Antioxidant effect induced by the essential oil of Pituranthos scoparius in a formulation of a whey spread emulsion

Anis Chikhoune, Jan Damjan Pavleca, Mikhail Shashkov, Zahra Berroua, Kaissa Chebbi, Hind Bougherra, Brahim Zeroual, Khellaf Aliane, Mohammed Gagaoua, Abdelghani Boudjellal, et al.

Received: 3 February 2016 | Revised: 15 June 2016 | Accepted: 20 July 2016

DOI: 10.1111/jfpp.13163

ORIGINAL ARTICLE

WILEY Journal of Food Processing and Preservation Institute of Food Science + Technology ifst

Antioxidant effect induced by the essential oil of *Pituranthos scoparius* in a formulation of a whey spread emulsion

Anis Chikhoune[1] | Jan Damjan Pavleca[2] | Mikhail Shashkov[3,4] | Zahra Berroua[5] | Kaissa Chebbi[5] | Hind Bougherra[6] | Brahim Zeroual[7] | Khellaf Aliane[7] | Mohammed Gagaoua[1] | Abdelghani Boudjellal[1] | Irena Vovk[2] | Mitja Križman[2]

[1]Equipe Maquav, Laboratoire BIOQUAL, I.N.A.T.A.A, Université Frères Mentouri Constantine, Route de Ain El-Bey, Constantine 25000, Algérie

[2]Laboratory for Food Chemistry, National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia

[3]Boreskov Institute of Catalysis SB RAS, Pr. Lavrentyeva. 5, Novosibirsk 630090, Russian Federation

[4]Novosibirsk state University, 630090, Pyrogova st, 2, Novosibirsk 630090, Russian Federation

[5]Département des Technologies Alimentaires, I.N.A.T.A.A, Université Frères Mentouri Constantine, Route de Ain El-Bey, Constantine 2500, Algérie

[6]Département de Génie des Procédés, Université Abderrahmane Mira, Laboratoire des Matériaux Organiques, Bejaia 06000, Algérie

[7]Cévital spa, nouveau quai, port de Bejaia, BP 334, Bejaia 06000, Algerie

Correspondence
Dr. Anis Chikhoune, Equipe Maquav, Laboratoire BIOQUAL, I.N.A.T.A.A, Université Frères Mentouri Constantine, Route de Ain El-Bey 25000 Constantine, Algeria.
Email: anis.chikhoune@gmail.com

Funding information
INATAA, University of Brothers Mentouri Constantine

Abstract

This study falls within the framework of industrial exploitation of the essential oil recovered from *Pituranthos scoparius*. Chromatographic techniques, namely TLC, HPTLC, MHE-GC-FID-MS, GC X GC, and GC-FID-MS have been used for the characterization, identification and quantification of 48 compounds, divided into three major terpene groups: monoterpenes, diterpenes, and oxygenated compounds. The essential oil displayed an antioxidant activity, presented by a radical scavenging activity and evaluated by the TLC–DPPH$^{\bullet}$ method. Tests conducted at laboratory scale showed high protection from lipid oxidation of the spread emulsion, with an oxidative stability of 20.82 h. A sensory evaluation was carried out on emulsions incorporated with the essential oil. The PCA analysis revealed the effect of some attributes on the segregation between the four emulsions formulated. These data suggest that the essential oil of *Pituranthos scoparius* is a possible and promising candidate as a biopreservative at an industrial scale for food preservation.

Practical applications

Essential oil of *Pituranthos scoparius* is traditionally used in folk medicine as a remedy for several illnesses. Its antibacterial properties are also exploited in the preservation of traditional cheese making. Whey is a by-product of cheese and casein industries, still considered as a waste. Valorization of this by-product in a whey spread emulsion is considered in the present work. Due to the bioactive compounds contained in the essential oil (such as terpinen-4-ol), the essential oil exhibits high antioxidant activity. As oxidation phenomena becomes a topical issue in fat and oil food industry, natural plants containing essential oils with high antioxidant properties are considered as good alternatives to synthetic antioxidants.

KEYWORDS

essential oil, monoterpenes, emulsion, oxidative stability

1 | INTRODUCTION

The occurrence of lipids in emulsified forms introduces the oxidative instability and foods become sensitive to lipid oxidation (Adachi, Minten, & Kobayashi, 2009). The latter is a main problem for both consumers and food industries since it affects several aspects of the food matrix such as: flavor, texture, shelf life and nutritional quality of the final product (Kiokias, Dimakou, Tsaprouni, & Oreopoulou, 2006; Ponginebbi, Nawar, & Chinachoti, 1999). Interfacial oxidation is of a great concern to the food industry, as it affects the stability of a large number of foods which exist as emulsions (Calligaris, Manzocco, & Nicoli, 2007). Many studies have now confirmed that exogenic antioxidants are essential for counteracting oxidative stress, especially those found in essential oils. These complex mixtures of several compounds are likely influenced by interactions among their structural components due to additive action and/or synergism between chemical classes

(Nenaah, 2014). Consumer awareness arises recently regarding synthetic chemical additives. Therefore, natural additives used for food preservation become increasingly popular (Holley & Patel, 2005). In fact, the demand for healthier and safer food products has encouraged to explore newer and more efficient preservation techniques. To reduce lipid oxidation, direct addition of natural antioxidants was applied among strategies of food preservation (Gómez-Estaca, López-de-Dicastillo, Hernández-Muñoz, Catalá, & Gavara, 2014).

Genus *Pituranthos* has more than 20 species, some of which are specific to North Africa and are often found in arid or desert areas (Nègre, 1961). *P. scoparius* is an endemic plant of North Africa and is widespread in Algeria, especially in the high plateau and in most parts of the Sahara. The traditional use of the plant in folk medicine concerns applying it in poultices to the head for headaches (Bellakhdar, 1997). Boutaghane, Nacer, Kabouche, and Ait-Kaki (2004) reported an antibacterial activity for the seeds and stems of *P. scoparius*. Reports of the chemical profile of the essential oils of P. *scoparius* are very limited. We report mainly the work of (Vérité, Nacer, Kabouche, & Seguin, 2004) on stems and seeds of *P. scoparius* (Boutaghane et al., 2004) on their antibacterial activities and more recently the work of (Lograda, Ramdani, Kiram, Chalard, & Figueredo, 2013) on the variation of the essential oils compositions of *P. scoparius* collected from different locations in Algeria.

To the best of our knowledge, this is the very first report on the potential use of *Pituranthos scoparius'* essential oil as a biopreservative in a whey spread-type emulsion. To reach this objective, an elegant approach using chromatography was used; including a fingerprinting of the bioactive compounds by high performance thin layer chromatography (HPTLC) densitometry, evaluation of the relative composition and quantification of volatiles by MHE–GC–FID–MS and an improved GC × GC procedure for the separation of the related compounds. Then, our efforts were focused on the study of oxidative stability of a whey spread emulsion formulation, incorporated with the essential oil and compared to a control one containing α-Tocopherol. In this context, a Rancimat test was carried out regarding the resistance to oxidation of the emulsions produced at a laboratory scale.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Formic acid (98-100%), n-hexane (≥99%) and isooctane were purchased from Merck (Darmstdat, Germany), ethylacetate (99.5%) from Sigma-Aldrich (St. Louis, USA) and methanol (99.9%) of HPLC grade from Sigma-Aldrich (St. Louis, USA). Bidistilled water (conductivity = 18.2 μs cm^{-1}) was used. Sulfuric acid (95–97%) and 4-methoxybenzaldehyde (anisaldehyde) were from Merck (Darmstdat, Germany), DPPH• (2,2-diphenyl-b-picrylhydrazyl) was from Sigma-Aldrich (St. Louis, USA). Standards of carvone (≥99%), p-cymene (95%), limonene (≥98%), α-phellandrene (≥99%), α-pinene (≥99%), β-pinene (≥99%) α-terpinen (85–90%), and γ-terpinen were from Fluka (Seelze, Germany), thymol from Carl Roth (Karlsruhe, Germany). Standards of gallic acid (≥99%), Quercetin (≥98%), Ascorbic acid (≥99%) were obtained from Sigma-Aldrich (St. Louis, USA), Folin–Ciocalteu reagent from Merck (Darmstdat, Germany) and the remaining reagents and solvents were of analytical grade.

