0.08	0.38 ± 0.01	0.51 ± 0.09
17	Camphor	95, 81, 41	1150	15.066	0.57 ± 0.06	2.21 ± 0.04	3.55 ± 0.03
18	α-Acetoxytoluene	108, 91, 43	1168	15.475	2.5 ± 0.2	–	–
19	Borneol	95, 41, 55	1176	15.645	2.6 ± 0.3	1.51 ± 0.02	2.4 ± 0.5
20	Pinocamphone	55, 69, 83	1174	15.603	–	–	0.28 ± 0.02
21	4-Terpineol	71, 111, 43	1185	15.847	0.682 ± 0.001	0.39 ± 0.03	0.561 ± 0.004
22	α-Terpineol	59, 93, 121	1199	16.151	10.14 ± 0.63	1.58 ± 0.05	1.23 ± 0.04
23	Verbenone isomer	107, 91, 135	1206	16.280	–	–	0.56 ± 0.01
24	*S*-Verbenone	107, 91, 135	1212	16.396	1.39 ± 0.07	2.61 ± 0.14	4.02 ± 0.05
25	Carvone	82, 54, 108	1249	17.051	4.9 ± 0.7	3.45 ± 0.15	0.16 ± 0.01
26	Unknown	93, 121, 138	1250	17.067	–	–	0.20 ± 0.01
27	Bergamiol	93, 43, 69	1255	17.150	1.4 ± 0.2	1.52 ± 0.03	0.8 ± 0.4
28	α-citral	69, 41, 39	1273	17.471	–	–	tr
29	Bornyl acetate	95, 43, 93	1288	17.729	1.1 ± 0.1	0.83 ± 0.03	1.85 ± 0.07
30	Thymol	135, 150, 91	1296	17.866	–	–	51.0 ± 0.7
31	Carvacrol	135, 150, 91	1300	17.949	5.3 ± 0.5	1.06 ± 0.13	4.53 ± 0.09
32	α-Terpineol acetate	121, 93, 136	1352	18.725	0.48 ± 0.05	0.41 ± 0.04	0.825 ± 0.003
33	Ylangene	105, 119, 93	1369	18.979	–	–	0.279 ± 0.005
34	Nerol acetate	69, 41, 93	1381	19.160	0.31 ± 0.04	–	–
35	β-Caryophyllene	93, 133, 41	1423	19.785	1.8 ± 0.1	0.75 ± 0.02	2.59 ± 0.07
36	Isocaryophyllene	41, 93, 69	1447	20.143	–	–	0.095 ± 0.002
37	α-Caryophyllene	93, 121, 41	1459	20.328	0.20 ± 0.03	–	0.174 ± 0.001
38	*p*-Benzoquinone, 2,6-di-*tert*-butyl	177, 165, 135	1464	20.396	0.7 ± 0.1	0.57 ± 0.14	0.49 ± 0.02
39	Unknown	97, 57, 41	1474	20.545	–	–	0.221 ± 0.001
40	β-Ionone	177, 43, 91	1485	20.712	0.41 ± 0.07	–	–
41	Unknown	189, 133, 57	1491	20.804	0.11 ± 0.03	–	–
42	Unknown	105, 93, 41	1497	20.895	0.23 ± 0.02	–	–
43	Unknown	107, 106, 161	1500	20.941	0.18 ± 0.02	–	–
44	BHT	205, 57, 220	1503	20.995	–	–	0.84 ± 0.02
45	Unknown	189, 93, 107	1605	21.025	0.48 ± 0.02	–	–
46	Unknown	141, 105, 91	1615	21.215	–	–	0.113 ± 0.004
47	Unknown	141, 105, 91	1646	21.762	–	–	0.11 ± 0.01
48	Elemol	59, 93, 161	1660	22.011	8.13 ± 0.01	–	–
49	Spathulenol	43, 205, 119	1688	22.518	2.13 ± 0.04	–	–
50	Unknown	149, 43, 57	1694	22.626	3.93 ± 0.02	2.80 ± 0.41	–
51	Veridiflorol	43, 69, 109	1703	22.798	1.64 ± 0.10	–	–
52	Guaiol	161, 59, 107	1725	23.312	2.66 ± 0.05	–	–
53	Methyl jasmonate	83, 153, 55	1733	23.499	0.853 ± 0.003	0.84 ± 0.01	–
54	Unknown	59, 149, 81	1738	23.617	12.1 ± 0.6	–	–
55	4-Eudesmenol	43, 81, 161	1743	23.732	5.0 ± 0.2	–	–
56	Isopropyl myristate	43, 60,102	1926	25.337	0.538 ± 0.002	–	0.17243 ± 0.00004
57	Isopropyl palmitate	40, 102, 69	2100	26.865	–	–	0.24471 ± 0.0001
No identified					**17.71**	**2.80**	**0.64**
Identified					**82.29**	**97.20**	**99.36**
Extraction yield (%)					1.4 ± 0.7[a]	1.6 ± 0.1[a]	2.2 ± 0.3[a]

Compound	Calibration curve	R^2	LOD ($g \bullet L^{-1}$)	LOQ ($g \bullet L^{-1}$)	Mass ($_{\mu gcompound} \bullet g_{plant}^{-1}$, SD)		
					P. dulcis	*C. avellana*	*J. regia*
D-Limonene	$y = 2.21 \times 10^{12}x - 1.12 \times 10^{7}$	0.9990	1.10×10^{-5}	3.33×10^{-5}	3.9 ± 0.3[a]	1.64 ± 0.03[b]	0.82 ± 0.01[c]
Eucalyptol	$y = 2.52 \times 10^{12}x + 8.97 \times 10^{6}$	0.9991	2.40×10^{-6}	7.28×10^{-6}	0.56 ± 0.04[a]	0.30 ± 0.02[b]	0.15 ± 0.01[c]
Thymol	$y = 2.09 \times 10^{12}x - 3.11 \times 10^{6}$	0.9985	1.09×10^{-5}	3.31×10^{-5}	–	–	5.6 ± 0.1

[1]LRI: linear retention indices calculated through Kovats retention index equation for series of alkanes C8-C40 using a cross bonded fused column in GC–MS. tr = traces. Averages with different letters in the same line indicate significant difference with α = 0.05.

