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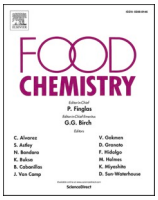

Chemical and organoleptic properties of bread enriched with *Rosmarinus officinalis* L.: The potential of natural extracts obtained through green extraction methodologies as food ingredients

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ABSTRACT

The potential of *R. officinalis* L. (RO) extracts as a source of aromas was accessed by hydrodistillation (HD) and supercritical fluid extraction using carbon dioxide (SFE-CO_2), followed by a series of analysis: quantification by GC-MS, sensory perception and description, and cytotoxicity against Vero cells. The extracts shown abundancy of α-pinene, eucalyptol, *S*-verbenone and camphor, contributing for the green, fresh, citric, and woody as main sensory notes. The odour threshold (ODT) value (less than 3.0×10^{-3} μg·mL^{-1}) and the cytotoxic potential (ca. 220 μg•mL^{-1}) defined the concentration range for food application. The most promising extract was added to bread doughs and the final volatile profile was characterised by GC-MS through HS-SPME over time. Among the 34 compounds found, furfural showed an evident contribution in the bread crust aroma, which persisted over four hours of storage, contributing to a pleasant bread fragrance according to the evaluators. This study aims to represent a stepping stone for the use of natural aromas as ingredients for the development of innovative food products.

1. Introduction

The flavour and fragrance industry has shown a significant increase in the demand for chemical aromas in recent years, reaching USD 5730 million in esters in 2018. The incorporation of natural ingredients, such as essential oils, is presented as a rising alternative, exotic and viable in the development of cosmetic, aromatherapeutic and pharmaceutical products. The sector continues to grow in popularity in the use of natural flavouring agents in food and wellness products (F&F, 2019).

Essential oils are the volatile apolar fraction obtained from plant materials, generally a complex mixture of compounds with varied properties, used to increase the added value of products, to extend their shelf life or to enhance their sensory quality (Borges et al., 2019). The incorporation of essential oils in costumer goods is approved by the European Commission and the US Food and Drug Administration (FDA) and is classified as "Generally Recognized as Safe" (GRAS) to use in edible commodities (Conde-Hernández et al., 2017; Gomes et al., 2007).

The extraction of essential oils is commonly performed by conventional methods such as hydrodistillation, steam distillation or using organic solvents (Borges et al., 2019; Conde-Hernández et al., 2017). Hydrodistillation (HD) allows the isolation of the extract up to its water solubility using a green solvent (Chemat et al., 2019). Nevertheless, this extraction method is responsible to promote chemical alterations and may have a negative impact on thermosensitive compounds (Pourmortazavi & Hajimirsadeghi, 2007), including the degradation of unsaturated or esterified molecules and hydrolytic effects (Okoh et al., 2010). In addition, the solubility in water of some molecules may affect and modify the perception of flavours (Chemat et al., 2019).

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Thus, to increase the selectivity and preserve the substances of interest, a suitable and greener technology has been reported for extraction of natural products, namely supercritical fluid extraction using carbon dioxide (SFE-CO_2) (Chemat et al., 2019). Safety, non-toxicity, non-carcinogenicity, non-flammability and selectivity of the extracted compound, are presented as some of the advantages of supercritical extraction of natural plant-based products (Conde-Hernández et al., 2017). In fact, the SFE-CO_2 technology appears on the top list of emerging green methodologies (Chemat et al., 2019).

SFE-CO_2 is adaptable to different separation processes, since modifying the pressure and temperature of the extraction system, the density of the carbon dioxide vapour changes, promoting higher selectivity of the target volatile substances. Furthermore, the use of relatively low temperatures during the extraction favours the achievement of thermal unstable and oxidative compounds (Machado et al., 2013). Fresh odour and flavour characteristics provided by SFE-CO_2 extracts, lower energy cost and the assurance of high-quality products are other advantages mentioned when compared with conventional techniques for extraction of essential oils (Gomes et al., 2007).

Using green extraction methodologies to obtain essential oil from *Rosmarinus officinalis* L. (RO) is promising to achieve products with high added value. RO is native from the Mediterranean basin and belongs to the Lamiaceae family (Okoh et al., 2010). The plant potential is widely recognised due to its antioxidant activity resulting from the high concentrations of phenolic compounds (Fornari et al., 2012) and also to its rich composition in aroma compounds. The main volatile compounds group listed in the literature are monoterpenoids, sesquiterpenoids, carbonyl, ketones, acids, phenols, ethers, aldehydes and alcohols chemical groups (Perestrelo et al., 2016).

A singular dependence of the sensory characteristics is noted according to its odour active compounds, usually analysed by gas chromatography-mass spectrometry (GC-MS) and sensory evaluations, or gas chromatography-olfactometer (GC-O) (Miyazaki et al., 2012). Kamath et al. (Kamath et al., 2001) carried out a qualitative descriptive analysis (QDA) and odour detection threshold (ODT) with selected essential oils and evaluated their volatile composition. The nature and quality of these substances obtained from *R. officinalis* and their respective concentration depends on the agricultural conditions of cultivation (Carvalho et al., 2005; Conde-Hernández et al., 2017), extraction parameters and sample pre-treatment (Pourmortazavi & Hajimirsadeghi, 2007).

Concerning food applications, a critical evaluation of the essential oils' chemical composition and their respective safe limits is necessary. Thus, several studies have been dedicated to HD and SFE-CO_2 extraction, including the process optimization to improve the bioactive compounds composition (Carvalho et al., 2005; Conde-Hernández et al., 2017; Fornari et al., 2012; Ibáñez et al., 1999).

Focusing on the aroma profile of fresh and dehydrated plant material of RO from northern Portugal, this work brings an innovative proposal: to improve the odour of bread dough through the incorporation of natural volatile compounds. To achieve the final goal, HD and SFE-CO_2 techniques were applied and compared regarding their final extraction yields, extract chemical composition and sensorial attributes, as well as cytotoxic potential. The best overall extract was studied as a flavouring agent by means of its aromatic properties. The sensorial analyses were performed with a discerning panel to appoint the intensity of natural extracts and their ODT values, and relationship between the major compounds and the sensory descriptive terms used, namely the "aroma map", was obtained. Cytotoxic evaluation defined a safe concentration range to incorporate the extracts into a food matrix.

The most promising extract was used to increase the bread dough natural flavour. Characterisation procedures without and with the added extract were performed to the odour provided from bread crusts and crumbs, and their respective sensory perception was also evaluated.

The aim of this work is to develop olfactory marketing strategies using natural extracts and bread as a case study.

2. Materials and methods

2.1. Chemicals

The analytical standard α-pinene (CAS 80-56-8, 98%), series of alkanes C8-C40 (ref. 40147-U) and *R. officinalis* commercial standard (CAS 8000-25-7, FG) were obtained from Sigma Aldrich (Madrid, Spain), while verbenone (CAS 1196-01-6, 99%) and *n*-hexane (CAS 110-54-3, 99%) were purchased from Supelco (Madrid, Spain). Eucalyptol (CAS 470-82-6, 99%) and camphor (CAS 76-22-2, 96%) were 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. Plant material

R. officinalis samples were collected in Póvoa de Lanhoso, Braga, Portugal, in May 2020. Leaves and flowers were separated from the branches and the aerial part was dried until a constant weight using an air circulation oven (Venticell, MMM Medcenter, Germany) at 40 °C, reaching a water content value of 63.45 ± 0.61% (w/w).

2.3. Extraction methodologies

2.3.1. Extraction by hydrodistillation

The plant material (75 g, fresh or dehydrated) was submitted to hydrodistillation extraction for 3 h, as suggested by the European Pharmacopoeia (COE, 2007). Then, the two volatile distillate phases were collected and separated to isolate the resulting essential oils, which were stored at 4 °C until further analysis.

2.3.2. Extraction by supercritical CO_2

R. officinalis samples (30 g) were subjected to SFE-CO_2 extraction at 80 bar, 50 °C for 2 h. The extractions were performed in a previously developed pilot-scale equipment (Gomes et al., 2007). The extraction cell is made of stainless steel with a capacity of 1 L and designed to work up to 200 bar, the ideal pressure range for most volatile compounds from plants (Gomes et al., 2007). The depressurization valves were maintained at 40 and 6 bar, respectively, to release the aromatic extracts. The separator was set at −15 °C to avoid the compounds volatilization. All extraction procedures were carried out in static mode, with a single CO_2 feed, and the extracts were kept at 4 °C until analysis.