For whey spread emulsion formulation, refined, bleached and deodorized oils, palm oil (PO), sunflower oil (SFO) and soybean oil (SBO) are obtained from a local refinery (CEVITAL SPA). Other ingredients included emulsifier (mono lactic acid) purchased from (Palsgaard, Denmark), a colorant: Anatto Curcumin OSS 3525 (Global Entreprise, Switzerland), filtered water, pasteurized liquid whey (generously donated by SAFILAIT food industry from cheese-making process) and vacuum-dried salt.

2.2 | Preparation of standard solutions

Working standard solutions of α-pinene, β-pinene, α-terpinen, α-phellandrene, and p-cymene were prepared in ethylacetate to a concentration of 0.1 mL/0.2 mL^{-1} in pure ethylacetate for HPTLC analysis.

For HS–GC–FID analysis, standard solution of β-pinene, limonene and *trans*-anethole was prepared in methanol at a concentration of 10 mg mL^{-1}, each. Ten microliters of the standard solution were put into 20 mL HS vial plus 20 mL of water.

2.3 | Preparation of spraying and dipping reagents

Anisaldehyde detection reagent was prepared by mixing glacial acetic acid (20 mL) and methanol (170 mL); the mixture was cooled in an ice bath and sulfuric acid (16 mL), and 4-methoxybenzaldehyde (1 mL) were added in a dropwise manner.

DPPH• (2,2-diphenyl-b-picrylhydrazyl) was prepared by dissolving 0.2 g of the reagent in 100 mL of pure methanol, so as to have a solution of 0.2% (m/v).

2.4 | Extraction of essential oil

Aerial parts (stems and leaves) of *Pituranthos scoparius* were collected from Sedrata, Souk Ahras situated in the northeast of Algeria. The samples were collected in April 2014. A part of the plant aerial material was used as freshly collected for MHE–GC–FID–MS analysis; the other part was dried at room temperature in laboratory and cut in small pieces. An amount of 150 g of the latter was subjected to hydrodistillation for 4 h 30 on a Clevenger type apparatus to isolate the essential oil. Two types of essential oil were obtained; namely, fresh plant (FP) and dried plant (DP) according to the drying process of the material plant: a yellowish-transparent essential oil corresponding to the fresh plant dried for 48 h and a yellowish-intense essential oil dried for approximately 1 month, at laboratory and at room temperature. The essential oils, obtained after the hydrodistillation process, were of a characteristic odor. They were allowed to be separated from the aqueous phase in a glass tube, then carefully and gently collected by a syringe and filtered through a 0.45 μm Millipore Millex-HV hydrophilic poly(vinyldiene difluoride)-PVDF membrane filter (Billerica, MA), then stored in ambered vials of 1.5 mL, protected from light at 4°C until use.

2.5 | Thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC)-densitometry analyses

The essential oil was diluted to 1:30 (1 + 29, v/v). For TLC screening of terpenes, TLC silica gel 60 plate 5 cm × 10 cm (Merck, Darmstadt, Germany) (Art. No. 1. 05553) was used. The plate was developed in twin-trough chamber (CAMAG, Muttenz, Switzerland). Five microliters of the working sample solution were applied in the form of 5 mm bands, at 8 mm from the bottom, and 15 mm from the left edge of the plate (the space between two bands was set to 10 mm) with a Camag microliter syringe (25 μL syringe size) using an ATS4 autosampler (CAMAG) on to the TLC plate, 1 cm from the bottom edge. The plate was first developed in isooctane throughout 8 cm length. The second development was done to 5 cm height with n-hexane-ethyl acetate (5 + 1, v/v). For both developments, 10 mL of the developing system was used without any preconditioning. After development, the plate was dried under warm stream before derivatization step. Postchromatographic derivatization was performed by respectively heating the plate at 110°C for 3 min on a TLC plate heater (CAMAG), then spraying for 1 min the plate with anisaldehyde detection reagent. Documentation of the chromatographic plate was performed by DigiStore 2 Documentation System (CAMAG) operated with winCATS Version 1.4.1.8154 software. For the HPTLC procedure, a HPTLC F_{254} silica gel 60 plate 10 cm × 10 cm (Merck, Darmstadt, Germany) (Art. No. 1. 05629) was developed and derivatized as previously, along with 10 μL of the working standard solutions then scanned at $\lambda = 500$ nm with a TLC scanner III (CAMAG), the slit dimension was kept at 3.00 mm × 0.30 mm with a scanning speed of 20 mm s^{-1} and a data resolution of 100 μm $step^{-1}$.

3 | ANALYSIS OF VOLATILES BY MULTIPLE HEADSPACE EXTRACTION (MHE)–GAS CHROMATOGRAPHY (GC)–FLAME IONIZATION DETECTOR (FID)–MASS SPECTROMETRY (MS)

3.1 | Instrumentation

MHS–GC–FID analyses were performed on a Focus GC (Thermo) coupled to a HS 40 XL (Perkin Elmer) headspace autosampler. GC–MS identification was done on a Trace Ultra GC coupled to a DSQ II single-quad mass spectrometer (Thermo). MHE and GC–FID conditions were the following: carrier gas (He), oven temperature (145°C), needle temperature (265°C), transfer temperature (190°C), pressurization (270 kPa), thermostat time (35 min), pressurization time (0.6 min), and injection time (0.04 min). MHE injections (3), flow mode (2.0 mL min^{-1}, constant flow), split ratio (1:25), inlet temperature (280°C), FID temperature (280°C), GC column: ZB-5MSi, 30 m × 0.25 mm, 0.25 μm film (Phenomenex), oven temperature program: 50°C (0.5 min) to 105°C at 15°C min^{-1} to 180°C at 30°C min to 250°C at 10°C/min (1 min).

3.2 | Analysis and quantification

GC–MS identification of volatile compounds was performed by injecting solutions of 1% (v/v) *Pituranthos* essential oil in ethyl acetate. Plant samples were first dried at 50–60°C, and then homogenized with a dismembrator to obtain a fine powder. Twenty milligrams of homogenized sample were put into a HS vial with the addition of 20 mL of water and 10 μL of methanol. MHE–GC analysis was performed by three sequential HS injections from standard and sample vials. Chromatographic peaks were classified into three peak groups, namely monoterpenes, diterpenes, and oxygenated compounds—the latter including all aromatic compounds, terpene alcohols, etc. Monoterpene and diterpene peak groups were quantified by means of β-pinene and limonene standards while the oxygenated compound group was quantified by means of *trans*-anethole standard. GC–MS conditions were the following: carrier gas (He), flow mode (1.0 mL min^{-1}, constant flow), split ratio (1:25), inlet temperature (280°C), injection volume (0.5 μL), transfer line temperature (280°C). MS source temperature (200°C), ionization energy (+70 eV), scan range (40–250 m/z), GC column J&W: ZB-5HT, 20 m × 0.18 mm, 0.18 μm film, oven temperature program: 50°C (0.5 min) to 105°C at 15°C min^{-1} to 180°C at 30°C min^{-1} to 250°C at 10°C min^{-1} (1 min).

3.3 | GC–MS analysis of the hydrodistilled essential oil

GC–MS identification was performed by injecting solutions of 1% (v/v) *Pituranthos* essential oil in hexane. Standard solutions of α-pinene, β-pinene, α-phellandrene, α-terpinene, p-cymene, limonene, γ-terpinene, and carvone were prepared in hexane, at a concentration of 200 ppm. Thermo Trace GC Ultra gas chromatograph equipped with a ZB-5HT fused silica capillary column (20 m × 0.18 mm × 0.18 μm) was interfaced to a Thermo DSQ II mass spectrometer. Temperature was programmed from 50°C (1 min) to 150°C at 3°C min^{-1} and then at a rate of 5°C min^{-1} till 260°C, with a 1 min hold. Helium was used as a carrier gas (0.6 mL min^{-1}); injection was in split mode (1:25); with injector temperature 280°C. The mass spectrometer worked in EI mode at 70 eV. The identification of components was based on computer matching of their mass spectra with those of NIST mass spectral library (NIST MS Search 2.0, 2005) as well as on comparison of their retention indices with indices from NIST database. Retention indices were calculated with Van den Dool and Kratz formula. Column and method was transferred to Thermo Focus GC with flame ionization detector for quantitative determination.