3. Results and discussion

3.1. Volatile composition of the potential new food flavouring ingredients

P. dulcis, C. avellana, and *J. regia* nuts samples were submitted to SFE-CO_2 extraction procedures. The extraction yield results are shown in Table 1, in which no significant difference was observed ($\alpha = 0.05$). *J. regia* have shown the highest extraction yield (2.2 ± 0.3%), followed by *C. avellana* (1.6 ± 0.1%) and *P. dulcis* (1.4 ± 0.7%). These results are a stepstone for the extraction of added-value molecules from nuts using supercritical technology at low pressures, ideal to obtain volatiles (Gomes et al., 2007). Previous works focused on the extraction of the lipidic fraction by applying higher pressures. For example, almond extraction resulted in 23.5% at 300 bar, recovering a slight amount of terpenoids within the oil rich in fatty acids (Mezzomo et al., 2010). Therefore, increases in the CO_2 solvation power promoted by the higher-pressure result in lower selectivity of the compounds and higher extraction yield. Higher pressures favour the extraction of lipidic molecules, which has higher molecule weights than volatile terpenoids. Higher pressures facilitate extraction, as the pressure forces supercritical CO_2 into the pores of the matrix samples and extracts non-polar or compounds of low polarity (Kessler et al., 2022b). As expected, aromatic plants have produced highly superior volatile compounds to those obtained in the present work. The different natural compositions of both raw materials, aromatic plants and nuts, result in noticeable variability of extraction yield of terpenoid molecules. Nevertheless, the terpenoid-enriched nut extracts have still been widely applied for edible and topical purposes as a natural flavouring agent in food and cosmetic products, respectively (Caputi & Aprea, 2011; Gomes et al., 2007).

The phytochemical identification was carried out by HS-SPME GC–MS, which revealed the abundance of terpenoids. After the optimisation procedure to define the ideal temperature and time of fibre exposition (data not shown), the volatile fraction was adsorbed from HS vials and desorbed in the GC–MS injector apparatus.

Regarding the volatile composition (Table 1), 36 compounds were identified in *P. dulcis* extract, a rich source of terpenoids such as D-limonene (15.1%), α-terpineol (10.1%), and elemol (8.1%). For the *C. avellana* extract, 23 molecules were found with 48.1% in D-limonene, 12.2% eucalyptol, and 8.4% *p*-cymene. Moreover, a higher complexity in the odour blend was attributed to the *J. regia* extract. Among the 37 identified molecules, thymol chemotype stood out with 51.0% of the relative composition. Additionally, D-limonene, *p*-cymene and *S*-verbenone were also present at 6.9%, 4.7% and 4.0% fractions, respectively. For better elucidation, the aromatic profile of each extract is shown in **Supplementary material 1**.

The three main compounds' mass composition (also shown in Table 1) was obtained from their respective calibration curves, expressed in $\mu g_{compound}.g_{plant}^{-1}$. Among the selected standards, *P. dulcis* extract was significantly richer ($\alpha = 0.05$) in D-limonene and eucalyptol molecules with 3.9 ± 0.3 $\mu g \bullet g^{-1}$ and 0.56 ± 0.04 $\mu g \bullet g^{-1}$, respectively. In addition, the thymol compound, with 5.6 ± 0.1 $\mu g \bullet g^{-1}$, prevailed in the *J. regia* extract. The achieved results are in good agreement with other literature data (Kesen et al., 2018), where various terpenes molecules were found in the volatile composition of *P. dulcis*, *C. avellana*, and *J. regia* oils, namely α-pinene, β-pinene, sabinene, 3-carene, D-limonene, γ-terpinene, *p*-cymene, and 4-terpineol. However, a significant difference was observed for D-limonene, with an eight times higher value than previously reported, namely 0.48 $\mu g \bullet g^{-1}$ (Kesen et al., 2018).

Different studies have explored the potential of nuts as sources of odours. Despite differences in extraction and sampling methodologies, which result into variable compositions, they all produced relevant clues about the inherent characteristics of the resulting extracts. In this sense, HS-SPME evaluation indicated the presence of 22 volatile compounds in almond samples, prevailing the compounds of 1,3-dimethyl-benzene, nonane, octanal, nonanal, dodecane, and decanal (Beltrán et al., 2011). No terpenoid molecule was identified, and temperature and time negatively influenced the adsorption of aldehyde compounds. However, they favoured hydrocarbon retention (*e.g.*, dodecane). In addition, 59 compounds described the odour-activity of raw, dried, and roasted almonds, analysed by gas chromatography–olfactometry (GCO) (Erten & Cadwallader, 2017). The aldehyde, ketones, furans, nitrogen-, and sulfur-containing molecules have influenced the volatile composition. Nevertheless, no terpene fraction was detected. Similarly, 58 compounds were found in roasted almonds, including aldehydes, ketones, pyrazines, and alcohols (Xiao et al., 2014). The prevailed odour substances have resulted from the Maillard lipid oxidation/degradation and caramelisation reactions due to the high temperature applied in the sample preparation (Erten & Cadwallader, 2017). Furthermore, only α-pinene and D-limonene terpenes were identified using DVB/CAR/PDMS HS-SPME fibre, commonly present in the volatile composition of almonds (Valdés et al., 2015; Xiao et al., 2014). Also, the fingerprint of dried-roasting hazelnut mapped the odoriferous compounds formed by their non-volatile precursors (Rosso et al., 2020). Once again, no terpenoid molecule was identified, prevailing aldehydes, ketones, pyrazines, acetic acids, and furans.