2.4. Chemical and sensorial characterisation of R. officinalis extracts

2.4.1. Characterisation of R. officinalis extracts by GC-MS

The chemical profile of *R. officinalis* extracts was performed by GC-MS (TQ8040 NX Triple Quadrupole, Shimadzu, Japan), 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 oven temperature was programmed isothermal 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}. The injector was set at 290 °C with 1 μL of the sample volume and, the ultrapure helium flowrate was set at 1 mL·min^{-1}. The analysis was carried out with ion and interface temperature at 250 °C and 260 °C, respectively, with the mass scanning range maintained at *m/z* 40–500. All samples were diluted in *n*-hexane (GC grade) and their composition was expressed in percentage values calculated through the GC peak areas for each compound identified. 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 alkanes (C8-C40) were analysed

under the same chromatographic conditions, and the homologous series was used to calculate the linear retention index values, which were compared with these reported in the literature. Finally, the selected molecules were quantified through calibration curves of each correspondent analytical standard.

2.4.2. Sensory odour evaluation of R. officinalis extracts

The resulting extracts of *R. officinalis* were evaluated by a panel of 12 panellists (10 females), with ages from 20 to 37 years old, who performed their evaluations individually. All panellists received a brief training under International Standard ISO 8586:2012 instructions (International Organization for Standardization 8586:2012, 2012). The main conditions and experimental procedures were introduced, and preliminary tests were performed to select the most accurate panellists (Kessler et al., 2022). 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.

Sensory analysis was performed by assessing two different parameters: 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).

2.4.3. Odour detection threshold (ODT)

For each extracted sample, a series of sequential water dilutions were prepared in a concentration range of 1.0×10^{-5} to 1.0×10^{-2} μg·mL^{-1} and presented for individual assessment. The sample solution odour was sniffed from polypropylene flasks covered with cotton. The evaluation was concluded when a minimal perception of the odour - the ODT - was detected by each individual. Finally, the results were grouped and the ODT values were calculated by linear interpolation and obtained by the concentration for which at least 50% of the evaluators were stimulated.

2.4.4. 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. Suitable terms for the main individual components (descriptors) were chosen from the literature (Kamath et al., 2001; Miyazaki et al., 2012), and a scorecard was developed through a preliminary session to discuss the aroma properties of RO extracts. The scorecard comprised an unstructured scale, where 0 represented "low intensity" and 9 corresponded to "high intensity". The perception assessment descriptors were woody, green, fresh, citrus, floral, sweet, spicy, fruity or oily. The number of descriptors used for each extract was at the discretion of the evaluator and the average score answers were considered in the results interpretation.

2.4.5. 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). Then, the sulforhodamine B assay was performed according to a previously established protocol (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 using triplicates.

2.5. Chemical and organoleptic properties of bread enriched with Rosmarinus officinalis L.

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 reception, the dough was frozen and stored at −20 °C until further analysis. For each assay, dough was defrosted overnight at 4 °C, sliced into 100 g pieces and baked at 240 °C for 15 min in a convection oven model (2000 W, O30-B Moulinex series). For the incorporated samples, the extracts were directly added to a known amount of dough to obtain the desired final concentration on each batch of bread (the concentrations applied will be detailed in section 3.2.) and mixed in a food processor (Thermomix® TM5) for 20 s. After cooling at room temperature for 30 min, a portion of the sample was immediately prepared for analysis (*t*0) and another one was kept in paper bags for four hours (*t*4). Samples of both bread crust and crumb were carefully separated, frozen with liquid nitrogen and grinded (Hr7762/90 Mini Chopper, Philips Walita) to obtain a particle size of > 1 mm. Each sample was prepared in triplicate.

2.5.2. Characterisation of bread aroma by GC-MS-SPME

About 1 g (±5 mg) of each sample was transferred into a 20 mL headspace vial and sealed with an aluminium cap with PTFE septum. Following the procedure presented by (Pico et al., 2015), samples were incubated at 50 °C for 5 min for temperature stabilization, prior to the SPME fibre (50/30 μm, 2 cm of divinylbenzene-carboxen-polydimethylsiloxane - DVB/CAR/PDMS) exposition for 30 min. The same procedure was applied to the stored bread (t4) in duplicate at the same day for three days (2 × 3).

After the sampling time, the fibre was removed and coupled into GC-MS (TQ8040 NX Triple Quadrupole, Shimadzu, Japan) for desorption of the volatiles in split injection mode. The injector was kept at 270 °C, split ratio at 2 and high-pressure injection at 200 kPa for 0.50 min.

To promote ion separation, the following temperature program was used: 40 °C isothermal for 6 min, then increased to 75 °C at 8.5 °C·min^{-1} for 2 min, from 75 to 150 °C at 10 °C·min^{-1} for 2 min, and reached 270 °C at 15 °C·min^{-1} for 3 min. The ion and interface temperature were set at 230 °C and 270 °C, respectively. Furthermore, the scan mode operated in a range of 20–500 *m/z*, ultrapure helium flow rate was set at 6.3 mL·min^{-1} and linear velocity flow control mode was applied. The injection volume was set at 1 μL.

The volatile composition was expressed in percentage values and their identification was performed as described in Section 2.4.1. Finally, the most abundant aromas of extract (*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 aroma

Following the International Standard ISO 8586:2012 instructions (International Organization for Standardization 8586:2012, 2012), 19 panellists (18 female, ages from 20 to 43 years old) were chosen to apply the sensory evaluation of bread odour. The sensory profile of bread crumb and crust was evaluated by a Multiple Comparison Test (MCT). In this evaluation, panellists were questioned about the odour deviation of unknown samples in comparison to a reference one (relative-to-reference rating), as suggested by the ISO 13299:2016 (International Organization for Standardization 13299:2016, 2016). Four concentrations of the *R. officinalis* extract were used: 0.0, 0.5, 1.0 and 2.0 g/ 100 g of bread. Random codified samples were introduced to the panellists for comparison against the control bread (without extract incorporation). Each panel member pointed out a description based on a structured scale from 1 meaning "much more intense odour of bread than control", to 5 meaning "much less intense odour of bread than control". Similarly, the presence of a distinct/unusual odour besides the bread odour was expressed though a second scale from 1 – "distinct odour much more intense than control" up to 5 – "distinct odour much less intense than

control" for both crust and crumb. The result was based on the average value indicated by the panel evaluators.

2.6. Statistical analysis

The significant differences between the extraction yields, the main compounds and the sensory odour analysis were evaluated by applying ANOVA and Tukey tests, with $\alpha = 0.05$ (significance level). To explain the relationship between chemical composition and sensory descriptions of the extracted products, the "odour map" was performed by the Principal Component Analysis (PCA) procedure based on the generalized inverse (Statistica StatSoft, version 12, USA) after data range standardizing.

The extraction yield is obtained by:

$$Yield(\%) = \left[\frac{Essentialoil(\mathrm{g})}{Sampleweight(DW)(\mathrm{g})}\right] \times 100 \quad (1)$$

3. Results and discussion

3.1. Chemical composition of R. officinalis extracts

Fresh and dried *R. officinalis* plant samples were submitted to HD and SFE-CO_2 extraction methods. The extraction yield results are shown in Table 1 where the values are expressed on a dry weight (DW) basis. The extraction yields of both fresh and dried essential oils, obtained by HD were statistically significantly lower than those obtained by SFE-CO_2. In fact, the fresh samples of *R. officinalis* plant presented extraction efficiencies of 0.75 ± 0.01% for EO-HD against 3.3 ± 0.3% for EX-SFE-CO_2, while dried samples resulted in 0.60 ± 0.04% and 3.03 ± 0.06% extraction yields, respectively. Conde-Hernández et al. (Conde-Hernández et al., 2017) gave lower values for both extraction methods from fresh RO, achieving 0.35% in HD extraction and less than 2.0% in supercritical fluid extraction using 174 bar and 40 °C. In contrast, Carvalho et al. (Carvalho et al., 2005), using pressures between 100 and 300 bar and temperature between 30 and 40 °C, achieved SFE-CO_2 extractions yielded up to 5.0%, while using the HD method the higher yield was 1.8%.