3.4 | GC × GC analysis of the essential oil

GC × GC analysis was performed using a 2D-chromatograhy system Agilent 7890N, equipped with flow modulator. The detector used was FID at 250°C. Oven temperature program was 70°C (2 min)—7°C min^{-1}—240°C (15 min). The inlet temperature was 250°C. The two dimensional column set was composed of an ionic liquid custom column (4MPyC6, 30 m × 0.25 mm × 0.2 m km; referred to as column 1) and a HP-5 (5 m × 0.25 mm × 0.2 m km, referred to as column 2). High purity helium was used as the carrier gas. The sample was injected using a 1:100 split mode. The flow velocities for column 1 and column 2 were respectively 1 and 25 mL min^{-1}, with a modulation time of 1.4 s.

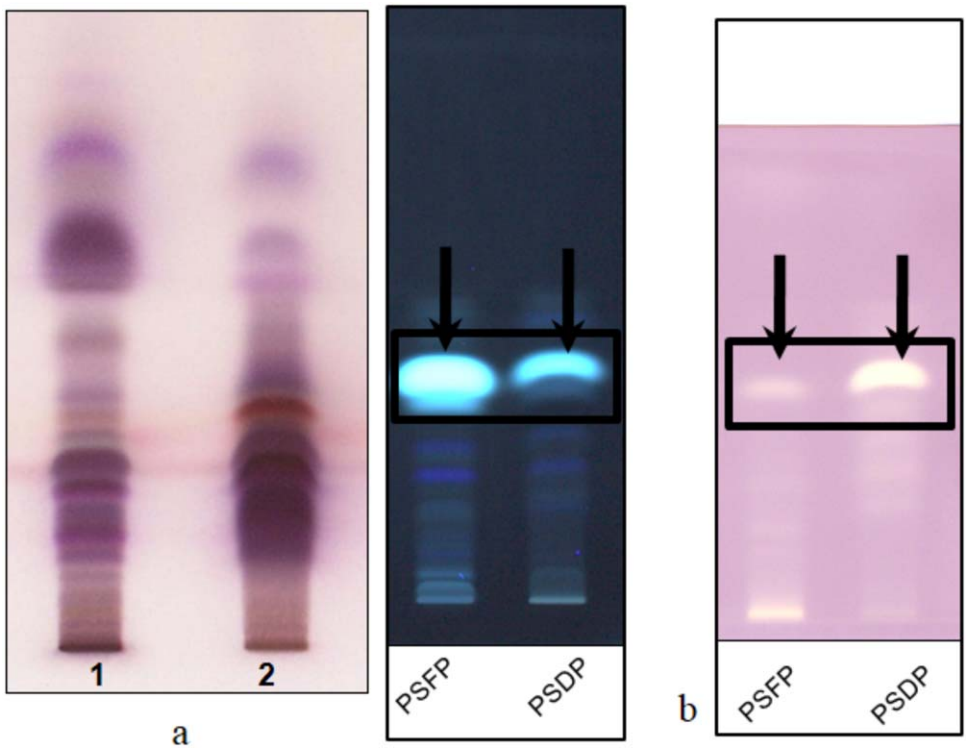

FIGURE 1 (a) TLC silica gel 60 plate developed in unsaturated twin-trough developing chamber using developing solvents isooctane (first development); n-hexane-ethyl acetate (5:1 v/v) (second development) after derivatization with anisaldehyde detection reagent; white light illumination (vis r). Tracks: 1 = FP; 2 = DP, (b) TLC silica gel 60 plate developed in unsaturated twin-trough developing chamber using developing solvents isooctane (first development); n-hexane-ethyl acetate (5:1 v/v) (second development), under 366 nm illumination before dipping in DPPH• (left) and after dipping (right) under remission light illumination. The rectangle and the arrows show the most active fractions in FP and DP essential oil of *P. scoparius* (PSFP: *P. scoparius* fresh plant; PSDP: *P. scoparius* dried plant)

3.5 | Antioxidant activity by the TLC–DPPH• dipping method

Fresh solution of DPPH• (2,2-diphenyl-b-picrylhydrazyl) was prepared by dissolving 0.2 g of the reagent in 100 mL of pure methanol, so as to have a solution of 0.2% (m/v), according to (Cieśla, Kryszeń, Stochmal, Oleszek, & Waksmundzka-Hajnos, 2012). TLC silica gel 60 plates without F 5 cm × 10 cm (Merck, Germany) (Art. No. 1. 05553) were developed following the TLC procedure described above, then immersed for 5 s using chromatogram immersion device III (CAMAG). The plate was photographed, protected from daylight by covering the immersion device with an aluminum foil, in 30 min after staining. The experiment was performed in an ambient temperature.

3.6 | Preparation of the emulsion

The whey spread emulsion formulated presented the global composition as following:

- 82% fatty phase;
- 16% aqueous phase;
- 2% additives.

The formulation was performed at a laboratory scale. The fatty phase contains a blend of three oils: palm oil (PO), sunflower oil (SFO), and soybean oil (SBO). Besides, amounts of fat soluble ingredients and the essential oil of *Pituranthos scoparius* were dissolved in a beaker and added to the fat blend. The aqueous phase contained pasteurized liquid whey, salt, potassium sorbate, citric acid, the colorant (Anatto Curcumin OSS 3525). These two phases were poured into a stainless steel tank where emulsification occurred with continuous stirring during 20 min, using a stirrer. At this stage, stability of the emulsion is incomplete; a crystallization is needed. This was carried out in a stainless steel tank containing a cold water and ice. Stirring was carried out during the whole process to ensure a homogeneous product. Two emulsions were prepared:

1 An emulsion containing the essential oil in the fatty phase as the biopreservative agent (working emulsion);
2 An emulsion containing α-Tocopherol as antioxidant in the fatty phase (control emulsion).

The emulsions produced were packed in sticks of 250 g and stored in a refrigerator at 4°C.

3.7 | Oxidative stability

The oxidative stability or the resistance to auto-oxidation of the emulsion incorporated by the essential oil of *P. scoparius* was determined using a Metrhom 743 Rancimat (Herisau, Switzerland) per the ISO 6886 method (ISO international standard method, 2006). In this test, a