The noticeable oil content of nuts makes them easily susceptible to thermal oxidation, and a decrease in stability is usually observed under high temperature and humidity conditions over storage time. This lipid degradation was not observed by the chemical characterisation due to the well-known stability of thermolabile compounds extracted by SFE-CO_2, and contrasting with previously reported extraction methods: Soxhlet with petroleum ether (Beltrán et al., 2011), maceration with diethyl ether (Erten & Cadwallader, 2017), and ultrasound (Rosso et al., 2020).

3.2. Defining a safe range concentration to introduce the nut extracts as a new flavouring ingredient in a food matrix

The odour profile can be tracked through the human nose receptors. By creating a series of nervous impulses from the nose to the brain, the physiological responses reflect memories, feelings, and sensations of a set of volatile molecules (Franklin & Mitchell, 2019). These stimuli are widely explored for commercial purposes, mainly for olfactory marketing strategies. Therefore, recovering odours from natural sources comprise a creative way to the acceptance by the most demanding consumers. Embracing the use of aromatic compounds extracted from raw materials as edible ingredients depends on their safety and quality characteristics, as indicated by the FDA. (Caputi & Aprea, 2011).

In this context, a cytotoxic test and a sensory ODT analysis were performed for a complete characterisation and to define safe and perceptible odour concentrations for nut extracts. The minimal odour perception was indicated at $8.3x10^{-4} \mu g \bullet mL^{-1}$ forJ. Regia extract, setting a lower limit of concentration where the sensory panellists evidenced some stimuli. *C. avellana* has shown an ODT value of $3.0x10^{-3} \mu g \bullet mL^{-1}$, while *P. dulcis* indicated a higher value ($6.9x10^{-3} \mu g \bullet mL^{-1}$). Despite this range of values, the ODT values have not shown a significant difference using $\alpha = 0.05$ (Table 2).

In contrast, the cytotoxic potential differed significantly among the three extracts. *P. dulcis* did not present cytotoxicity under the ranged experimental conditions ($GI_{50} > 400 \mu g \bullet mL^{-1}$); consequently *P. dulcis* is the one with a higher screen to be used as a food ingredient. Higher

Table 2
Resulting safe concentration range of *P. dulcis, C. avellana*, and *J. regia* products, defined by the cytotoxic GI_{50} measurements and ODT values.

Test	*P. dulcis*	*C. avellana*	*J. regia*
GI_{50} ($\mu g \cdot mL^{-1}$)	$> 400^{a}$	254 ± 14^{b}	177 ± 12^{c}
ODT value ($\mu g \cdot mL^{-1}$)	6.9×10^{-3a}	3.0×10^{-4a}	8.3×10^{-4a}

Averages with different letters in the same line indicate significant difference with $\alpha = 0.05$.
GI_{50} (ellipticine) = 1,41 ± 0,06 $\mu g \cdot mL^{-1}$.

citotoxicity values were found in *J. regia* (GI_{50} = 177 μg•mL^{-1}) and *C. avellana* (GI_{50} = 254 μg•mL^{-1}). All results are presented in Table 2. To the best of the authors' knowledge, this is the first time that this approach was performed in these types of extracts, except for a previous study on a polyphenol-enriched extract obtained from almond skin that showed cytotoxicity effects over Vero cells at a concentration of 604 μg•mL^{-1} (Musarra-Pizzo et al., 2019).

Most of the obtained extracts are suitable to apply into foods to create new blends as they act as a flavouring agent. In this context, aiming to characterise the odour of the extracted products, a panel of 12 members defined their primary sensory descriptors. Results (Fig. 1) show that *J. regia* presented a higher intensity of fresh (4.4), green (2.4), and spicy (1.5) notes, with significant difference (α = 0.05) only for the fresh and spicy notes in comparison to the other extracts (data not shown). *P. dulcis* samples were described as woody (1.7), green (1.7), and oily (0.6) notes, while *C. avellana* showed similar intensity to woody (1.0), fresh (0.9), and green (0.8) descriptors. Although the influence of the main compounds, the sensory profile was dominated by the mixed terpene molecules, according to the panel's judgment.

It is challenging to dimension the importance of each volatile compound among the hundreds of odour-active molecules from natural products (Franklin & Mitchell, 2019). Indeed, the human ability to distinguish odours is unquestionable. The best sensory performance indicates that 350–400 volatile receptors can detect thousands of odours (Rinaldi, 2007). The receptors are activated by several chemical compounds or functional groups and need to be stimulated over time, resembling and training the olfactory nerve cells (Franklin & Mitchell, 2019).

Literature studies have reported the odour-active compounds of nuts. *P. dulcis* odour has been distinctly identified for raw, roasted, and dried roasted samples. Previously referred notes include mushroom odour, baked potato, earthy, floral, and caramel (Erten & Cadwallader, 2017). Furthermore, *C. avellana* odour was described as nutty, fruity, sweet, oily, and green, mostly correlated to the contribution of ketones, pyrazines, and furan compounds as revealed by the odour activity value (OAV) (Kiefl & Schieberle, 2013).

The extracts obtained in the present study preserve their natural odour, mainly observed by the dominance of fresh and green descriptors, predominantly attributed for the identified terpene compounds. Aiming to depict the influence of the main compounds over the sensory notes, the odour map (Fig. 2) explains>76% of these correlations. Eucalyptol and D-limonene molecules resemble flower and citrus notes, with no correlation to fresh, green, and woody attributes. Although previous works have described the eucalyptol odour as green, herbal, and spicy (Caputi & Aprea, 2011; Miyazaki et al., 2012), and D-limonene as citrus, fresh, and sweet notes (The Good Scents, 2022), the PCA model reflects the mixture of compounds instead the individual odour itself (Kessler et al., 2022a). The fragrance perception is both related to the ODT and relative concentration, but also to the effects of the vapour-liquid equilibrium resulting from the physicochemical properties of each molecule, such as vapour pressure, molar weight, and molar composition (Teixeira et al., 2011). Nevertheless, the thymol compound - highly identified in *J. regia* - has revealed a strong correlation to woody and green notes, previously reported as herbaceous (The Good Scents, 2022).