These results clarify the differences between the extractions

Table 1
Volatile composition of essential oil and extract of the *R. officinalis* material plant, obtained by hydrodistillation and supercritical fluid extraction, respectively.

n°	Compounds	RT (min)	LRI	Base peak	Fresh EO-HD (%)	Dried EO-HD (%)	Fresh EX-SFE-CO_2 (%)	Dried EX-SFE-CO_2 (%)
1	Tricyclene	5.913	922	93, 41, 91	0.058 ± 0.004	0.36 ± 0.02	0.14 ± 0.02	0.225 ± 0.003
2	α-Thujene	5.988	926	93, 77, 91	0.11 ± 0.01	0.068 ± 0.004	-	-
3	α-Pinene	6.140	933	93, 92, 91	42.8 ± 0.3	33.5 ± 0.2	29 ± 9	38 ± 3
4	Camphene	6.480	949	93, 121, 79	2.40 ± 0.03	2.93 ± 0.04	3.8 ± 0.6	5.9 ± 0.7
5	Linalool oxide	6.906	969	43, 69, 68	1.6 ± 0.2	1.494 ± 0.001	-	-
6	β-Pinene	7.067	977	93, 41, 69	2.0 ± 0.2	0.73 ± 0.02	3.4 ± 0.5	2.2 ± 0.4
7	β-Myrcene	7.313	988	41, 93, 69	2.80 ± 0.01	4.4 ± 0.8	5 ± 1	3.7 ± 0.3
8	α-Phellandrene	7.681	906	93, 91, 77	-	0.200 ± 0.002	-	-
9	3-Carene	7.908	1016	93, 121, 136	0.39 ± 0.01	0.7 ± 0.1	0.4 ± 0.2	0.6 ± 0.1
10	*p*-Cymol	8.067	1023	119, 134, 91	0.72 ± 0.04	1.03 ± 0.03	1.5 ± 0.4	1.5 ± 0.2
11	D-Limonene	8.180	1029	68, 93, 67	23 ± 9	10.123 ± 0.005	2.7 ± 0.8	2.7 ± 0.3
12	Eucalyptol	8.239	1031	43, 81, 108	5.4 ± 0.6	16.6 ± 0.2	31 ± 6	21 ± 2
13	Ocimene quintoxide	8.506	1044	139, 43, 55	0.5 ± 0.2	0.19 ± 0.01	-	-
14	γ-Terpinene	8.809	1058	93, 91, 136	0.95 + 0.08	1.4 ± 0.2	1.3 ± 0.1	1.20 ± 0.04
15	β-Terpineol	9.062	1070	71, 43, 93	0.82 + 0.06	0.68 ± 0.05	0.3 ± 0.2	-
16	Terpinolene	9.414	1086	93, 121, 91	1.18 ± 0.05	1.04 ± 0.04	0.5 ± 0.2	0.33 ± 0.03
17	β-Linalool	9.695	1099	71, 93, 55	11.3 + 3.9	7.9 ± 0.2	0.83 ± 0.01	0.40 ± 0.01
18	Camphor	10.719	1047	95, 41, 81	1.1 ± 0.1	6 ± 1	7.7 ± 0.4	8.5 ± 0.6
19	Borneol	11.263	1172	95, 110, 41	0.13 ± 0.04	1.4 ± 0.2	0.9 ± 0.3	1.7 ± 0.4
20	Pinocamphone	11.340	1175	55, 83, 41	0.19 ± 0.06	-	0.73 ± 0.05	0.50 ± 0.05
21	4-Terpineol	11.444	1180	71, 111, 43	tr	0.84 ± 0.08	0.3 ± 0.2	0.52 ± 0.01
22	α-Terpineol	11.743	1194	59, 93, 121	0.6 ± 0.2	1.49 ± 0.01	0.4 ± 0.1	0.90 ± 0.02
23	Isobornyl formate	11.912	1102	95, 93, 41	tr	-	-	-
24	*S*-Verbenone	11.993	1206	107, 91, 135	0.31 ± 0.06	3.6 ± 0.3	7 ± 2	4 ± 1
25	Bornyl acetate	13.574	1284	95, 43, 93	1.38 ± 0.07	3.3 ± 0.6	2.1 ± 0.3	4.4 ± 0.9
26	Ylangene	15.265	1371	105, 93, 120	-	-	-	0.26 ± 0.03
27	Isoeugenol methyl ether	15.760	1397	178, 163, 107	tr	-	-	-
28	β-Caryophyllene	16.220	1422	93, 133, 41	0.21 ± 0.02	0.15 ± 0.04	0.7 ± 0.2	1.1 ± 0.3
29	Caryophylene oxide	19.088	1585	79, 43, 69	0.10 ± 0.03	-	-	-
30	Methyl dihydrojasmonate	20.109	1624	83, 153, 55	-	0.60 ± 0.05	-	-
Identified total					**100.00**	**100.00**	**100.00**	**100.00**
Extraction yield (%, DW)					0.75 ± 0.01[a]	0.60 ± 0.04[a]	3.3 ± 0.3[b]	3.03 ± 0.06[b]

Compound	Calibration curve	R^2	LOD (g•L^{-1})	LOQ (g•L^{-1})	Mass (ugcompound/gplant (DW)) Fresh EO-HD	Dried EO-HD	Fresh EX-SFE-CO_2	Dried EX-SFE-CO_2
α-Pinene	$y = 1.02 \times 10^{10}x - 2.16 \times 10^{6}$	0.9987	8.73×10^{-4}	2.64×10^{-3}	5699 ± 33[a]	2550 ± 17[b]	87 ± 28[c]	89 ± 7[c]
Camphor	$y = 9.03 \times 10^{9}x - 3.05 \times 10^{6}$	0.9974	1.11×10^{-3}	3.37×10^{-3}	407 ± 50[b]	565 ± 96[a]	44 ± 2[c]	39 ± 3[c]
Eucalyptol	$y = 1.05 \times 10^{10}x - 4.86 \times 10^{6}$	0.9972	1.29×10^{-3}	3.91×10^{-3}	1028 ± 107[b]	1343 ± 18[a]	108 ± 21[c]	69 ± 7[c]
γ-Terpinene	$y = 7.75 \times 10^{9}x - 4.89 \times 10^{6}$	0.9966	1.44×10^{-3}	4.35×10^{-3}	635 ± 54[a]	470 ± 52[b]	45 ± 4[c]	41 ± 1[c]
***p*-Cymol**	$y = 7.13 \times 10^{9}x - 2.66 \times 10^{6}$	0.9997	4.05×10^{-4}	1.23×10^{-3}	415 ± 23[a]	321 ± 9[a]	30 ± 8[b]	27 ± 3[b]
***S*-Verbenone**	$y = 7.60 \times 10^{9}x - 6.96 \times 10^{6}$	0.9946	1.80×10^{-3}	5.47×10^{-3}	740 ± 144[b]	810 ± 66[a]	83 ± 30[c]	67 ± 20[c]
Total					**8924**	**6059**	**397**	**332**

RT: retention time, 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, EO-HD: essential oil obtained by hydrodistillation, EX-SFE-CO_2: extract obtained by carbon dioxide supercritical fluid extraction, SD: standard deviation, tr: traces, LOD: limit of detection, LOQ: limit of quantification.

methods. Regarding hydrodistillation, an accelerated process of rearrangement reactions is promoted by using the aqueous medium, a polar solvent, and alteration of essential oil compounds may be a consequence of the combination of temperature and pH, which can lead to losses of the volatile portion (Okoh et al., 2010). On the other hand, supercritical CO_2 is a relatively non-polar solvent capable to extract non-polar compounds and a few polar volatile substances with low molecular weight. The variation of supercritical fluids thermodynamic properties influences, mass transfer between the solute to extract and the CO_2 and, consequently, in the selectivity of the target compounds (Chemat et al., 2017).