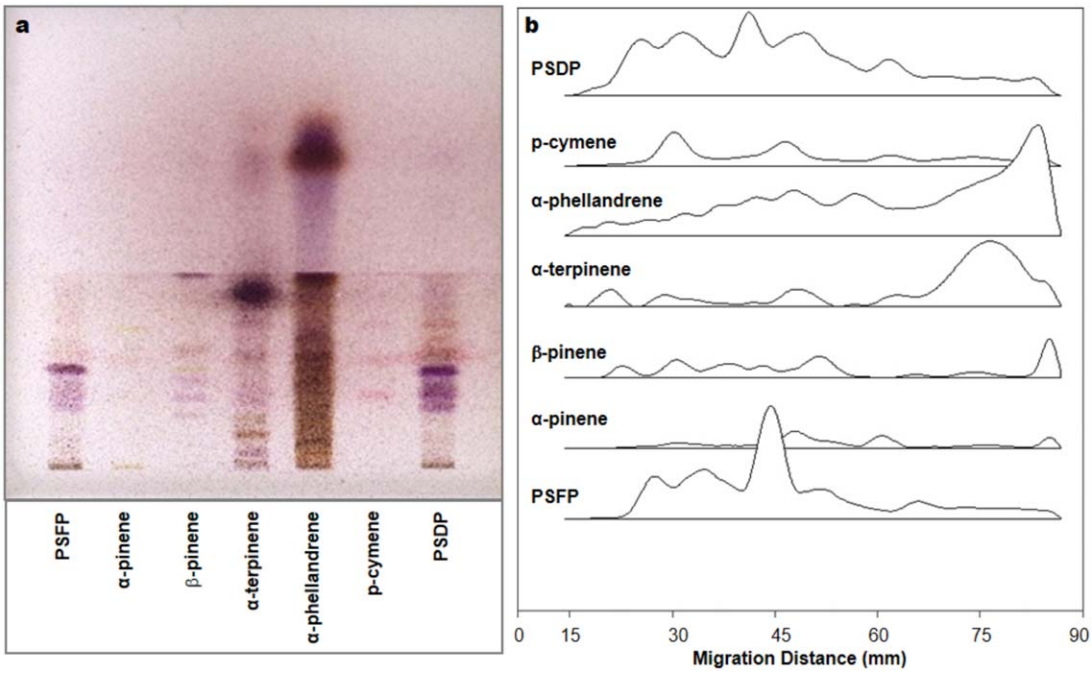

FIGURE 2 (a) HPTLC silica gel 60 F254 PLATE, (b) densitograms recorded at 500 nm representing standards of monoterpenes and *P. scoparius*' essential oils FP and DP. The developing solvents were isooctane (first development); n-hexane–ethyl acetate (5:1 v/v) (second development) after derivatization with anisaldehyde detection reagent; white light illumination (vis T)

10 L h^{-1} stream of dry air is bubbled into 3 g samples maintained at 98°C. Volatile oxidation products are carried through the detector chamber containing deionized water. The change in conductivity is measured and recorded. The increase of the latter is measured as a function of time until maximal change which reflects the IP. The test was carried out until the endpoints of the samples were reached, with a maximum allowable limit of 48 h.

3.8 | Sensory evaluation

The sensory evaluation was carried out as recently described by (Chikhoune et al., 2014). Subjects, number of 31 tasters, were staff of an agribusiness food industry (CEVITAL), Algeria. Four spread emulsions were prepared, as previously described, coded from 1 to 4: Emulsion A (control emulsion), Emulsion B (working emulsion containing 100 ppm of the essential oil), Emulsion C (working emulsion containing 300 ppm of the essential oil) and Emulsion D (working emulsion containing 600 ppm). They were presented simultaneously to the subjects together with slices of apples. A hedonic test was performed to characterize the emulsions in terms of ten attributes: spreadability, creaminess, sweetness, melting, appearance, texture, butter flavor, margarine flavor, salty, and aftertaste.

3.9 | Statistical analysis

Data of oxidative stability are means ± SD of two measurements. Differences between groups were tested by an analysis of variance (ANOVA) using the STATISTICA software (5.5). The ρ values ≤ 0.05 were considered significant. Principal component analysis (PCA) on the data of sensory evaluation was performed using XLstat Software 7.5.

4 | RESULTS

4.1 | Screening of terpenes on TLC and HPTLC densitometry

The screening profile of the hydrodistilled essential oil from the fresh (FP) and dried (DP) plant material, is depicted in Figure 1a. Several bands with different colors and different intensities were revealed using the Anisaldehyde detection reagent. For the application volume used (5 μL) and under the remission visible light (VisR), the essential oils exhibited numerous differences; first, the upper part of (FP) shows a richer region in terms of compounds detected compared to (DP). Bands in this region spread out, probably due to the particle size of the stationary phase precoated on TLC plate, but also the effect of the first developing solvent (isooctane) used. On the contrary, the lower part shows more intense bands for (DP), where shape of the bands is more characteristic and lead to form more pronounced curved structures. According to Pothier, Galand, El Ouali, and Viel (2001), TLC technique is limited because the compounds of essential oils are volatile, so at the top of the plate a diffusion can be observed as an edge effect.

The monoterpenes profiles of the essential oil (Figure 1a), studied following the HTPLC procedure, were scanned by densitometry at 500 nm and the densitogramms obtained are presented (Figure 2). The densitogramms obtained are not well sharply resolved on the base line, on track 1 FP (fresh plant material) showed peaks matching with the main peaks of standards: p-cymene, β-pinene and α-phellandrene. Whereas on track 7, peaks of DP (dry plant material) are matching more with the main peaks of p-cymene, α-pinene, α-terpinene, and α-phellandrene. This rapid screening of the essential oil (FP and DP) gave an overview of the

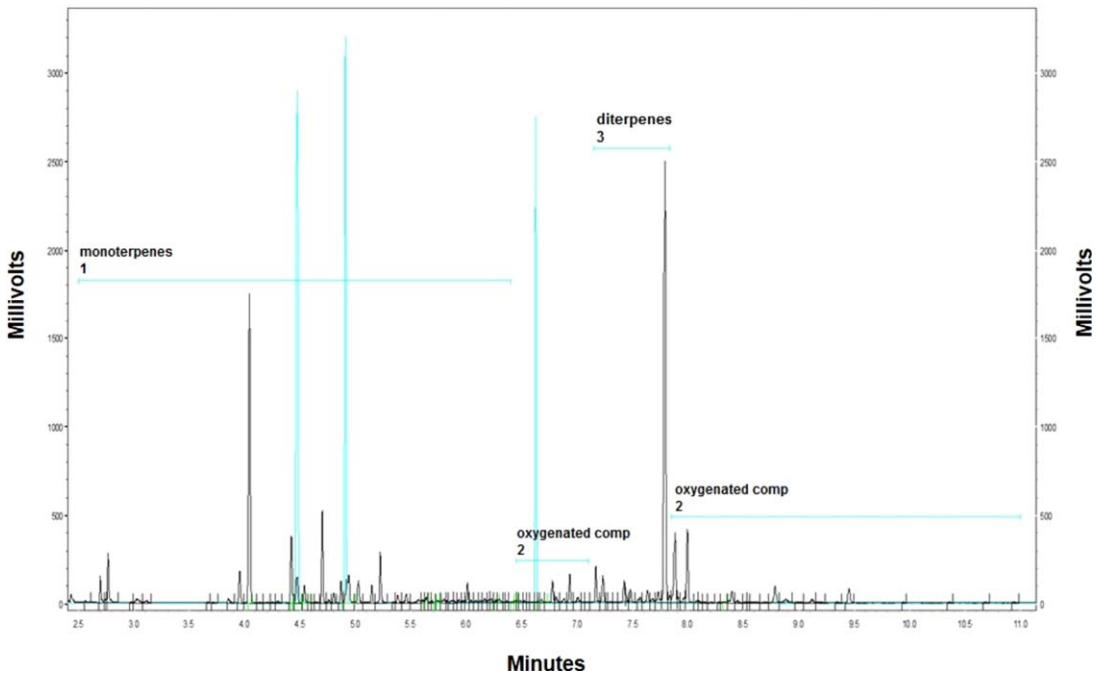

FIGURE 3 Chromatogram of *Pituranthos scoparius* sample with analyte groups indicated. The overlaid is the chromatogram of β-pinene, limonene, and trans-anethole standards, respectively

complexity of the plant matrix, due to the several compounds present (Figure 1a), but also the dilemma of their separation (Figure 2).

4.2 | MHE–GC–FID–MS analysis

MHE–GC analysis was performed by three sequential HS injections from standard and sample vials. Chromatographic peaks were classified into three peak groups, namely monoterpenes, diterpenes, and oxygenated compounds; the latter including all aromatic compounds, terpene alcohols, etc. Monoterpene and diterpene peak groups were quantified by means of β-pinene and limonene standards while the oxygenated compound group was quantified by means of *trans*-anethole standard.

The chromatogram of *Pituranthos scoparius* sample is presented (Figure 3). This analysis enabled the identification of 110 peaks. Among them, over 50 peaks can be classified as monoterpenes and about half of that represents the majority of volatile constituents. The oxygenated compound group is mainly composed of terpene alcohols but also some aromatic compounds (e.g., phenylpropanoids). Diterpenes, in contrast, represent only a minor group.

The analytical results from MHE calculations were reported (Table 1). The total volatiles' contents of the three identified groups are in the range (0.203–0.399)%. The correlation coefficients are also given for the three samples analyzed and for every single group of compounds identified. The best correlation coefficients were obtained for sample 3 and are of $R^2 = 0.9969$ for monoterpenes, $R^2 = 0.9999$ for diterpenes and $R^2 = 0.9989$ for oxygenates.