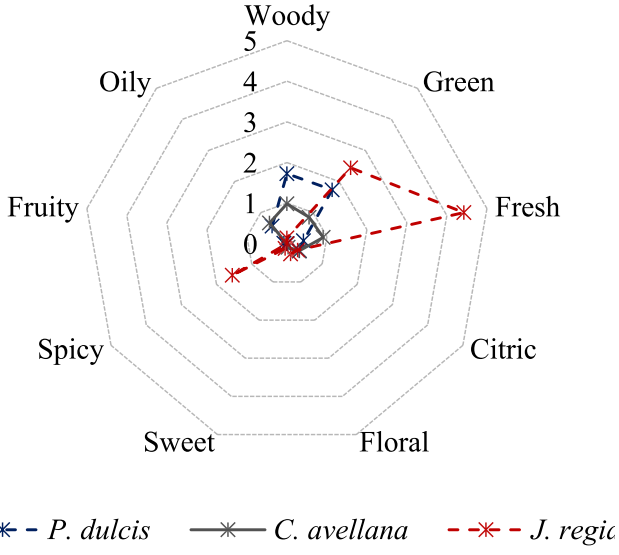

Fig. 1. Sensory profile of *P. dulcis*, *C. avellana*, and *J. regia* products obtained by Quantitative Descriptive Analysis (QDA) method.

3.3. Chemical characterisation of bread food matrix added the most promising flavouring ingredient

Due to the selectivity of *P. dulcis* extract for thymol, its organoleptic properties, and the absence of cytotoxicity, almonds extracts were chosen as an attractive candidate as a flavouring additive.

As a case study, bread was chosen as the food matrix. Bread is a complex food matrix, and some studies have related its odour characteristics (Birch et al., 2013; Cho & Peterson, 2010; Heenan et al., 2009; Jensen et al., 2011; Pico et al., 2015). Bread dough has a three-dimensional structure, formed by chains of gluten proteins, and can retain volatile compounds (Gan et al., 1990). A preliminary sensory test (data not shown) indicated the minimal concentration of *P. dulcis* extract to be added and perceived in bread samples. A concentration range of 2 to 8 μL•100 $g^{-1}$of bread dough was used in MCT analysis, corresponding to three orders of magnitude higher than the ODT determined for the pure oil. According to the sensory panellists, a significant difference (α = 0.01) was found in the odour provided from bread crust at 4 μL•100 $g^{-1}$of bread dough. This concentration indicates the highest intensity of natural bread odour and the most distinct odour compared to the control sample (without extract). However, none of the tested concentrations presents significant differences for the bread crumb compared to the control sample, with 90% confidence.

A previous work (Kessler et al., 2022a) reported the control bread volatile characterisation and the potential of rosemary extracts on bread's chemical and sensory profile. The study focused on incorporating natural extracts in bread doughs and displayed a guidance procedure for the sequential odour intensification in foods. Using the same approach, Table 4 and **Supplementary material 2** show the relative odour fractions of bread crust and crumb added of *P. dulcis* extract in two different timeframes: *t0* or 30 min after the baking cooling, and *t4* or 4 h of storage in paper bags after the *t0* step. Among the 39 identified compounds by HS-SPME GC–MS analysis, 9 aldehydes, 6 furans, 6 pyrazines, 5 alcohols, 5 hydrocarbons, 5 terpenoids, 2 unknowns, and 1 ketone molecules, were identified.

Concerning the volatile complexity, previous results on control bread and rosemary extract as a flavouring ingredient (Kessler et al., 2022a) support the behaviour of the odour composition of the *P. dulcis* enriched bread. The chemical profile of the control bread and those incorporated with the natural extracts have evidenced similar conclusions: (i) the furfural compound reduces its intensity over time; (ii) dihydro-2-methyl-3-furanone and 2-methylpyrazine molecules were found only in the crust samples with low interference of the storage time; (iii) similar observation for 1-heptanol, which appears only in the bread crumb; and (iv) terpenoids compounds prevailed in the bread crumb.

The intense bread odour promoted by the incorporation ofP. dulcisextract is a consequence of the key compounds release, largely reported in the literature, mainly referring to the presence of hexanal, benzaldehyde, nonanal, heptanal, 1-hexanol, 1-pentanol, and 2-heptanone in bread crumb. On the other hand, bread crust has shown an abundance in furfural, 2-pentylfuran, 2-furanmethanol, 5-methyl furfural, and styrene molecules, for example. This volatiles mixture expresses the natural and