Concerning the extracts composition (Table 1), GC-MS analysis revealed α-pinene as the prevailing molecule, above 29% relative concentration for all samples, among 30 other substances (Fig. S1, Supplementary Information). The EO-HD showed α-pinene concentration at 5699 $\mu g \cdot g^{-1}$ and 2550 $\mu g \cdot g^{-1}$ for fresh and dried plant material, respectively. Also, the eucalyptol and *S*-Verbenone molecules were found with high contribution in both samples. Eucalyptol achieved 1028 $\mu g \cdot g^{-1}$ in fresh EO-HD and 1343 $\mu g \cdot g^{-1}$ in dried EO-HD, while verbenone presented 740 $\mu g \cdot g^{-1}$ and 810 $\mu g \cdot g^{-1}$ for fresh and dried *R. officinalis* essential oils, respectively. The mass concentration in the extracts obtained by SFE-CO_2 were lower than those obtained by HD and showed similar values regardless of the hydration state of the raw material: SFE-CO_2 extracts values were 397 $\mu g \cdot g^{-1}$ and 330 $\mu g \cdot g^{-1}$ and HD extractions achieved 8924 $\mu g \cdot g^{-1}$ and 6059 $\mu g \cdot g^{-1}$, for fresh and dried samples, respectively.

Previous works pointed out camphor, eucalyptol, verbenone, borneol and α-pinene as the main compounds provided from *R. officinalis* herb extracted by SFE-CO_2 (Ibáñez et al., 1999) and hydrodistillation processes (Boutekedjiret et al., 2003; Elyemni et al., 2019; Jamshidi et al., 2009; Mena et al., 2016). Thus, despite the effect of the extraction method, pre-treatment, nature and environmental conditions of the plant material production, similar chemical composition was found.

3.2. Study of the concentration range to be incorporated in food samples

The range of extract concentrations to be added in bread was defined by two main factors: the odour perception of the volatile oils and their cytotoxic potential (Table 2). In this context, the odour threshold measurement was applied to the *R. officinalis* products. Within a concentration range of 1.0×10^{-5} to 1.0×10^{-2} $\mu g \cdot mL^{-1}$, the panel members defined the minimal value of the odour perception. The products obtained from the dried plant material showed higher concentration for both extraction methods, 3.0×10^{-3} $\mu g \cdot mL^{-1}$ and 2.3×10^{-4} $\mu g \cdot mL^{-1}$ in SFE-CO_2 and HD, respectively. Regarding the fresh samples, the judges asserted values of 3.0×10^{-4} $\mu g \cdot mL^{-1}$ for EX-SFE-CO_2 and 4.0×10^{-5} $\mu g \cdot mL^{-1}$ for OE-HD, the smallest ODT achieved. No significant difference was found between samples ($\alpha = 0.05$).

Fig. 1 shows the aromatic profile of the four extracts obtained by the average of the scored notes. Green, fresh and citric attributes were the most pronounced, especially for the dried samples. Fresh EX-SFE-CO_2 was described also as spicy, sweet and floral, at a similar score. Oily odour sensory perception was mentioned only for fresh essential oil obtained by HD. Previously, rosemary sensory profile was reported with camphoraceous, herbal, citrus, spicy and woody notes (Kamath et al., 2001).

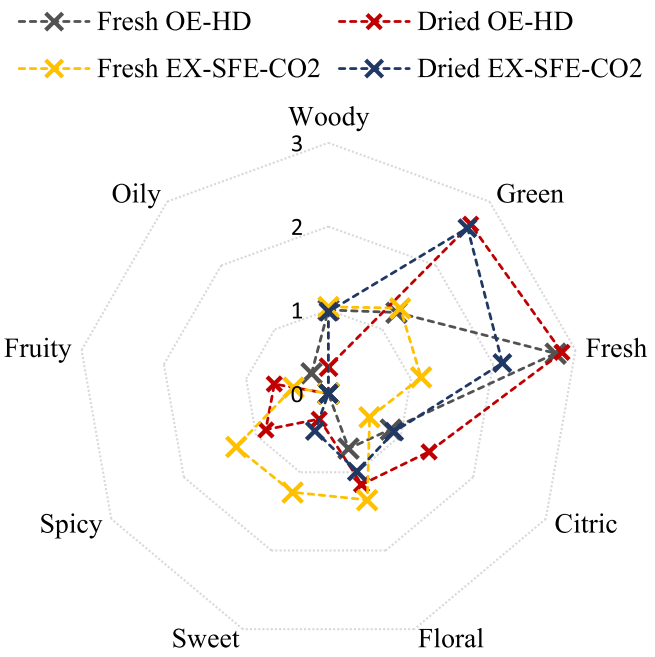

Fig. 1. Sensory profile of *R. officinalis* products achieved by Quantitative Descriptive Analysis (QDA) method.

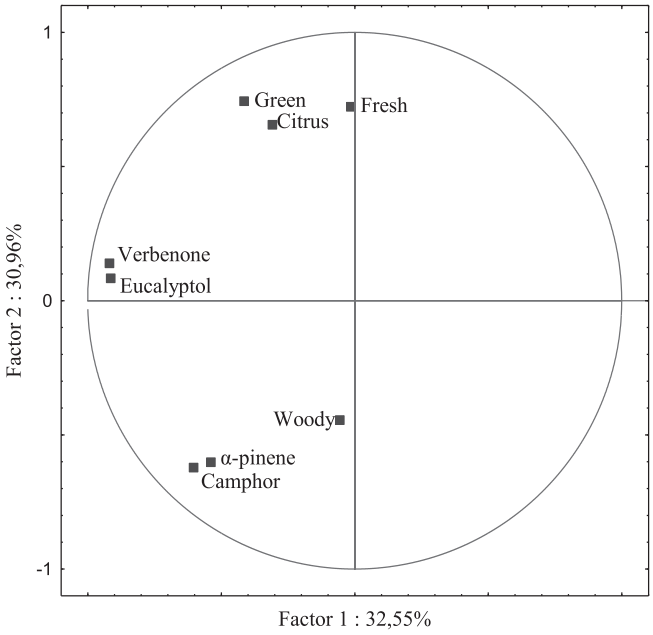

Fig. 2. Odour map of the *R. officinalis* products, according to the volatile fraction and sensory descriptors.

PCA model was used to clarify the contributes of main volatile compounds and the key odour attributes of the *R. officinalis*, namely the "odour map". Fig. 2 plots Factor 1 versus Factor 2, which explains 63.51% of the correlation between the volatile molecules identified in

Table 2
Safe concentration ranges of the *R. officinalis* L. products, defined by the cytotoxic GI_{50} measurements and ODT values.

Test	Fresh EO-HD	Dried EO-HD	Fresh EX-SFE-CO_2	Dried EX-SFE-CO_2
GI_{50} ($\mu g \cdot mL^{-1}$)	263 ± 17^{a}	219 ± 3^{a}	248 ± 23^{a}	227 ± 10^{a}
ODT value ($\mu g \cdot mL^{-1}$)	$4.0 \times 10^{-5\ a}$	$2.3 \times 10^{-4\ a}$	$3.0 \times 10^{-4\ a}$	$3.0 \times 10^{-3\ a}$

*Averages with different letters in the same line indicate significant difference with $\alpha = 0.05$.
EO-HD: essential oil obtained by hydrodistillation, EX-SFE-CO_2: extract obtained by carbon dioxide supercritical fluid extraction.

the extracts and the attributed sensory descriptors. Eucalyptol and *S*-verbenone molecules were correlated with citrus (fresh and light odour), green (typical botanical note with fresh odour) and fresh (clean, refreshing, and new odour) sensory notes. Previous works have described eucalyptol odour as green, herbal, and spicy, and *S*-verbenone as a camphoraceous type with herbal and citrus notes (The Good Scents Company, 2021; Miyazaki et al., 2012). According to the panellists, *α*-pinene and the camphor compounds, as well as the woody characteristic, are negatively correlated in relationship to other compounds (*e.g.*, *S*-verbenone and eucalyptol). These compounds revelled no correlation in relation to the fresh note. Pine earthy, turpentine, fresh, sweet and woody were the odour attributes used for α-pinene, while camphoraceous and herbal described the camphor molecule (The Good Scents Company, 2021; Xiao et al., 2016a; Xiao et al., 2016b).