From these results, MHE–GC–FID–MS allowed the characterization and quantitation of the volatiles of *P. scoparius* directly from the plant material and without any pretreatment. A very good linearity of the method was also obtained.

4.3 | GC × GC analysis

Two-dimensional chromatography is one of the most effective analytical methods for complex multicomponent samples. This is especially true in the case of mixtures containing several compounds with different chemical classes. An example of such complex samples are essential oils, *Pituranthos* in particular. Achieving good separation for

TABLE 1 Analytical results from MHE calculations. The analysis was performed by three sequential hs injections from standard and sample vials

	Sample 1			Sample 2			Sample 3		
Analyte group	Content (%)	MHE curve slope	MHE curve R^2	Content (%)	MHE curve slope	MHE curve R^2	Content (%)	MHE curve slope	MHE curve R^2
Monoterpenes	0.192	1.5788	0.9963	0.178	1.5506	0.9960	0.134	1.5775	0.9969
Diterpenes	0.028	1.5719	0.9986	0.122	1.3570	0.9967	0.011	1.8130	0.9999
Oxygenates	0.064	1.4120	0.9845	0.099	1.1679	0.9898	0.058	1.4283	0.9989
Total volatiles	0.284			0.399			0.203		

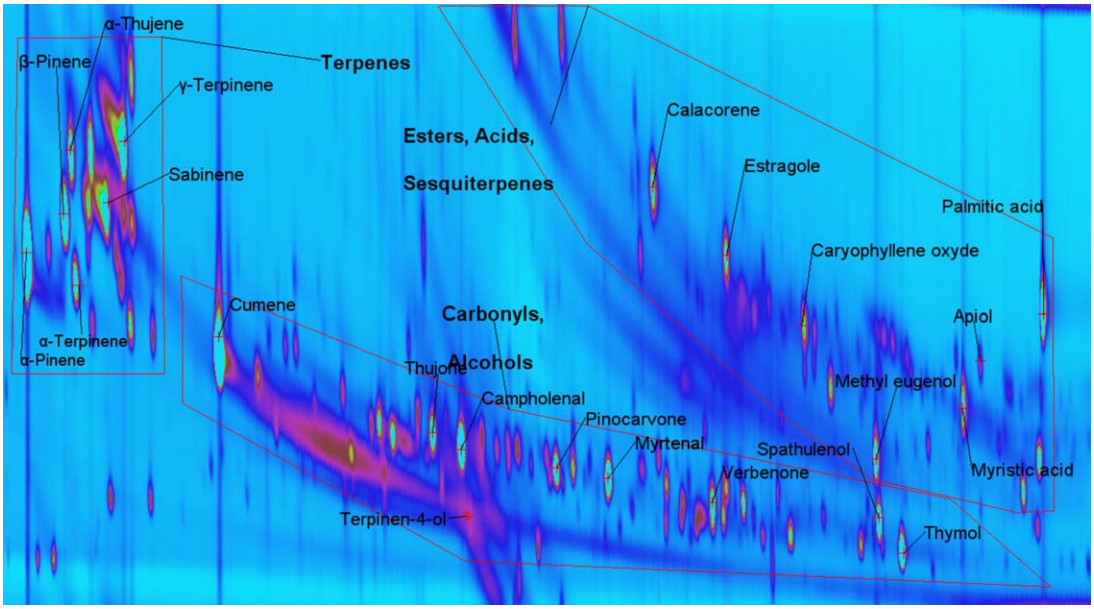

FIGURE 4 GC × GC chromatogram of *Pituranthos scoparius*' essential oil. The red circled zones highlighted the three main groups separated

such samples by traditional GC is most of the time a very difficult task, because it is almost impossible to get the resolution for all peaks of multiple oxygen compounds by one-dimensional GC, while 2D-chromatography offers quite better separation. Therefore, it is necessary in GC × GC to use two columns with different selectivity. Moreover, its common to use high-polar ionic liquid columns in GC × GC (Seeley, Seeley, Libby, Breitbach, & Armstrong, 2008). It is worth noting that using ionic liquid columns as the first column achieves more quality and complete separation of complex mixtures (Purcaro, Cordero, Liberto, Bicchi, & Conte, 2014; Seeley et al., 2008). For the separation of *Pituranthos scoparius'* components, it has been suggested to use pyridinium ionic liquids developed by (Shashkov and Sidelnikov, 2013), because this class of stationary phases has shown high-polarity and selectivity for oxygen-containing compounds. As the first column was suggested to use dicationic pyridinium ionic liquid 4MPyC6, the second one was nonpolar siloxane HP-5. By careful selection of the conditions, an improved resolution was achieved (Figure 4). From the latter, certain zones depicted correspond clearly to different chemical classes. Particularly, they can be divided into three groups: group of low-boiling components (monoterpenes), group of the most polar components (carbonyls, alcohols) and group of less polar compounds (sesquiterpenes, high acids, aromatic, and terpene esters). Contour plot peak identification was successfully achieved through information derived from monodimentional GC separation, as reported by GC–MS (Table 2) and MHE–GC–MS (Figure 3). As it can be seen from the red circled zones, the three groups highlighted all fall within specific zones in the bidimensional chromatogram. In addition, the distribution of peaks in the rightmost part of the chromatogram confirms again the need for a 2-D separation. The effectiveness of the 2-D comprehensive GC approach is undoubtedly confirmed for the essential oil of *Pituranthos scoparius*. GC × GC has an enormous potential and superiority compared to traditional mono dimensional and multidimensional techniques (Mondello, Casilli, Tranchida, Dugo, & Dugo, 2005).

4.4 | Characterization and quantification of the volatiles of the hydrodistilled essential oil by GC–FID–MS

The percentage composition of individual compounds of *P. scoparius'* essential oil (FP and DP) obtained by hydrodistillation are listed in Table 2. A total of 48 compounds, representing 93.5% (FP) and 92.8% (DP) of the essential oil were identified by GC–MS and compared with those of reference substances. Quantitative data were based on peak area calculations. The average yield in essential oil was (0.502 ± 0.091)% (w/w). Monoterpenes are the most abundant compound group of the essential oil, as emphasized by the MHE–GC–FID–MS analysis and GC × GC analysis. The major constituents of the oil were mainly belonging to the monoterpenes hydrocarbons class: sabinene, α-pinene, p-cymene, α-thujene, and β-pinene. But also oxygenated monoterpenes like terpinen-4-ol and spathulenol are present in the essential oil. As also reported previously, other components such as: sesquiterpene hydrocarbons, oxygenated sesquiterpenes and aromatic derivatives of monoterpenes were also detected (Table 2).

From Table 2, it can be seen that the major components were detected in both FP and DP, mainly the monoterpenes hydrocarbons, with slight differences in their contents. This is in agreement with the densitogramms obtained with HPTLC (Figure 2), where major constituents were detected in both FP and DP.