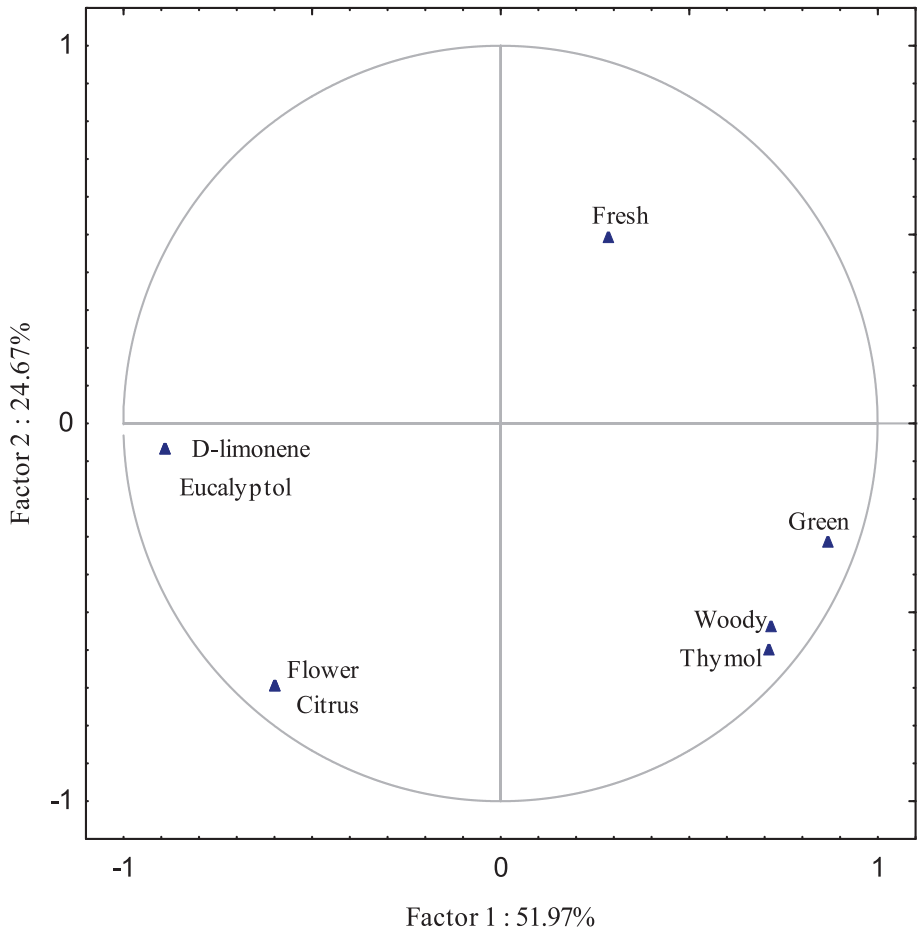

Fig. 2. Odour map of the nuts extracts, according to the volatile fraction and sensory descriptors.

pleasant odour of the food product (Birch et al., 2014).

After introducing the flavouring agent, positive effects were also observed over time, by increasing or sustaining the compounds' presence. Comparing the results with those previously reported (Kessler et al., 2022a) for the control bread, a tendency is observed in that the incorporation of the extracts enhances the pleasant odour compounds (*e.g.*, furans, pyrazines, and some aldehydes) and decreases the relative composition of molecules responsible for the off-flavours (*e.g.*, alcohols and some aldehydes). Related to the sensory profile (Table 3) and the effect over time, the relative furfural composition of bread crust showed an improvement at *t4*, as well as dihydro-2-methyl-3-furanone, 2-furanmethanol, 2-acetylfuran, and 5-methylfurfural. These phenomena repeat for most of the pyrazine compounds (*e.g.*, 2,5-dimethylpyrazine, 2-ethylpyrazine, 2,3-dimethylpyrazine, 2,5-dimetilpyrazine, 2-ethyl-5-methylpyrazine, and 2-ethyl-3-methylpyrazine) and for the desirable aldehyde molecules (*e.g.*, benzaldehyde, nonanal, 2-nonenal, and decanal).

Furthermore, a decreasing effect from *t0* to *t4* was noticed for hexanal, heptanal, methional, benzene acetaldehyde, and 2-octenal, frequently responsible for off-flavour in baked bread (Pico et al., 2015). Increase in hexanal concentration was previously observed in wheat bread over storage time, resulting from the lipid oxidative reactions initiated during baking (Jensen et al., 2011; Kessler et al., 2022a; Valdés et al., 2015). Mostly, the odours' formation happens along with the fermentation and heat processes, and biochemically comprises three routes: lipid oxidation, caramelisation, and Strecker degradation into Maillard reaction. Their set synthesis and odour formation (*e.g.*, pyrazines, furans, aldehydes, and amino ketones) have been widely discussed (Birch et al., 2014; Cho & Peterson, 2010). Therefore, the odour-key of bread is a blend of volatile compounds, in which the contribution of each molecule is achieved by the synergy between lipids and heat (oxidation), carbohydrates and heat (caramelisation) and/or proteins and carbohydrates under relatively high temperatures (Maillard reaction) (Heenan et al., 2009).

Meanwhile, alcohol, aldehyde, ketone, and acid groups are naturally present in relatively high concentrations in bread crumb due the fermentation of sugars. These compounds also affect the odour mixture (Birch et al., 2014; Jensen et al., 2011). Isopentyl alcohol, 1-pentanol, 1-hexanol, 1-heptanol, and 1-octen-3-ol associated with acid compounds usually influence negatively the pleasant odour of bread and show a tendency to increase over time (Paraskevopoulou et al., 2012). However, no acid fraction was identified in bread incorporated with P. dulcis

Table 3
Average scores and significant differences in sensory evaluation of the control bread odour and the bread incorporated with *P. dulcis* extract, obtained by carbon dioxide supercritical fluid extraction (SFE-CO_2).

Concentration (μL•100 g^{-1} of bread)	Q1	Q2	Q3	Q4
Control (0)	3.32^{a}	3.16^{a}	3.42^{a}	2.89^{a}
2.0	3.05^{a}	2.79^{a}	3.11^{a}	2.95^{a}
4.0	**2.58^{b}**	**2.63^{a}**	**2.74^{b}**	**2.68^{a}**
8.0	2.79^{a}	3.21^{a}	3.16^{a}	2.95^{a}

Averages with different letters in the same column indicate significant difference from control sample with $\alpha = 0.05$.
Scale: 1- much more intense; 2- slightly more intense; 3- same as the control; 4- less intense; 5- much less intense.
Q1: Regarding of the odour in the bread crust; Q2: Regarding of the odour in the bread crumb; Q3: Regarding of the distinct odour in the bread crust and Q4: Regarding of the distinct odour in the bread crumb.

extract, and the main alcohol molecules decreased their relative concentration over time, mainly observed in the bread crust (Table 4). The odour from each compound results from the total concentration of the respective volatile compounds and odour threshold (odour activity value, OAV), as well as from the interactions between the extract volatiles and the bread matrix (Jensen et al., 2011). However, to explain the full behaviour of the bread odour enriched with extract, it is necessary to consider more than its relative volatile composition. The flavouring agent can act as an enhancer of the volatilisation process and improve their perception by the human nose. Some observations are proposed to understand this phenomenon: (i) the terpene compounds from SFE-CO_2 extract perform as a carrier of the natural odour from bread; (ii) the higher vapour pressure of terpenoid compounds opens adjacent ways through the three-dimensional structure of bread crumb to diffuse their natural aromatic molecules; and/or (iii) physicochemical interactions between the surface of bread crust and the extract molecules improve their volatility.