This behaviour is affected by the mixture of compounds itself, mainly due to the ODT volatile impact and not necessarily due to the relative concentration contribution (Münch & Schieberle, 1998). The effect of fragrance intensity and their sensory perception is a combination of the vapour pressure, molar weight and molar composition of each molecule component, among other physicochemical properties, namely vapour-liquid equilibrium (Teixeira et al., 2011).

The cytotoxic potential of the extracts was estimated by measuring the inhibition of Vero cells proliferation at 50% (GI_{50}). Table 2 summarizes the results; no statistically significant differences were observed between samples. The GI_{50} values were 248 ± 23 μg•mL^{-1} (EX-SFE-CO_2) and 263 ± 17 μg•mL^{-1} (EO-HD) for fresh *R. officinalis* extracts. Similar results were obtained for the dried product: 227 ± 3 μg•mL^{-1} and 219 ± 3 μg•mL^{-1}, respectively to SFE-CO_2 and HD methods. According to the cytotoxic test, all extracts showed safe potential to ensure edible products.

3.3. Sensory evaluation of incorporated samples of bread and chemical characterisation of bread crumb and crust

The potential of *R. officinalis* extracts as aromatic ingredients was evaluated by incorporation in bread dough samples, as bread represents one of the simplest matrix and most consumed food in the world (Pico et al., 2015). Therefore, as a case study, EX-SFE-CO_2 from dried samples of *R. officinalis* was selected due to its overall profile regarding its odour characteristics, cytotoxicity, as well as being obtained through an emerging greenest technique of extraction. Furthermore, extracts from dried samples were preferred aiming to act nearly to industrial preferences.

Preliminary sensory tests (data not shown) indicated that, to be perceived in bread, it is necessary to increase the concentration of aroma about four orders of magnitude compared to the ODT previously determined in water. Therefore, the extract concentration range tested was 2.0 to 14.0 μg·mL^{-1} and the results regarding the MCT analysis are presented in Table S1 (Supplementary Information). No significant differences were noted in average scores ($\alpha = 0.05$) but, at concentrations of 6.0 μg·mL^{-1} a higher odour note was reported for all questions (**Table S1**). Consequently, according to the sensory evaluators, both bread crust and crumb presented the most intense and distinct odour comparing to the control bread.

The human olfactory system allows a good sensory perception and a great ability to discriminate the odorant compounds. Differences about 7% can be identified, even at low concentrations (Cain, 1977). However, in this work no statistical differences (at 95% of confidence) were found, even though olfactory detection may be influenced by physiological conditions, such as sex, age, and experience regarding the sample and evaluation methodology (Sela & Sobel, 2010).

Table 3 shows the chemical composition of bread crust and crumb samples in two different timeframes (Fig. S2, Supplementary Information). Time *t*0 was set at 30 min after baking, allowing the bread samples to cool. Aiming to reproduce the time of exposure of bread in a store, other bread samples were kept in paper bags for 4 h more (time *t*4). The samples were then prepared for the HS-SMPE-GC-MS analysis.

The relative abundance of each detected molecule is summarized in Table 3. In general, crust samples presented a greater complexity in terms of its composition in volatiles compared to crumb samples (higher number of molecules; Table 3).

It is challenging to discuss the set of results related to the volatile composition of the analysed samples. Even so, some conclusions can be drawn from the analysis of Table 3. Regarding the molecules typically present in bread samples (Pico et al., 2015), furfural (6) tends to disappear with time, even for samples with incorporated extract. On the other hand, dihydro-2-methyl-3-furanone (4) only appears in bread crust samples with incorporated extract, and similarly for styrene (9) and 2-pentylfuran (21) in crumb samples.

The identification of additional molecules in the final product, namely the furans (dihydro-2-methyl-3-furanone and 2-pentylfuran) and aldehydes (furfural) could be the key for revealing the potential that the incorporation of EX-SFE-CO_2 aroma extracts in food products may represent as their pleasant effect on the odour of bread is recognized (Budryn et al., 2016).

In addition, laboratory tests indicate that (except for furfural) these pleasant aromas linger over time (at least 4 h; Table 3). Thus, the incorporation of natural ingredients, such as EX-SFE-CO_2, can represent an asset in the durability of organoleptic properties (odour) of food matrices (such as bread), as indicated by preliminary sensory evaluation studies (Table S1, Supplementary Information).

Aroma molecules from the incorporated extracts were also identified. Through incorporation of EX-SFE-CO_2 it was possible to detect α-pinene, camphene, sabinene, β-pinene, *p*-cymol, D-limonene, eucalyptol, γ-terpinene, and camphor, both in crust and crumb samples. A significant decrease is observed from *t*0 to *t*4 (Fig. S2, Supplementary Information).

The most abundant volatiles are D-limonene and eucalyptol, and for this reason, they were quantified (Table 3, $r2 > 0.999$). The bread crust volatiles showed higher concentrations for both molecules at *t*0, as well as higher decrease rate after storage time (*t*4). Eucalyptol ranged 2.68 to 0.45 μg/100 g, while D-limonene reduced of 0.68 to 0.10 μg/100 g. The bread crumb presented a similar behaviour, but with lower values overall.

Previous studies (Barbarisi et al., 2019; Pico et al., 2018) highlighted that the volatile compounds of baked bread are derived especially from the Maillard reaction, caramelisation, and thermal degradation process. Initially, the main furan and pyrazine groups are formed due to the Maillard non-enzymatic reaction between amino acids and reducing sugars, at a temperature range of 110 to 150 °C. At 150 to 200 °C, the caramelisation of sugars produces the carbonyl compounds and some furan molecules. Finally, the thermal degradation of both sugars and amino acids occurs around 220 °C, which is the main responsible for the formation of aldehyde compounds. All reactions are most evident on the surface of the bread, this is because the crumb temperature reach around 100 °C and their volatile compounds are provided from fermentation, lipids oxidation and enzymatic reactions, for example, or even an odour diffusion by the crust (Pico et al., 2015).

Commonly, aldehyde (3, 9, 10, 11, 27, 30, 32), as well as furan (4, 6, 7, 12, 17, 21) and pyrazine (5, 22) molecules families are recognized as the pleasant odour in baked bread. In contrast, alcohol (1, 2, 20) and acids (8) chemical groups show less pleasant volatile sensory characteristics (Budryn et al., 2016). Esters, ethers, ketones, lactones, and sulphur compounds also play a key role in the originate odour of the bread (Pico et al., 2015).

The present work presents a positive correlation between sensory evaluations and the increase in the relative concentration of the target compounds, combined with the decrease in isopentyl alcohol. These effects remain with the addiction of AD extract, which promoted a favourable intensification of the bread odour compared to the control sample. In fact, despite the decrease in extract aroma compounds, the intensity of key aromas belonging to the bread profile such as peaks 3

Table 3
Chemical volatile fraction of bread crust and crumb, without and with dried *R. officinalis* extract (AD) incorporated, obtained by SFE-CO_2.