The chemical profile of the essential oil from the present study is similar to that reported by (Vérité et al., 2004), but the contents of major components are different, so that those reported in our study are higher. Also, sabinene is reported to be the most abundant compound in our study, whereas no trace of this compound was reported

TABLE 2 Chemical profile of the essential oil extracted by hydrodistillation from *P. scoprius*

	Compound[a]	RI[b]	MW[c]	Fresh plant (FP) (%)	Dried plant (DP) (%)
1	α-Thujene	924	136	5.3	5.7
2	α-Pinene	931	136	34.4	23.6
3	Dehydrosabinene	940	134	–	<0.1
4	Camphene	944	136	0.2	<0.1
5	Verbenene	949	134	<0.1	0.1
6	Sabinene	971	136	16.3	26.5
7	β-Pinene	973	136	5.5	2.4
8	Myrcene	990	136	1.4	1.0
9	α-Phellandrene	1003	136	0.3	0.4
10	δ-Carene	1008	136	1.9	–
11	α-Terpinene	1014	136	<0.1	0.7
12	p-Cymene	1022	134	10.1	8.6
13	Limonene	1026	136	1.9	1.3
14	*trans*-β-Ocimene	1037	136	3.9	1.2
15	γ-Terpinene	1055	136	0.2	1.9
16	*cis*-Sabinene hydrate	1063	154	0.3	0.2
17	Terpinolene	1085	136	0.2	0.7
18	α-Thujone	1113	152	<0.1	0.2
19	p-Menth-2-en-1-ol	1117	154	0.3	0.5
20	α-Campholene aldehyde	1122	152	<0.1	0.1
21	Alloocimene	1128	136	0.3	<0.1
22	Pinocarveol	1134	152	0.3	0.2
23	*cis*-Verbenol	1141	152	0.6	0.5
24	Sabina ketone	1152	138	<0.1	0.2
25	Terpinen-4-ol	1174	154	4.1	9.7
26	p-Cymen-8-ol	1182	150	0.6	0.9
27	α-Terpinoeol	1187	154	0.3	0.5
28	Myrtenal	1191	150	0.4	0.6
29	Estragole	1195	148	0.1	0.9
30	Verbenone	1204	150	0.4	0.5
31	*trans*-Carveol	1218	152	<0.1	<0.1
32	*cis*-Carveol	1222	152	<0.1	<0.1
33	Cuminaldehyde	1239	148	<0.1	<0.1
34	Carvone	1244	150	0.2	–
35	Phellandral	1278	152	<0.1	<0.1
36	Carvacrol	1311	150	0.2	–
37	α-Copaene	1374	204	0.2	–
38	β-Damascenone	1382	190	<0.1	<0.1
39	β-Cubebene	1387	204	0.1	–
40	Methyl eugenol	1403	178	0.2	–
41	α-Humulene	1447	204	–	<0.1
42	α-Muurolene	1496	204	<0.1	–
43	δ-Cadinene	1519	204	0.2	0.2
44	α-Calacorene	1536	200	<0.1	0.1
45	Spathulenol	1571	220	1.5	0.8
46	Caryophyllene oxide	1575	220	0.3	0.5

TABLE 2 (Continued)

	Compound[a]	RI[b]	MW[c]	Fresh plant (FP) (%)	Dried plant (DP) (%)
47	τ-Muurolol	1638	222	0.7	1.1
48	β-Eudesmol	1644	222	0.2	0.6
	Total			93.5	92.8
	Monoterpene hydrocarbons			71.7	65.7
	Oxygenated monoterpenes			7.2	13.4
	Aromatic monoterpenes			11.3	10.4
	Sesquiterpene hydrocarbons			0.6	0.4
	Oxygenated sesquiterpenes			2.8	3.0

[a]Compounds listed in order of elution from a ZB-5HT fused silica capillary column.
[b]Retention indices of the compounds calculated on the basis of Van den Dool and Kratz formula.
[c]Molecular Weight for MW.

in the study of the previous authors. The latters stipulated that the qualitative and quantitative differences could be due to climatic and geographical conditions (difference of period and of geographic area of collection or both), or to the existence of different chemotypes. Indeed (Lograda et al., 2013), confirmed the existence of three chemotypes in Algerian populations of *P. scoparius* and that the variability of terpenoids reflects the heterogeneity of the genetic structure of *Pituranthos scoparius*. The chemical profile of the essential oil in our study falls in the Sabinene chemotype, as this chemotype is widespread among populations found in northeastern Algeria.

4.5 | TLC–DPPH• dipping method of the essential oil

Recently, a new technique combining DPPH• with TLC was reported by (Cieśla et al., 2012). This technique was carried out on (FP) and (DP) essential oil, after developing the plate as previously described. The images were taken before (366 nm) and after (Vis R) dipping in DPPH• and the result is depicted (Figure 1b). From the latter, two intense and bright bands were observed for the essential oil. FP showed a higher intensity under 366 nm, while DP showed a higher intensity under remission illumination light (Vis R). This can be explained likely by the fact that compounds of DP interact highly with DPPH• after dipping. Due to their redox properties, essential oils and their bioactive constituents can act as antioxidants (Kasrati, Jamali, Bekkouche, Wohlmuth, Leach, & Abbad, 2014) when their chemical structure allows a free radical scavenging reaction and/or the chelation of redox-active metals (Fraga, Oteiza, & Galleano, 2014). The brightening intensity of the bands observed may suggest the power of the radical scavenging activity related to the compounds present in the essential oil studied and/or a synergistic phenomena among the bioactive compounds contained. The presence of oxygenated monoterpenes in DP (13.4%) may play a major role and contribute significantly to the antioxidant activity. Terpenes of several essential oils, flavonoids, and phenolics exhibit significant antioxidant effects (Raut & Karuppayil, 2014). An oxygenated monoterpene (terpinen-4-ol), present as a major compound in DP (9,7%), can be considered as a potential cantributor to the antioxidant activity. Due to its high structural similarity to thymol and carvacrol (well known as the best antioxidants in several essential oils), it is believed to act efficiently against lipid oxidation. Two schemes of interaction between the antioxidant species can be proposed: hydrogen donation of the more active antioxidant to regenerate the other antioxidant and formation of heterodimer from the moieties of the antioxidant during autoxidation (de Guzman, Tang, Salley, & Ng, 2009). The antioxidant capacity of the plant extracts is due to the presence of bioactive compounds acting as hydrogen donors to the radical involved in the oxidation reaction (Cordeiro et al., 2013).

4.6 | Oxidative stability by the rancimat method

The effect of the essential oil incorporation, with different concentrations on oxidative stability of the whey spread emulsion was evaluated by Rancimat equipment. The hydrodistilled essential oil from the dried state of the plant material (DP) is chosen for the incorporation, based on the results of TLC–DPPH• method and its content in oxygenated monoterpenes (Table 2).

The essential oil of *P. scoparius* was found to be more effective in stabilizing the emulsion against oxidative deterioration as compared to the control emulsion containing the α-tocopherol as antioxidant. The recorded induction period for the emulsion added with 100 mg kg^{-1} of the essential oil of *P. scoparius* (20.80 ± 0.03)h is higher than the latter (14.41 ± 0.04) h ($\rho \leq 0.05$). The blend of the control emulsion contains PO, SFO, and SBO. According to Lee, Jeung, Park, Lee, & Lee (2010) and Seppanen, Song, & Saari Csallany (2010), the degradation of tocopherols was temperature sensitive in sunflower and soybean oils and even for the α-tocopherol added as an antioxidant. The concentration of 100ppm of the essential oil added into the emulsion is determined according to the internal standards of the food industry. Therefore, the addition of antioxidants in low concentrations into a fat system was very important for its oxidative stability (Rios, Santos, Maia, & Mazzetto, 2013).

These results are strongly supported by the data previously obtained, displaying the occurrence of bioactive molecules in the essential oil (terpenes), responsible for the antioxidant activity. Indeed, it is suggested from this study that the essential oil of *P. scoparius* could improve significantly the preservation process, delay lipid oxidation phenomena and therefore ensure a high oxidative stability of fat