The contribution of both major terpene compounds, D-limonene and eucalyptol, was measured through their respective calibration curves, expressed in $\mu g_{compound} \bullet 100\ g_{bread}^{-1}$. Table 4 shows a decrease in mass concentration with storage time. As expected, the bread crumb sustains higher quantities of volatiles without significant reduction and shows a constant release rate over time (1.52 ± 0.05 to 1.48 ± 0.02 μg•100 g^{-1} for D-limonene and 1.4 ± 0.1 to 1.26 ± 0.02 μg•100 g^{-1} for eucalyptol, $\alpha = 0.05$), mainly due to the transfer mass barrier created by the bread matrix (Onishi et al., 2011). Therefore, the volatile compounds were significantly higher in the bread crumb than in the bread crust samples. In addition, the eucalyptol fraction in the bread crust was significantly lower under the *t4* condition compared to the initial evaluation (*t0*) (0.95 ± 0.02 to 0.79 ± 0.01 μg•100 g^{-1}). The volatile potential is conditioned by the surface area and the heat exposition (Birch et al., 2014), which explains the higher aromatic content in the bread crumb even past 4.5 h of storage, besides the presence of the β-linalool compound.

Five types of mass transfer characteristics between the bread crust and crumb based on experimental evaluation have been suggested (Onishi et al., 2011): (i) the total amount of volatile compounds released from the bread crust to the surroundings by the convective mechanism; (ii) the total amount of volatile compounds from the crust surface released to the bread crumb and the surroundings by the diffusive and convective mechanisms, respectively; (iii) a fraction of volatile compounds remains in the crust surface, while another fraction diffuses to the bread crumb and the third fraction is released to the surroundings by the convective mechanism; (iv) the total amount of volatile compounds released from the bread crumb to the surroundings by diffusive and convective mechanisms; and (v) a fraction of volatile compounds remains into the bread crumb, while another fraction diffuses to the crust surface and to the surroundings. Therefore, based on the behaviour observed in the analysed bread crust and crumb and their respective composition, aldehydes compounds could have assumed mechanisms (i) and (v), pyrazines may be classified in the (ii) definition, and furans may represent the (iii) mass transfer characteristic.

The formation and release of volatiles comprise the food quality and consumer acceptability (Valdés et al., 2015). According to the sensory analysis and the volatile profile, the contribution of the flavouring agent has positively influenced the improvement of the pleasant bread's odour. The incorporation of *P. dulcis* extract of 4 μL•100 g^{-1} of bread dough masks off-flavours commonly originated by the fermentation process, Maillard reaction, caramelisation process, and/or lipid degradation in the bread crumb and crust (Pico et al., 2015).

The main compounds responsible for the sensory attributes of bread correspond to aldehydes, furans, and pyrazines. Nonanal and decanal are recognised for the citrus fragrance (Pico et al., 2015), while 2-nonenal is described as fatty and green, and benzaldehyde resembles the almond and caramel notes (Pico et al., 2015). 2-pentylfuran shows green, waxy, metallic fragrancies (Jensen et al., 2011), furfural reminds almond and toasted odours (Barbarisi et al., 2019), dihydro-2-methyl-3-furanone presents spicy, green, and fatty characteristics (Pico et al., 2015), 2-acetylfuran has smoky, roasted, and fermented sensory descriptors (Barbarisi et al., 2019; Pico et al., 2015), and 5-methylfurfural was mentioned as butter, caramel, and musty (Pico et al., 2015). Regarding the pyrazines, the main compounds identified and their respective sensory attributes were: 2-methylpyrazine (roasted (Barbarisi et al., 2019)), 2,5-dimethylpyrazine (nutty, musty, coffee and roasted (The Good Scents, 2022)) and 2-ethyl-5-methylpyrazine (baked (Pico et al., 2015)).

4. Conclusion

This work has proposed a new approach for obtaining natural and safe sources of flavouring food ingredients. As a case study, the pleasant odour characteristics of *P. dulcis*, *C. avellana*, and *J. regia* were investigated as potential additives to enhance the odour of the bread crust and crumb.

The volatile compounds from nuts were extracted by SFE-CO_2 under optimised conditions and further analysed by HS-SPME GC–MS. According to the trained sensory panel members, the recovered extracts showed a distinctive terpene composition, resembling woody, fresh, and green odour notes. Also, the extracts present low or non-cytotoxic effect against Vero cells (IG_{50}) and no significant difference was found between their ODT values.

The chemical composition of the extracts, sensory evaluation, yield extraction, odour perception, and safe properties have indicated *P. dulcis* extract as the most promising ingredient to be added into bread doughs; therefore, samples enriched with 4 μL•100 $g^{-1}$of bread dough were analysed at *t0* and *t4* storage time. The *P. dulcis* extract incorporation resulted in an increasing odour perception of bread. The crust and crumb pleasant and natural odour also increased over time, noticed by the relative concentration of furans (furfural, dihydro-2-methyl-3-furanone, 2-furanmethanol, 2-acetylfuran, and 5-methylfurfural), pyrazines (2,5-dimethylpyrazine, 2-ethylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-5-methylpyrazine, 2-ethyl-3-methylpyrazine), and aldehydes (benzaldehyde, nonanal, 2-nonenal and decanal). On the other hand, off-flavours molecules such as alcohols (*e.g.*, isopentyl alcohol and 1-pentanol) and aldehydes (*e.g.*, hexanal and heptanal) have lowered their influence in the total composition when analysed at *t4* condition.