n°	Compound	LRI	RT (min)	MS/MS	Sensory description	Bread crust (control)		Bread crust (AD-SFE-CO_2)		Bread crumb (control)		Bread crumb (AD-SFE-CO_2)	
						t0	t4	t0	t4	t0	t4	t0	t4
						(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
1	Isopentyl alcohol	735	2.672	55, 42, 70	Alcoholic and fermented (The Good Scents Company, 2021)	14.1 ± 1.4	15.7 ± 0.1	8.52 ± 0.43	13.0 ± 0.1	51.83 ± 0.01	50.59 ± 0.07	23.3 ± 0.8	29.4 ± 0.6
2	1-Pentanol	769	3.303	42, 55, 70	fruity, alcoholic, plastic and pungent (Jensen et al., 2011)	0.49 ± 0.01	0.41 ± 0.01	0.35 ± 0	0.67 ± 0.01	1.41 ± 0.02	1.70 ± 0.03	2.2 ± 0.1	1.87 ± 0.05
3	Hexanal	802	3.959	44, 56, 41	fresh, green and fatty(Barbarisi et al., 2019; Jensen et al., 2011; Kirchhoff & Schieberle, 2001; Pico et al., 2015)	13.5 ± 0.7	13.30 ± 0.02	11.5 ± 0.4	16.1 ± 0.5	18.67 ± 0.02	19.68 ± 0.01	24.1 ± 0.1	22.5 ± 0.6
4	Dihydro-2-methyl-3-furanone	808	4.183	43, 28, 72	spicy, rancid and butter(Pico et al., 2015)	–	–	0.23 ± 0	0.13 ± 0	–	–	– –	– –
5	2- Methylpyrazine	822	4.707	94, 67, 40	roasted(Barbarisi et al., 2019)	5.68 ± 0.06	6.4 ± 0.2	8.8 ± 0.6	5.93 ± 0.08	–	–	– –	– –
6	Furfural	830	5.016	96, 39, 29	almond, toasted and bread-like(Barbarisi et al., 2019; Jensen et al., 2011)	25.6 ± 0.2	25.3 ± 0.7	28.6 ± 1.1	25.2 ± 0.4	2.51 ± 0.01	–	2.6 ± 0.2	– –
7	2-Furanmethanol	861	6.212	98, 41, 81	faint burning(Barbarisi et al., 2019; Budryn et al., 2016)	5.5 ± 0.1	5.8 ± 0.2	10.8 ± 0.9	6.3 ± 0.4	–	–	– –	– –
8	1-Hexanol	875	6.742	56, 43, 70	pungent(The Good Scents Company, 2021)	6.2 ± 1.0	7.78 ± 0.03	4.53 ± 0.08	7.23 ± 0.09	18.44 ± 0.03	19.66 ± 0.01	25 ± 1	23.3 ± 0.2
9	Styrene	890	7.287	104, 78, 51	Sweet, balsamic and floral(The Good Scents Company, 2021; Jensen et al., 2011)	1.64 ± 0.02	2.8 ± 0.1	4.8 ± 0.1	3.67 ± 0.07	–	–	1.08 ± 0.05	2.84 ± 0.01
10	Heptanal	905	7.814	44, 70, 55	fatty, green, rancid, citrus and malty(Pico et al., 2015)	3.65 ± 0.03	3.95 ± 0.01	2.9 ± 0.2	3.88 ± 0.06	3.42 ± 0.01	3.92 ± 0.01	4.82 ± 0.06	4.28 ± 0.07
11	Methional	911	7.994	48, 104, 76	boiled-potato, cooked-potato, malty and waxy(Pico et al., 2015)	0.55 ± 0.05	0.51 ± 0.01	0.32 ± 0.01	0.49 ± 0.02	–	–	– –	– –
12	2-Acetylfuran	913	8.077	95, 110, 39	smoky, roasty, yeasty and fermented(Barbarisi et al., 2019; Pico et al., 2015)	1.16 ± 0.02	1.22 ± 0.04	1.81 ± 0.04	1.35 ± 0.06	–	–	– –	– –
13	2,5-Dimethylpyrazine	917	8.175	108, 42, 39	nutty, roasty and woody(The Good Scents Company, 2021)	3.883 ± 0.002	1.10 ± 0.40	1.30 ± 0.08	0.8 ± 0.06	–	–	– –	– –
14	2-Ethylpyrazine	919	8.233	107, 80, 53	Nutty, musty, coffee and roasted(The Good Scents Company, 2021)	3.881 ± 0.004	1.92 ± 0.07	0.40 ± 0.01	0.42 ± 0.02	–	–	– –	– –
15	α-Pinene	935	8.726	93, 91, 77	pine earthy, turpentine, fresh, sweet and woody(The Good Scents Company, 2021; Xiao et al., 2016a)	–	–	0.84 ± 0.06	0.65 ± 0.05	–	–	1.79 ± 0.04	2.68 ± 0.05
16	Camphene	951	9.193	98, 121, 79	camphor, woody and herbal(The Good Scents Company, 2021; Xiao et al., 2016a)	–	–	0.10 ± 0.01	tr	–	–	0.26 ± 0.02	0.29 ± 0.01
17	5-Methylfurfural	969	9.760	110, 53, 27	butter, caramel and musty(Pico et al., 2015)	0.99 ± 0.03	1.08 ± 0.02	0.78 ± 0.03	1.84 ± 0.09	–	–	– –	– –
18	Sabinene	975	9.931	93, 77, 41	pine, turpentine, woody, terpenic, spicy and citrus(The Good Scents Company, 2021; Xiao et al., 2016a)	–	–	–	–	–	–	0.13 ± 0.01	0.21 ± 0.01
19	β-Pinene	978	10.015	93, 41, 69	green, pine and woody(The Good Scents ; Miyazaki et al., 2012)	–	–	tr	tr	–	–	– –	– –
20	1-Octen-3-ol	988	10.326	57, 43, 72	mushroom, earthy, green and herbal(Jensen et al., 2011)	0.69 ± 0.01	0.80 ± 0.01	0.73 ± 0.04	0.82 ± 0.04	0.88 ± 0.02	1.04 ± 0.03	1.01 ± 0.02	0.80 ± 0.03
21	2-Pentylfuran	994	10.491	81, 138 ,53	butter, green bean, floral, fruity, mushroom and raw nuts(Jensen et al., 2011)	3.87 ± 0.02	4.06 ± 0.09	3.1 ± 0.1	3.8 ± 0.2	–	–	2.6 ± 0.2	2.58 ± 0.06
22	2-Ethyl-3-methylpyrazine	1002	10.748	121, 67, 39	baked(Pico et al., 2015)	0.39 ± 0.02	0.42 ± 0.01	0.60 ± 0.01	0.47 ± 0.03	–	–	– –	– –
23	*p*-Cymol	1026	11.535	119, 134, 91	green, fresh, rubber, terpenic, woody and spicy(The Good Scents Company, 2021; Miyazaki et al., 2012)	–	–	0.33 ± 0.02	0.18 ± 0.01	0.44 ± 0.01	0.47 ± 0.01	0.35 ± 0.01	0.55 ± 0.03
24	D-Limonene	1032	11.731	68, 93, 136	citrus, fresh and sweet(The Good Scents Company, 2021; Xiao et al., 2016a)	–	–	0.85 ± 0.02	0.75 ± 0.01	–	–	1.20 ± 0.01	1.18 ± 0.04
25	Unknown	1033	11.751	67, 95, 43	–	1.87 ± 0.01	1.21 ± 0.02	–	–	–	–	– –	– –
26	Eucalyptol	1033	11.770	93, 68, 43	green, herbal and spicy(Miyazaki et al., 2012)	–	–	2.60 ± 0.02	1.82 ± 0.03	1.24 ± 0.03	1.49 ± 0.01	3.6 ± 0.2	3.03 ± 0.04
27	Benzene acetaldehyde	1048	12.238	91, 120, 65	fruity, honey and sweet(Kirchhoff & Schieberle, 2001)	3.85 ± 0.02	3.36 ± 0.01	2.8 ± 0.1	2.3 ± 0.2	–	–	– –	– –
28	γ-Terpinene	1063	12.743	93, 77, 136	herbal, minty, pine, terpene and fruity(The Good Scents Company, 2021; Miyazaki et al., 2012)	–	–	tr	–	–	–	– –	– –
29	β-Linalool	1102	14.010	71, 43, 55	floral, lavender, citrus, woody and green(The Good Scents Company, 2021; Miyazaki et al., 2012; Xiao et al., 2016a)	–	–	–	–	–	–	0.18 ± 0.02	0.14 ± 0.01
30	Nonanal	1109	14.154	57, 41, 98	citrus, floral, fruity and fatty(Budryn et al., 2016; Jensen et al., 2011; Kirchhoff & Schieberle, 2001; Pico et al., 2015)	1.01 ± 0.03	1.11 ± 0.02	1.1 ± 0.1	0.86 ± 0.04	0.87 ± 0.03	0.94 ± 0.01	1.23 ± 0.11	0.61 ± 0.05
31	Camphor	1150	15.063	96, 81, 41	camphoraceous and herbal (The Good Scents Company, 2021)	–	–	–	–	–	–	2.55 ± 0.05	2.85 ± 0.17
32	2-Nonenal	1167	15.451	43, 55, 70	beans, green, oil and cucumber(Kirchhoff & Schieberle, 2001)	0.77 ± 0.09	0.91 ± 0.02	0.81 ± 0.07	0.77 ± 0.03	0.29 ± 0.01	0.49 ± 0.02	0.34 ± 0.02	0.50 ± 0.03
33	Bornyl acetate	1286	17.703	95, 43, 121	camphor, woody, pine, balsamic, herbal and spicy (The Good Scents Company, 2021)	–	–	0.22 ± 0.01	0.19 ± 0.01	–	–	1.30 ± 0.02	0.40 ± 0.02
34	Tetradecane	1303	17.989	57, 43, 71	mild and waxy (The Good Scents Company, 2021)	0.74 ± 0.06	0.83 ± 0.06	0.32 ± 0.02	0.51 ± 0.03	–	–	–	–

