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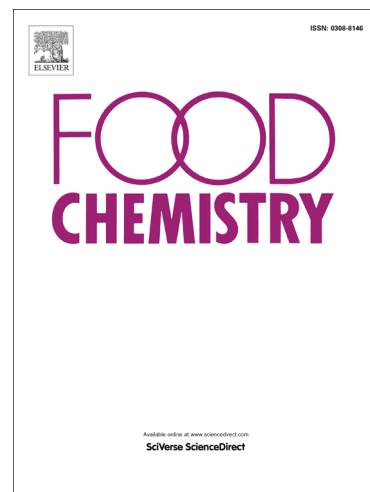
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Composition, antibacterial and antioxidant activities of *Pimpinella saxifraga* essential oil and application to cheese preservation as coating additive

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Abstract

The effect of *Pimpinella saxifraga* essential oil (PSEO) addition (1-3%) in sodium alginate coating on the bacterial and oxidative stability of cheese was studied during refrigerated storage. The GC-HRMS analysis of PSEO showed that anethole, pseudoisoeugenol and *p*-anisaldehyde were the main components. The PSEO exhibited strong *in vitro* DPPH radical scavenging activity ($IC_{50} = 6.81 \mu\text{g/mL}$), β -carotene bleaching inhibition ($IC_{50} = 206 \mu\text{g/mL}$), ferric reducing power ($EC_{50} = 35.20 \mu\text{g/mL}$), total antioxidant activity ($213.96 \pm 11.12 \mu\text{mol/mL}$ α -tocopherol equivalent) and notable DNA protection potential. Additionally, PSEO displayed potent antibacterial activity against 3 Gram-positive and 3 Gram-negative bacteria (MICs = 0.78-3.12 mg/mL). The acute toxicity of PSEO was determined using mice model ($LD_{50} = 976.2 \text{ mg/kg}$). The enrichment of sodium alginate coating with PSEO, particularly at 3%, improved cheese preservation by reducing the weight loss, preserving the pH and color and enhancing oxidative and bacterial stability without unpleased flavor for consumers.

Keywords: *Pimpinella saxifraga* essential oil, chemical composition, anethole, antibacterial and antioxidant activities, acute toxicity, coating, bacterial and oxidative stability, sensory analysis

1. Introduction

Essential oils are natural mixtures of volatile and versatile compounds characterized by a strong odor. They are secondary metabolites synthesized by plants to protect them against viruses, microorganisms and insects. They can be produced by all the organs of aromatic plants namely buds, flowers, leaves, stems, seeds, fruits and roots, then stored in secretory epidermal cells, canals, glandular trichomes and cavities (Sangwan, Farooqi, Shabih, & Sangwan, 2001). Essential oils were basically used in folk medicine as analgesic, antispasmodic, anti-rheumatic and anti-epileptic (Adorjan & Buchbauer, 2010). Nowadays, there is a growing interest in the use of the essential oils, endowed with biological activities, in pharmaceutical, cosmetic and food fields in order to respond to critical problems such as bacterial resistance and synthetic antioxidants (Burt, 2004). In fact, controlling bacterial infections is becoming an important issue as a result of the developed resistance of bacteria against a large spectrum of commercial antibiotics (Fair & Tor, 2014). Another matter of concern is the use of synthetic antioxidants which has been suspected to have harmful effects on human health such as toxic and carcinogenic risks (Augustyniak et al., 2010). Thus, the increasing bacterial resistance and the intensive demands for natural antioxidants have motivated the researchers to explore plants as a source of new components with antimicrobial and antioxidant potential such as essential oils.

In plant kingdom, the essential oil is a very complex combination of almost 20 to 60 compounds at considerably different concentrations which influences their biological properties. For example, the Apiaceae family is the focus of many studies due to its large number of aromatic plants producing essential oils with antibacterial and antioxidant activities like caraway, cumin, parsley, anise, dill, fennel and coriander (Khalil, Ashour, Fikry, Singab, & Salama, 2017). In this case, anethole, a terpenoid widely found in Apiaceae essential oils and usually used as sweet flavoring agent, is known to be highly effective against bacteria and fungi (Marinov & Valcheva-

Kuzmanova, 2015). Hence, the analysis of the essential oil composition is primordial to predict its biological effects.

Essential oils are conventionally known as natural safe components with antimicrobial effect but once the required doses are exceeded, they can become toxic and damage the human health (Tisserand & Young, 2013). Consequently, the study of the acute toxicity of essential oil is crucial to a non-toxic use especially in food industry.