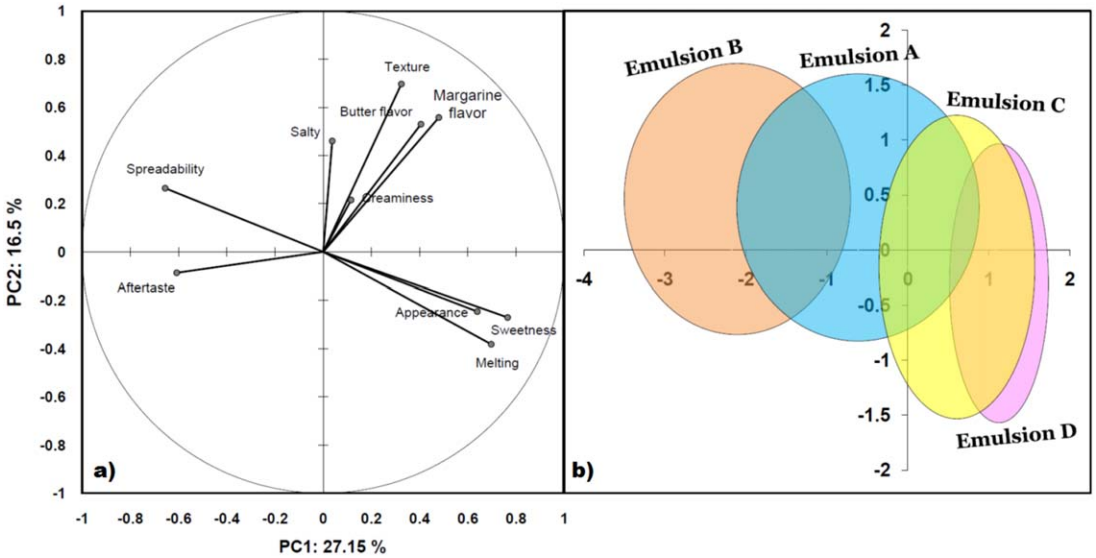

FIGURE 5 Principal component analysis (PCA) of four emulsions: emulsion A (control emulsion), emulsion B (emulsion containing 100 ppm essential oil), emulsion C (emulsion containing 300 ppm essential oil), and emulsion D (emulsion containing 600 ppm essential oil). The attributes assessed were spreadability, creaminess, sweetness, melting, appearance, texture, butter-like flavor, margarine-like flavor, salty, and aftertaste

products. There is a strong evidence from recent reserches that antioxidants play a very important role in increasing the oxidative stability of food emulsions (Lante & Friso, 2013). This can be also translated by improving the shelf life of these products, acting even better than α-tocopherol, which is widespreadly used in fat and oil industry.

MHE–GC–FID–MS allowed to quantify and identify the major terpene groups of the essential oil from the fresh plant material. Since antioxidants are commonly used under conditions of high temperature in food processing and cooking (Nakatani, Tachibana, & Kikuzaki, 2001), Rancimat test can be representative of the extent at which these antioxidants can protect the food emulsion from oxidation. Highest oxidative stability was obtained for the emulsion incorporated with the essential oil. From Table 2 and Figure 4, an oxygenated monoterpene, namely terpinen-4-ol, was detected in the essential oil and accounts for the major components of DP essential oil. This latter is likely responsible for the antioxidant activity of the essential oil, due to the hydroxyl group located on the aromatic ring, as the CHO fraction is the most potent antioxidant (Kulisic, Radonic, Katalinic, & Milos, 2004). Its structure is close to other two phenol terpenes: thymol and carvacrol, responsible for the antiocxidant activity of most of essential oils. Practically, these phenols take part in chain initiation during oxidation with an order of magnitude higher than α-tocopherol, then reacting faster with lipids (Ruberto & Baratta, 2000; Yanishlieva, Marinova, Gordon, & Raneva, 1999). Also, from Table 2, two abundant components were detected: γ-terpinene and p-cymene. In fact, The mechanism of chain termination during the oxidation of γ-terpinene has been investigated in detail by (Amorati, Foti, & Valgimigli, 2013), hypothesizing that the driving force for the overall process is the formation of aromatic p-cymene and the very fast decay of neutral superoxide radical. In other terms, the presence of γ-terpinene will cause an overall increase in the rate of oxidative chain termination, thereby shortening the chain length and reducing the overall rate of oxidation. From our findings, the essential oil of *P. scoparius* gave a high protection for the emulsion from oxidation, even better than α-tocopherol. Moreover, Prakash, Kedia, Mishra, and Dubey (2015) reported that most of the essential oils are kept under the "GRAS" category by the FDA (Food and Drug Administration) and are approved as flavors and food additives. Taking into account these data, it can be postulated that the addition of antioxidant properties of each individual antioxidant could benefit from a synergistic effect. Moreover, the synergy amoung minor oxygen containing compounds has a determining role in the overall antioxidant activity expressed by the essential oil (Chougui et al., 2015; Kulisic et al., 2004).

4.7 | Sensory evaluation

Principal component analysis (PCA) is a multivariate data compresssion technique that allows multiple treatments to be graphically displayed as they are differentiated by multiple variables. Therefore, it is usually applied to assess how several products were differentiated by several sensory descriptors (Drake, 2007).

For a better visualization of the relationships between the sensory attributes evaluated, a principal component analysis (PCA) was performed (Figure 5a). The two first axes explained 43.6% of the variability and allowed to visualize the projection of the four studied emulsions (Figure 5b). The first principal component (PC) explaining 27.15% of variability was mainly characterized by sweetness, melting and appearance on the right positive side of the axis and spreadability and aftertaste on the left negative side. The second PC explaining 16.5% of variability was mainly characterized by margarine flavor, butter flavor and texture on the right positive side of the axis. The PCA allowed the separation of emulsions B (100 ppm

essential oil) and C (300 ppm essential oil), D (600 ppm essential oil) in comparison to the emulsion A (control emulsion, 100 ppm α-tocopherol). Emulsion B was appreciated for its spreadability and aftertaste as these both attributes are projected on the right side of the PCA. In contrast, emulsion C and D are characterized by sweetness, melting and appearance. This tendency can be explained by the concentration of the essential oil in each emulsion. Since emulsions C and D are positioned close to each other, we can assume that the tasters did not differenciate between the two emulsions containing 300 and 600 ppm as they did for the emulsion containing 100 ppm. As the essential oil displayed the presence of volatiles (data obtained above), their perception by the tasters differ according to their content in the emulsions.

According to Moskowitz and Krieger (1995) foods are complex matrices and it is difficult to isolate the effect of a sensory input without confounding with other sensory inputs. Taste/flavor, texture and appearance are respectively the most important attributes in terms of attribute liking on an aggregate basis.

One advantage from the PCA study is that the influence of some attributes can be highlighted, therefore taken as choice indicators for tasters (Chikhoune et al., 2014).

5 | CONCLUSION

From the present study, we can conclude that *P. scoparius* is a recoverable source of antioxidants in food industry, particularily in fat and oil industry. The results of antioxidant activity of the essential oil are encouraging to enhance its use in the field of food preservation. Indeed, its traditional use for health benefits, preservation from bacterial adulteration and aromatization in traditional cheese-making offers an opportunity to use it in fat and oil products. The results of Rancimat proved the valuable antioxidant power of the essential oil as a natural plant preservative and a green alternative to synthetic antioxidants. Its monoterpenes composition explains, for a great part, its radical-scavenging properties. Our results are promising when considered in light that these antioxidants are excellent biopreservative candidates, to replace the commonly used α-tocopherol. It would be intresting to perform an intensive cultivation of the plant in the locations of high plateau, to develop a local economic strategy for the use and the valorization of the plant. Also, it is prominent to set up pilot plants for the extraction of the essential oil together with the adaptation of the set in a food processing chain. Other studies are needed also to improve the large scale cultivation of the plant, through the involvement of agronomists and botanists in order to encourage its use as a food additive.

ACKNOWLEDGMENTS

The authors deeply thank the staff of CEVITAL for their support and assistance. Special thanks to Mr. Abderrahmane TOUNSI, head of laboratory of Quality Control for Fat Products, Mr. Samir HADJAL, Director of Research and Development and Azeddine MAOUCHE, Head of Department for Fat and Oils Quality Control for their valuable help and assistance. The authors thank also INATAA, University of Brothers Mentouri Constantine for financial support and the National Institute of chemistry, together with the Slovenian Research Agency for their contribution to achieve this work.

REFERENCES

Adachi, S., Minten, S., & Kobayashi, T. (2009). Oxidation of lipid in bulk and dispersion systems. *Japan Journal of Food Engineering*, *10*, 9–15.

Amorati, R., Foti, M. C., & Valgimigli, L. (2013). Antioxidant activity of essential oils. *Journal of Agricultural and Food Chemistry*, *61*, 10835–10847.

Bellakhdar, J. (1997). La pharmacopée Marocaine Traditionnelle. Médecine arabe ancienne et savoirs populaires. Edition Ibis Press. Paris. pp. 164-165.