Compound	Calibration curve	R^2	LOD ($g \cdot L^{-1}$)	LOQ ($g \cdot L^{-1}$)	Quantification (µg/100 g bread)			
					Crumb		Crust	
					t0	t4	t0	t4
D-Limonene	$y = 1.52 \times 10^{9}x + 1.07 \times 10^{7}$	0.9997	1.80×10^{-3}	5.46×10^{-3}	0.45 ± 0.01^{b}	0.23 ± 0.02^{c}	0.68 ± 0.03^{a}	0.10 ± 0.00^{d}
Eucalyptol	$y = 1.09 \times 10^{9}x + 1.67 \times 10^{7}$	0.9993	6.21×10^{-3}	1.88×10^{-2}	1.9 ± 0.1^{b}	0.86 ± 0.04^{c}	2.68 ± 0.01^{a}	0.45 ± 0.04^{d}

RT: retention time, 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, EO-HD: essential oil obtained by hydrodistillation, EX-SFE-CO_2: extract obtained by carbon dioxide supercritical fluid extraction, tr: traces, LOD: limit of detection, LOQ: limit of quantification.

(hexanal) and 8 (1-hexanol) in bread crumb, increase with the extract concentration (Fig. S2 - f and g; Supplementary Information).

Focusing on the sensory description of odorous compounds, there are many works that define them with some discordant characteristics. 2-Methylpyrazine (5) was mentioned as roasted and sweet notes, while furfural (6) as almond, toasted and bread-like odours. Faint burning was the attribute used to 2-furanmethanol (7) volatile substance. 1-Hexanol (8) was defined as pungent and 2-pentylfuran (21) with butter, green bean, floral, fruity, mushroom, and raw nuts odours (Barbarisi et al., 2019; Budryn et al., 2016; Pico et al., 2015).

Finally, this study reveals the potential of natural strategies to improve the sensory attributes of foods, namely the odour of baked bread over time. To the best of the authors knowledge, this is the first time that a complete description of the volatile profile of embedded bread samples has been reported. Therefore, this study plays an interesting role in the olfactory marketing of bakery-related products. In addition, it would be interesting to continue research on the nutritional and bioactive properties of this type products, as well as their production on a larger scale in industrial ovens, aiming at proof of concept.

4. Conclusion

Essential oils and supercritical extracts from *R. officinalis* L. plant material exhibited an abundancy of terpenes being a rich source of these molecules. A relationship between the amount of *S*-verbenone and eucalyptol molecules and the green, citrus, and fresh olfactive notes, was observed, as well as the presence of α-pinene and camphor compounds which reminded the woody fragrance. Furthermore, strategic characteristics such as a low cytotoxicity value, high reproducibility and extraction efficiency were considered for using rosemary extracts. Thus, by defining a safe concentration range by ODT and GI_{50} values, the extract can be used as an aromatic compound for food products with large potential to improve the pleasant odour of bread dough. The dried rosemary extract provided by SFE-CO_2 had the best overall results and was chosen for a proof of concept. Its positive influence was confirmed through the chemical evaluations of bread crust and crumb, even after a storage time of four hours.

In this way, the innovative product encourages its future reproducibility with studies aimed at the scaling-up of the process and other information according to the characterisation of the final product (*e.g.,* physical properties and nutritional value). Also, this work could represent a steppingstone for the improvement of bread sensorial properties using novel ingredients, namely natural aromas. In this regard, much more could be done by exploring natural raw materials. Finally, the studied extracts show an interesting potential for many other options, namely in the olfactory marketing field.

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 – review & editing, 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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.132514.

References

Barbarisi, C., De Vito, V., Pellicano, M. P., Boscaino, F., Balsamo, S., Laurino, C., & Volpe, M. G. (2019). Bread chemical and nutritional characteristics as influenced by food grade sea water. *International Journal of Food Properties, 22*(1), 280–289. https://doi.org/10.1080/10942912.2019.1579837

Barros, L., Pereira, E., Calhelha, R. C., Dueñas, M., Carvalho, A. M., Santos-Buelga, C., & Ferreira, I. C. F. R. (2013). Bioactivity and chemical characterization in hydrophilic and lipophilic compounds of Chenopodium ambrosioides L. *Journal of Functional Foods, 5*(4), 1732–1740. https://doi.org/10.1016/j.jff.2013.07.019

Borges, R. S., Ortiz, B. L. S., Pereira, A. C. M., Keita, H., & Carvalho, J. C. T. (2019). Rosmarinus officinalis essential oil: A review of its phytochemistry, anti-inflammatory activity, and mechanisms of action involved. *Journal of Ethnopharmacology, 229*, 29–45. https://doi.org/10.1016/j.jep.2018.09.038

Boutekedjiret, C., Bentahar, F., Belabbes, R., & Bessiere, J. M. (2003). Extraction of rosemary essential oil by steam distillation and hydrodistillation. *Flavour and Fragrance Journal, 18*(6), 481–484. https://doi.org/10.1002/ffj.1226

Budryn, G., Zaczyńska, D., & Oracz, J. (2016). Effect of addition of green coffee extract and nanoencapsulated chlorogenic acids on aroma of different food products. *LWT - Food Science and Technology, 73*. https://doi.org/10.1016/j.lwt.2016.06.019

Cain, W. S. (1977). Differential sensitivity for smell:“ noise” at the nose. *Science, 195* (4280), 796–798. https://doi.org/10.1126/science.836592

Carvalho, R. N., Moura, L. S., Rosa, P. T. V., & Meireles, M. A. A. (2005). Supercritical fluid extraction from rosemary (Rosmarinus officinalis): Kinetic data, extract's global yield, composition, and antioxidant activity. *The Journal of Supercritical Fluids, 35*(3), 197–204. https://doi.org/10.1016/j.supflu.2005.01.009

Chemat, F., Abert Vian, M., Ravi, H. K., Khadhraoui, B., Hilali, S., Perino, S., & Tixier, A. F. (2019). Review of Alternative Solvents for Green Extraction of Food and Natural Products: Panorama, Principles, Applications and Prospects. *Molecules, 24* (16). https://doi.org/10.3390/molecules24163007

Chemat, F., Rombaut, N., Meullemiestre, A., Turk, M., Perino, S., Fabiano-Tixier, A.-S., & Abert-Vian, M. (2017). Review of Green Food Processing techniques. Preservation, transformation, and extraction. *Innovative Food Science & Emerging Technologies, 41*, 357–377. https://doi.org/10.1016/j.ifset.2017.04.016

COE. (2007). *European Directorate for the Quality of Medicines*. Strasbourg: European Pharmacopoeia, France.

Conde-Hernández, L. A., Espinosa-Victoria, J. R., Trejo, A., & Guerrero-Beltrán, J.Á. (2017). CO2-supercritical extraction, hydrodistillation and steam distillation of essential oil of rosemary (Rosmarinus officinalis). *Journal of Food Engineering, 200*, 81–86. https://doi.org/10.1016/j.jfoodeng.2016.12.022

Elyemni, M., Louaste, B., Nechad, I., Elkamli, T., Bouia, A., Taleb, M., Eloutassi, N. (2019). Extraction of Essential Oils of Rosmarinus officinalis L. by Two Different Methods: Hydrodistillation and Microwave Assisted Hydrodistillation. *The Scientific World Journal, 2019*, 3659432. https://doi.org/10.1155/2019/3659432.