The results highlight that nuts can be used as sources of aromatic ingredients with potential as food additives. This study revealed that the incorporation of terpenes rich extract enhanced the volatilisation process of the bread pleasant odoriferous compounds. This phenomenon is not yet fully understood but opens a path to the development of olfactive marketing devices.

CRediT authorship contribution statement

Júlia C. Kessler: Methodology, Validation, Investigation, Writing – original draft. **Vanessa Vieira:** Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Supervision. **Isabel M. Martins:** Methodology, Investigation, Writing – original draft, Supervision. **Yaidelin A. Manrique:** Methodology, Writing – review & editing. **Patrícia Ferreira:** Investigation, Writing – review & editing. **Ricardo C. Calhelha:** Methodology, Formal analysis. **Andreia Afonso:** Conceptualization, Supervision. **Lillian Barros:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Alírio E. Rodrigues:** Conceptualization, Writing – review & editing. **Madalena M. Dias:** Conceptualization, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

Table 4
Volatile composition of bread crust and crumb with *P. dulcis* extract added (4 μL•100 g^{-1} of bread dough).

N°	Compound	MS/MS	LRI	RT (min)	Crust *t*0 (%, SD)	Crust *t*4 (%, SD)	Crumb *t*0 (%, SD)	Crumb *t*4 (%, SD)	Sensory description
1	Isopentyl alcohol	55, 42, 70	735	2.672	13.6 ± 0.3	11 ± 1	27.5 ± 0.8	26.5 ± 0.6	alcoholic and fermented (The Good Scents, 2022)
2	1-pentanol	42, 55, 70	769	3.303	0.49 ± 0.01	0.29 ± 0.02	1.46 ± 0.09	1.61 ± 0.08	fruity, alcoholic, plastic, and pungent (Jensen et al., 2011)
3	Hexanal	44, 56, 41	802	3.959	13.7 ± 0.9	11.5 ± 0.2	23.0 ± 0.4	21.7 ± 1.8	fresh, green, and fatty (Barbarisi et al., 2019; Birch et al., 2013; Jensen et al., 2011; Pico et al., 2015)
4	Dihydro-2-methyl-3-furanone	43, 28, 72	808	4.183	0.19 ± 0.01	0.211 ± 0.003	–	–	spicy, rancid, and butter (Pico et al., 2015)
5	2-Methylpyrazine	94, 67, 40	822	4.707	6.4 ± 0.6	6.0 ± 0.5	–	–	Roasted (Barbarisi et al., 2019)
6	Furfural	96, 39, 29	830	5.016	19.9 ± 1.9	21.4 ± 0.9	0.91 ± 0.05	–	almond, toasted, and bread-like (Barbarisi et al., 2019; Jensen et al., 2011)
7	2-Furanmethanol	98, 41, 81	861	6.212	6.626 ± 0.001	8.9 ± 0.7	–	–	faint burning (Barbarisi et al., 2019)
8	1-Hexanol	56, 43,70	875	6.742	6.4 ± 0.5	5.2 ± 0.2	24.9 ± 1.7	23.3 ± 0.8	pungent (The Good Scents, 2022)
9	Styrene	104, 78, 51	890	7.287	6.76 ± 0.02	5.6 ± 0.5	2.28 ± 0.09	5.1 ± 0.3	sweet, balsamic, and floral (The Good Scents, 2022; Jensen et al., 2011)
10	2-Heptanone	43, 58, 71	892	7.391	0.21 ± 0.01	0.3 ± 0.2	–	–	soapy, fruity, and cinnamon (Pico et al., 2015)
11	Unknown	81, 53, 39	902	7.735	–	0.62 ± 0.05	–	–	–
12	Heptanal	44, 70, 55	905	7.814	3.61 ± 0.15	2.87 ± 0.04	5.0 ± 0.2	4.98 ± 0.03	fatty, green, rancid, citrus, and malty (Pico et al., 2015)
13	Methional	48, 104, 76	911	7.994	0.85 ± 0.16	0.74 ± 0.05	–	–	boiled-potato, cooked-potato, malty, and waxy (Pico et al., 2015)
14	2-Acetylfuran	95, 110, 39	913	8.077	1.46 ± 0.51	1.80 ± 0.15	–	–	smoky, roasty, yeasty, and fermented (Barbarisi et al., 2019; Pico et al., 2015)
15	2,5-Dimethylpyrazine	108, 42, 39	917	8.175	1.203 ± 0.001	1.47 ± 0.06	–	–	nutty, roasty, and woody (The Good Scents, 2022)
16	2-Ethylpyrazine	107, 108, 80	919	8.233	0.363 ± 0.004	0.45 ± 0.04	–	–	nutty, musty, coffee, and roasted (The Good Scents, 2022)
17	2,3-Dimethylpyrazine	67, 108, 40	922	8.344	–	0.30 ± 0.01	–	–	popcorn and roasted (Pico et al., 2015)
18	Camphene	98, 121, 79	935	9.193	–	tr	–	–	camphor, woody, and herbal (The Good Scents, 2022)
19	Benzaldehyde	77, 105, 51	951	9.654	0.49 ± 0.02	0.67 ± 0.05	–	–	almond and caramel (Pico et al., 2015)
20	Unknown	67, 95, 43	966	9.684	–	–	0.43 ± 0.02	0.76 ± 0.01	–
21	5-Methylfurfural	110, 53, 27	967	9.760	0.7931 ± 0.0005	2.6 ± 0.3	–	–	butter, caramel, and musty (Pico et al., 2015)
22	1-Heptanol	70, 56, 43	969	10.09	–	–	0.992 ± 0.002	0.98 ± 0.03	green (Pico et al., 2015)
23	β-Pinene	93, 41, 69	975	10.015	–	tr	tr	–	green, piney, and woody (The Good Scents, 2022; Miyazaki et al., 2012)
24	1-Octen-3-ol	57, 43, 72	980	10.326	0.96 ± 0.08	0.93 ± 0.01	1.19 ± 0.07	1.5 ± 0.1	mushroom, earthy, green, and herbal (Birch et al., 2013; Jensen et al., 2011)
25	2-Pentylfuran	81, 138, 53	978	10.491	4.04 ± 0.01	3.3 ± 0.2	3.3 ± 0.2	3.7 ± 0.2	butter, green bean, floral, fruity, mushroom, and raw nuts (Jensen et al., 2011)
26	2-Ethyl-5-methylpyrazine	121, 39, 56	988	10.748	0.43 ± 0.01	0.76 ± 0.03	–	–	baked (Pico et al., 2015)
27	2-Ethyl-3-methylpyrazine	121, 67, 39	994	10.853	0.20 ± 0.02	0.28 ± 0.02	–	–	Nutty, roasted, and sweet (Pico et al., 2015)
28	D-Limonene	68, 93, 136	1002	11.731	1.19 ± 0.08	0.95 ± 0.07	2.42 ± 0.07	2.32 ± 0.07	citrus, fresh, and sweet (The Good Scents, 2022)
29	Eucalytol	93, 68, 43	1005	11.770	0.70 ± 0.07	0.56 ± 0.04	1.03 ± 0.02	1.05 ± 0.06	green, herbal, and spicy (Caputi & Aprea, 2011; Miyazaki et al., 2012)
30	Benzene acetaldehyde	91, 120, 65	1026	12.238	5.07 ± 0.05	4.9 ± 0.3	–	–	fruity, honey, and sweet (Birch et al., 2013)
31	2-Octenal	41, 55, 70	1032	12.842	0.20 ± 0.01	0.148 ± 0.003	0.35 ± 0.01	0.38 ± 0.02	fatty, nutty, and roasted (Pico et al., 2015)
32	β-Linalool	71, 93, 55	1033	14.010	–	–	0.23 ± 0.02	0.243 ± 0.005	floral, lavender, citrus, woody, and green (The Good Scents, 2022; Miyazaki et al., 2012)