Boutaghane, N., Nacer, A., Kabouche, Z., & Ait-Kaki, B. (2004). Comparative antibacterial activities of the essential oils of stems and seeds of Pituranthos scoparius from Algerian septentrional Sahara. *Chemistry of Natural Compounds*, *40*, 606–607.

Calligaris, S., Manzocco, L., & Nicoli, M. C. (2007). Modelling the temperature dependence of oxidation rate in water-in-oil emulsions stored at sub-zero temperatures. *Food Chemistry*, *101*, 1019–1024.

Chikhoune, A., Bedjou, F., Oubouzid, S., Boukefoussa, R., Bechri, B., Tarmoul, H., Abdeladim, T., . . . Kouadri, L. (2014). Development of sugar cane molasses in formulations of madeleines, mini croissants, and buns incorporated with interesterified oil. *Journal of Chemistry*, *2014*, 8.

Chougui, N., Djerroud, N., Naraoui, F., Hadjal, S., Aliane, K., Zeroual, B., & Larbat, R. (2015). Physicochemical properties and storage stability of margarine containing Opuntia ficus-indica peel extract as antioxidant. *Food Chemistry*, *173*, 382–390.

Cieśla, Ł., Kryszeń, J., Stochmal, A., Oleszek, W., & Waksmundzka-Hajnos, M. (2012). Approach to develop a standardized TLC-DPPH test for assessing free radical scavenging properties of selected phenolic compounds. *Journal of Pharmaceutical and Biomedical Analysis*, *70*, 126–135.

Cordeiro, A. M. T. M., Medeiros, M. L., Santos, N. A., Soledade, L. E. B., Pontes, L. F. B. L., Souza, A. L., Queiroz, N., & Souza, A. G. (2013). Rosemary (Rosmarinus officinalis L.) extract. *Journal of Thermal Analysis and Calorimetry*, *113*, 889–895.

de Guzman, R., Tang, H., Salley, S., & Ng, K. S. (2009). Synergistic effects of antioxidants on the oxidative stability of soybean oil-and poultry fat-based biodiesel. *Journal of the American Oil Chemists' Society*, *86*, 459–467.

Drake, M. A. Invited review: Sensory analysis of dairy foods. *Journal of Dairy Science*, *90*, 4925–4937.

Fraga, C. G., Oteiza, P. I., & Galleano, M. (2014). *In vitro* measurements and interpretation of total antioxidant capacity. *Biochimica Et Biophysica Acta (BBA)-General Subjects*, *1840*, 931–934.

Gómez-Estaca, J., López-de-Dicastillo, C., Hernández-Muñoz, P., Catalá, R., & Gavara, R. (2014). Advances in antioxidant active food packaging. *Trends in Food Science and Technology*, *35*, 42–51.

Holley, R. A., & Patel, D. (2005). Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. *Food Microbiology*, *22*, 273–292.

Kasrati, A., Jamali, C. A., Bekkouche, K., Wohlmuth, H., Leach, D., & Abbad, A. (2014). Comparative evaluation of antioxidant and insecticidal properties of essential oils from five Moroccan aromatic herbs. *Journal of Food Science and Technology*, *52*, 2312–2319.

Kiokias, S., Dimakou, C., Tsaprouni, I., & Oreopoulou, V. (2006). Effect of compositional factors against the thermal oxidative deterioration of novel food emulsions. *Food Biophysics*, *1*, 115–123.

Kulisic, T., Radonic, A., Katalinic, V., & Milos, M. (2004). Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chemistry*, *85*, 633–640.

Lante, A., & Friso, D. (2013). Oxidative stability and rheological properties of nanoemulsions with ultrasonic extracted green tea infusion. *Food Research International*, *54*, 269–276.

Lee, S. W., Jeung, M. K., Park, M. H., Lee, S. Y., & Lee, J. (2010). Effects of roasting conditions of sesame seeds on the oxidative stability of pressed oil during thermal oxidation. *Food Chemistry*, *118*, 681–685.

Lograda, T., Ramdani, M., Kiram, A., Chalard, P., & Figueredo, G. (2013). Variation of essential oils composition of Pituranthos scoparius in Algeria. *Global Journal of Research on Medicinal Plants and Indigenous Medicine*, *2*, 1–9.

Mondello, L., Casilli, A., Tranchida, P., Dugo, P., & Dugo, G. (2005). Comprehensive two-dimensional GC for the analysis of citrus essential oils. *Flavour and Fragrance Journal*, *20*, 136–140.

Moskowitz, H. R., & Krieger, B. (1995). The contribution of sensory liking to overall liking: An analysis of six food categories. *Food Quality and Preference*, *6*, 83–90.

Nakatani, N., Tachibana, Y., & Kikuzaki, H. (2001). Establishment of a model substrate oil for antioxidant activity assessment by oil stability index method. *Journal of American Oil Chemists Society*, *78*, 19–23.

Nègre, R. (1961). Petite flore des régions arides du Maroc occidental. Paris: CNRS.

Nenaah, G. E. (2014). Chemical composition, toxicity and growth inhibitory activities of essential oils of three Achillea species and their nano-emulsions against Tribolium castaneum (Herbst). *Industrial Crops and Products*, *53*, 252–260.

Ponginebbi, L., Nawar, W. W., & Chinachoti, P. (1999). Oxidation of linoleic acid in emulsions: Effect of substrate, emulsifier, and sugar concentration. *Journal of American Oil Chemists Society*, *76*, 131–138.

Pothier, J., Galand, N., El Ouali, M., & Viel, C. (2001). Comparison of planar chromatographic methods (TLC, OPLC, AMD) applied to essential oils of wild thyme and seven chemotypes of thyme. *Il Farmaco*, *56*, 505–511.

Prakash, B., Kedia, A., Mishra, P. K., & Dubey, N. (2015). Plant essential oils as food preservatives to control moulds, mycotoxin contamination and oxidative deterioration of agri-food commodities—Potentials and challenges. *Food and Control*, *47*, 381–391.

Purcaro, G., Cordero, C., Liberto, E., Bicchi, C., & Conte, L. S. (2014). Toward a definition of blueprint of virgin olive oil by comprehensive two-dimensional gas chromatography. *Journal of Chromatography A*, *1334*, 101–111.

Raut, J. S., & Karuppayil, S. M. (2014). A status review on the medicinal properties of essential oils. *Industrial Crops and Products*, *62*, 250–264.

Rios, M. S., Santos, F. P., Maia, F. N., & Mazzetto, S. (2013). Evaluation of antioxidants on the thermo-oxidative stability of soybean biodiesel. *Journal of Thermal Analysis and Calorimetry*, *112*, 921–927.

Ruberto, G., & Baratta, M. T. (2000). Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chemistry*, *69*, 167–174.

Seeley, J., Seeley, S., Libby, E., Breitbach, Z., & Armstrong, D. (2008). Comprehensive two-dimensional gas chromatography using a high-temperature phosphonium ionic liquid column. *Analytical and Bioanalytical Chemistry*, *390*, 323–332.

Seppanen, C., Song, Q., & Saari Csallany, A. (2010). The antioxidant functions of tocopherol and tocotrienol homologues in oils, fats, and food systems. *Journal of American Oil Chemists Society*, *87*, 469–481.

Shashkov, M., & Sidelnikov, V. (2013). Properties of columns with several pyridinium and imidazolium ionic liquid stationary phases. *Journal of Chromatography: Part A*, *1309*, 56–63.

Vérité, P., Nacer, A., Kabouche, Z., & Seguin, E. (2004). Composition of seeds and stems essential oils of Pituranthos scoparius (Coss. & Dur.) Schinz. *Flavour and Fragrance Journal*, *19*, 562–564.

Yanishlieva, N. V., Marinova, E. M., Gordon, M. H., & Raneva, V. G. (1999). Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. *Food Chemistry*, *64*, 59–66.

How to cite this article: Chikhoune A, Damjan Pavleca J, Shashkov M, et al. Antioxidant effect induced by the essential oil of *Pituranthos scoparius* in a formulation of a whey spread emulsion. *J Food Process Preserv*. 2017;41:e13163. https://doi.org/10.1111/jfpp.13163