Essential oils are usually lipophilic, volatile and almost insoluble in water. As it is a challenge to incorporate them directly in food, they need to be introduced in a matrix that is compatible with food applications, serving as vector of the bioactive components of essential oils and reducing their possible toxicity (Ju et al., 2018). Sodium alginate, a polysaccharide obtained from seaweeds, is film-forming compound with great mechanical capacities and usually used as potential coating material in foods and drugs (Tavassoli-Kafrani, Shekarchizadeh, & Masoudpour-Behabadi, 2016). In order to improve the shelf life of food, active sodium alginate coatings endowed with antibacterial effect have been developed by the addition of essential oils (Tavassoli-Kafrani et al., 2016).

The plant *Pimpinella saxifraga* of the Apiaceae family was screened for essential oil extracted from aerial parts and roots (Grys et al., 2009; Masoudi, Rustaiyan, & Mazloomifar, 2009), while the present work focuses on the essential oil of *P. saxifraga* seeds. Thus, the purposes of the current study are to investigate the chemical composition of *P. saxifraga* essential oil (PSEO) and evaluate its antibacterial and antioxidant activities. The acute toxicity of PSEO is also studied by the assessment of the median lethal dose (LD₅₀) on mice model. Furthermore, this work describes the effect of PSEO incorporation in sodium alginate coating on the bacterial and oxidative stability of cheese during refrigerated storage. This study will contribute to the progress of the use of new natural bioactive compounds in food preservation.

2. Materials and methods

2.1. Essential oil extraction

The seeds of *P. saxifraga* were collected from Sidi Bouzid in Tunisia between April and November 2016 and identified in Faculty of Sciences of Sfax (Tunisia). The essential oil was extracted by hydrodistillation of ground seeds (25 g) for 3 h in a Clevenger-type apparatus as described by Lo Cantore, Iacobellis, De Marco, Capasso, & Senatore (2004). This operation was repeated ten times and PSEO fractions were pooled to obtain enough material for experiments. The essential oil was dried using anhydrous sodium sulfate and stored in a dark glass vial at 4 °C until analysis.

2.2. GC-HRMS Orbitrap analysis

The volatile compounds were identified by GC-MS/MS (QExactive™ GC Orbitrap™, Thermo-Fisher, Villebon, France), using BP5 MS capillary column (SGE, 30 m x 0.25 mm, 0.25 μm). The carrier gas was helium at a flow rate of 1.2 mL/min. The oven temperature was initially held at 40 °C for 2 min after injection, then increased to 350 °C with a 3 °C/ min heating ramp and kept at 350 °C for 2 min. The PSEO (1 μL) was injected at 250 °C on mode splitless or with split ratio of 1/5. Mass spectrometer monitoring and spectral processing were released by Xcalibur software (Ver. 4.0.27) and the 2014 NIST database and an internally elaborated HRMS library. Mass spectra of electronic ionization and chemical ionization were recorded. The HRMS system acquired data in the range of 50-550 m/z at 60,000-resolution mode with an energy of 70 eV for electronic ionization. Methane was used at a flow rate of 1.5 mL/min in chemical ionization. The spectrometer was calibrated externally.

2.3. Antioxidant activity

2.3.1. DPPH radical scavenging capacity

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging ability of PSEO was assessed as described by Bersuder et al. (1998). The DPPH radical scavenging capacity was determined by the following formula:

$$\text{DPPH radical scavenging activity (\%)} = (\text{OD}_0 - \text{OD}_1) / \text{OD}_0 \times 100$$

where OD_0 was the absorbance of the control (without the PSEO sample) and OD_1 was the absorbance of the test sample (PSEO).

The inhibitory concentration (IC_{50}) of PSEO at which DPPH radicals were scavenged by 50% was assessed by linear regression of the concentration-response curve. The synthetic commercial antioxidant Butylated hydroxyl-anisole (BHA) was used as standard. The test was conducted in triplicate.

2.3.2. β -carotene bleaching assay

The inhibition of β -carotene bleaching by PSEO was assessed as described by Marco (1968). First, a β -carotene/linoleic acid emulsion was prepared by dissolving 0.5 mg of β -carotene, 25 μL of linoleic acid and 200 μL of Tween 40 in 1 mL of chloroform. The chloroform was then totally evaporated under vacuum using a rotatory evaporator at 50 °C. A volume of 100 mL of distilled water was added and the resulting emulsion was vigorously stirred. Thereafter, 2.5 mL of the β -carotene/linoleic acid emulsion were mixed with 0.5 mL of PSEO at different concentrations (100-700 $\mu\text{g}/\text{mL}$). The absorbance was measured at 470 nm before and after incubation at 50 °C for 2 h by UV-Vis spectrophotometry (Agilent, USA). BHA was the positive control. Tests were conducted in duplicate and the β -carotene bleaching inhibition was calculated using the following formula:

$$\text{Inhibition of } \beta\text{-carotene bleaching (\%)} = [1 - ((OD_0 - OD_t) / (OD'_0 - OD'_t))] \times 100$$

where OD_0 and OD_t were the absorbances of the test sample (PSEO) measured before and after incubation, respectively; and OD'_0 and OD'_t were the absorbances of the control measured before and after incubation.