(*continued on next page*)

Table 4 (*continued*)

N°	Compound	MS/MS	LRI	RT (min)	Crust *t*0 (%, SD)	Crust *t*4 (%, SD)	Crumb *t*0 (%, SD)	Crumb *t*4 (%, SD)	Sensory description
33	Nonanal	57, 41, 98	1048	14.154	1.53 ± 0.14	1.63 ± 0.09	1.10 ± 0.04	1.9 ± 0.1	citrus, floral, fruity, and fatty (Jensen et al., 2011; Pico et al., 2015)
34	2-Nonenal	43, 55, 70	1063	15.451	1.37 ± 0.06	2.22 ± 0.01	0.82 ± 0.07	1.49 ± 0.08	beans, green, oil, and cucumber (Birch et al., 2013)
35	3-Methylundecane	57, 43, 71	1066	15.641	0.069 ± 0.004	0.37 ± 0.01	1.27 ± 0.02	0.37 ± 0.03	–
36	Dodecane	57, 43, 71	1102	16.249	0.42 ± 0.04	0.66 ± 0.04	0.59 ± 0.03	0.52 ± 0.01	–
37	Decanal	41, 55, 70	1109	16.367	0.20 ± 0.01	0.60 ± 0.01	–	0.42 ± 0.01	citrus (Pico et al., 2015)
38	Tetradecane	57, 43, 71	1150	17.985	0.71 ± 0.01	0.69 ± 0.07	0.56 ± 0.04	0.53 ± 0.02	mild and waxy (The Good Scents, 2022)
39	Pentadecane	57, 43, 71	1167	19.469	0.62 ± 0.06	0.95 ± 0.04	0.68 ± 0.05	0.74 ± 0.04	–
No identified					0.00	0.62	0.43	0.76	
Identified					100.00	99.38	99.57	99.24	

Compound	Calibration curve	R^2	LOD ($g \bullet L^{-1}$)	LOQ ($g \bullet L^{-1}$)	Mass ($\mu g_{compound} \bullet 100\ g_{bread}^{-1}$)			
					Crust *t*0	Crust *t*4	Crumb *t*0	Crumb *t*4
***D*- Limonene**	$y = 1.53 \times 10^9 x + 1.07 \times 10^7$	0.9997	1.80×10^{-3}	5.46×10^{-3}	0.61 ± 0.05[b]	0.58 ± 0.02[b]	1.52 ± 0.05[a]	1.48 ± 0.05[a]
Eucalyptol	$y = 1.09 \times 10^9 x + 1.66 \times 10^7$	0.9993	6.21×10^{-3}	1.88×10^{-2}	0.95 ± 0.02[b]	0.79 ± 0.01[c]	1.4 ± 0.1[a]	1.26 ± 0.02[a]

[a]LRI: linear retention indices calculated through Kovats retention index equation for series of alkanes C8-C40 using a cross bonded fused column in GC–MS. tr = traces. Averages with different letters in the same line indicate significant difference with α = 0.05.

the work reported in this paper.

Data availability

The data that has been used is confidential.

Acknowledgements

The authors are thankful for the samples provided by the Portuguese "Centro Nacional de Competências de Frutos Secos" and M. Ferreira & Filhas Lda.

Funding

This work was financially supported by LA/P/0045/2020 (ALiCE), UIDB/50020/2020 and UIDP/50020/2020 (LSRE-LCM), funded by national funds through FCT/MCTES (PIDDAC), within the scope of Project Mobilizador Norte-01-0247-FEDER-024479: ValorNatural®. The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES (PIDDAC) to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2021). L. Barros and R. Calhelha also thank the national funding by FCT, P.I. through the institutional scientific employment and individual program-contract, and Júlia Cristiê Kessler acknowledges her Ph.D. scholarship by FCT (2020.06656.BD).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.135845.

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