F&F. (2019). *Flavours and Fragrances - Share & Trends Analysis Report By Product (Natural, Aroma), By Application (Flavors, Fragrances), By Region, And Segment Forecasts, 2019 - 2025*. Dublin: Research and Markets, Ireland, 2019.

Fornari, T., Vicente, G., Vázquez, E., García-Risco, M. R., & Reglero, G. (2012). Isolation of essential oil from different plants and herbs by supercritical fluid extraction. *Journal of Chromatography A, 1250*, 34–48. https://doi.org/10.1016/j.chroma.2012.04.051

Gomes, P. B., Mata, V. G., & Rodrigues, A. E. (2007). Production of rose geranium oil using supercritical fluid extraction. *The Journal of Supercritical Fluids, 41*(1), 50–60. https://doi.org/10.1016/j.supflu.2006.08.018

Ibáñez, E., Oca, A., de Murga, G., López-Sebastián, S., Tabera, J., & Reglero, G. (1999). Supercritical Fluid Extraction and Fractionation of Different Preprocessed Rosemary Plants. *Journal of Agriculture and Food Chemistry, 47*(4), 1400–1404. https://doi.org/10.1021/jf980982f

International Organization for Standardization 8586:2012, I. (2012). *Sensory analysis — General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors* https://www.iso.org/standard/45352.html.

International Organization for Standardization 11035:1994, I. (1994). *Sensory analysis — Identification and selection of descriptors for establishing a sensory profile by a multidimensional approach* https://www.iso.org/standard/19015.html.

International Organization for Standardization 13301:2018, I. (2018). *Sensory analysis — Methodology — General guidance for measuring odour, flavour and taste detection thresholds by a three-alternative forced-choice (3-AFC) procedure* https://www.iso.org/standard/68901.html.

International Organization for Standardization 13299:2016, I. (2016). *Sensory analysis — Methodology — General guidance for establishing a sensory profile* https://www.iso.org/standards.

Jamshidi, R., Afzali, Z., & Afzali, D. (2009). Chemical composition of hydrodistillation essential oil of rosemary in different origins in Iran and comparison with other countries. *American-Eurasian Journal of Agricultural & Environmental Sciences, 5*(1), 78–81.

Jensen, S., Oestdal, H., Skibsted, L. H., Larsen, E., & Thybo, A. K. (2011). Chemical changes in wheat pan bread during storage and how it affects the sensory perception of aroma, flavour, and taste. *Journal of Cereal Science, 53*(2), 259–268. https://doi.org/10.1016/j.jcs.2010.11.007

Kamath, A., Asha, M. R., Ravi, R., Narasimhan, S., & Rajalakshmi, D. (2001). Comparative study of odour and GC-olfactometric profiles of selected essential oils. *Flavour and Fragrance Journal, 16*(6), 401–407. https://doi.org/10.1002/ffj.1020

Kessler, J. C., Vieira, V. A., Martins, I. M., Manrique, Y. A., Afonso, A., Ferreira, P., & Dias, M. M. (2022). Obtaining Aromatic Extracts from Portuguese Thymus mastichina L. by Hydrodistillation and Supercritical Fluid Extraction with CO2 as Potential Flavouring Additives for Food Applications. *Molecules, 27*(3), 694. https://www.mdpi.com/1420-3049/27/3/694.

Kirchhoff, E., & Schieberle, P. (2001). Determination of Key Aroma Compounds in the Crumb of a Three-Stage Sourdough Rye Bread by Stable Isotope Dilution Assays and Sensory Studies. *Journal of Agriculture and Food Chemistry, 49*(9), 4304–4311. https://doi.org/10.1021/jf010376b

Machado, B. A. S., Pereira, C. G., Nunes, S. B., Padilha, F. F., & Umsza-Guez, M. A. (2013). Supercritical Fluid Extraction Using CO2: Main Applications and Future Perspectives. *Separation Science and Technology, 48*(18), 2741–2760. https://doi.org/10.1080/01496395.2013.811422

Mena, P., Cirlini, M., Tassotti, M., Herrlinger, K. A., Dall'Asta, C., & Del Rio, D. (2016). Phytochemical profiling of flavonoids, phenolic acids, terpenoids, and volatile fraction of a rosemary (Rosmarinus officinalis L.) extract. *Molecules*, *21*(11), 1576. https://doi.org/10.3390/molecules21111576.

Miyazaki, T., Plotto, A., Baldwin, E. A., Reyes-De-Corcuera, J. I., & Gmitter, F. G., Jr (2012). Aroma characterization of tangerine hybrids by gas-chromatography–olfactometry and sensory evaluation. *Journal of the Science of Food and Agriculture, 92*(4), 727–735. https://doi.org/10.1002/jsfa.4663

Münch, P., & Schieberle, P. (1998). Quantitative Studies on the Formation of Key Odorants in Thermally Treated Yeast Extracts Using Stable Isotope Dilution Assays. *Journal of Agriculture and Food Chemistry, 46*(11), 4695–4701. https://doi.org/10.1021/jf980511t

Okoh, O. O., Sadimenko, A. P., & Afolayan, A. J. (2010). Comparative evaluation of the antibacterial activities of the essential oils of Rosmarinus officinalis L. obtained by hydrodistillation and solvent free microwave extraction methods. *Food Chemistry, 120*(1), 308–312. https://doi.org/10.1016/j.foodchem.2009.09.084

Perestrelo, R., Silva, C. L., Rodrigues, F., Caldeira, M., & Câmara, J. S. (2016). A powerful approach to explore the potential of medicinal plants as a natural source of odor and antioxidant compounds. *Journal of Food Science and Technology, 53*(1), 132–144. https://doi.org/10.1007/s13197-015-2022-x

Pico, J., Antolín, B., Román, L., Gómez, M., & Bernal, J. (2018). Analysis of volatile compounds in gluten-free bread crusts with an optimised and validated SPME-GC/QTOF methodology. *Food Research International, 106*, 686–695. https://doi.org/10.1016/j.foodres.2018.01.048

Pico, J., Bernal, J., & Gómez, M. (2015). Wheat bread aroma compounds in crumb and crust: A review. *Food Research International, 75*, 200–215. https://doi.org/10.1016/j.foodres.2015.05.051

Pourmortazavi, S. M., & Hajimirsadeghi, S. S. (2007). Supercritical fluid extraction in plant essential and volatile oil analysis. *Journal of Chromatography A, 1163*(1), 2–24. https://doi.org/10.1016/j.chroma.2007.06.021

Sela, L., & Sobel, N. (2010). Human olfaction: a constant state of change-blindness. *Experimental Brain Research*, *205*(1), 13-29. https://doi.org/10.1007/s00221-010-2348-6.

Teixeira, M. A., Rodríguez, O., Mota, F. L., Macedo, E. A., & Rodrigues, A. E. (2011). Evaluation of Group-Contribution Methods To Predict VLE and Odor Intensity of Fragrances. *Industrial & Engineering Chemistry Research, 50*(15), 9390–9402. https://doi.org/10.1021/ie200290r

The Good Scents Company. (2021). Available online: https://doi.org/http://www.thegoodscentscompany.com/ (accessed 15 September 2021).

Xiao, Z., Fan, B., Niu, Y., Wu, M., Liu, J., & Ma, S. (2016a). Characterization of odor-active compounds of various Chrysanthemum essential oils by gas chromatography-olfactometry, gas chromatography-mass spectrometry and their correlation with sensory attributes. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences, 1009–1010*, 152–162. https://doi.org/10.1016/j.jchromb.2015.12.029

Xiao, Z., Ma, S., Niu, Y., Chen, F., & Yu, D. (2016b). Characterization of odour-active compounds of sweet orange essential oils of different regions by gas chromatography-mass spectrometry, gas chromatography-olfactometry and their correlation with sensory attributes. *Flavour and Fragrance Journal, 31*(1), 41–50. https://doi.org/10.1002/ffj.3268

Zellner, B. d. A., Bicchi, C., Dugo, P., Rubiolo, P., Dugo, G., & Mondello, L. (2008). Linear retention indices in gas chromatographic analysis: a review. *Flavour and Fragrance Journal*, *23*(5), 297-314. https://doi.org/https://doi.org/10.1002/ffj.1887.