2.3.3. Reducing power assay

The capacity of PSEO to reduce iron (III) was evaluated using the approach of Yildirim et al. (2001). The absorbance was recorded at 700 nm. Higher absorbance of test sample indicated higher iron reducing capacity. The commercial antioxidant BHA was used as positive control. The test was conducted in duplicate. The EC_{50} value, defined as the effective concentration at which the absorbance was 0.5 at 700 nm, was obtained by linear regression analysis.

2.3.4. Total antioxidant activity

The total antioxidant capacity was determined on the basis of the reduction of Mo (VI) by PSEO to Mo (V) leading to the formation of a green phosphate-Mo (V) complex in acid solution (Prieto, Pineda, & Aguilar, 1999). The total antioxidant capacity was considered in terms of α -tocopherol equivalents ($\mu\text{mol/mL}$). The test was done in duplicate.

2.3.5. DNA nicking assay

DNA nicking assay was performed using the method of Lee et al. (2002). The PSEO at the concentration of 0.625, 1.25 and 2.5 mg/mL was mixed with plasmid DNA, respectively. Native plasmid was used as control. Fenton's reagent was added after incubation. The mixture was then incubated for 5 min at 37 °C. The DNA was analyzed on 0.8% (w/v) agarose gel electrophoresis and visualized under ultraviolet light. The intensity of DNA was detected by Bio-Rad's Image Lab software (version 2.0). The retention of supercoiled plasmid DNA strand (%) was calculated by the following formula:

$$\text{Retention (\%)} = \frac{\text{Intensity of supercoiled DNA of sample}}{\text{Intensity of supercoiled DNA of control}} \times 100$$

2.4. Antibacterial activity

2.4.1. Bacterial strains

The antibacterial activity was studied against 6 bacterial strains from the American Type Culture Collection: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella* Typhimurium (ATCC 19430), *Listeria monocytogenes* (ATCC 19117), *Micrococcus luteus* (ATCC 4698) and *Bacillus cereus* (ATCC 11778) which were obtained from the microbial collection of the Centre of Biotechnology of Sfax (Tunisia).

2.4.2. Agar diffusion method

The antibacterial activity was assessed as described by Valgas, Souza, Smânia, & Smânia Jr. (2007). Culture suspensions (300 µL) of test microorganisms (10^6 colony forming units (CFU/mL) estimated by absorbance measurement at 600 nm) were spread on Luria-Bertani agar (LB, Thermo-Fisher, Villebon, France). Then, 60 µL of PSEO (1% w/v in dimethyl sulfoxide (DMSO)) were loaded into wells (6 mm in diameter) already punched in the agar layer. The Petri dishes were kept at 4 °C for 1 h and then incubated for 24 h at 37 °C. Antimicrobial activity was estimated by measuring the zone of growth inhibition (diameter expressed in millimeters) around the wells. Gentamicin was used as positive standard to determine the sensitivity of bacterial strains and DMSO was used as negative control. The antibacterial activity was performed in triplicate.

2.4.3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of PSEO which inhibits the visible bacterial growth after overnight incubation (Andrews, 2001). It was determined as reported by Valgas, Souza, Smânia, & Smânia Jr. (2007) in sterile 96-well

microplates with a final volume of 200 μL per well. Two fold serial dilution of PSEO (dissolved in Tween 80 (1% v/v)) was performed in LB broth medium. Thereafter, 20 μL of each bacterial suspension was inoculated. Each well included 100 μL of PSEO diluted in LB broth medium, 80 μL of the growth medium (LB) and 20 μL of cell suspension (10^6 CFU/mL). Bacteria only in LB medium and Tween 80 (1% v/v) were used as positive and negative controls, respectively. After the incubation of the plates for 24 h at 37 °C, 25 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (0.5 mg/mL) were added to the wells. The reaction of viable cells with MTT generates violet color. To estimate the MBC, an aliquot of 20 μL from each well presented no visible growth were spread on LB dishes. The minimum bactericidal concentration (MBC) was the lowest concentration of antibacterial agent that killed at least 99.9% of the starting inoculum after 24 h of incubation at 37° C (Andrews, 2001). The determination of MIC and MBC values was assessed in duplicate (expressed in mg/mL).

To determine the MBC, aliquots of 20 μL from all dilutions not showing any bacterial growth were spread onto LB agar plates and incubated (37 °C, 24 h). The minimum concentration for which there is no visible growth on agar plate was recorded as MBC

2.5. Acute toxicity

2.5.1. Animals

In this study, a population of Balb/C mice (24–29 g) of female sex was divided into 8 groups of 8 mice each. The mice were fasted for 12 h before measuring weight and they were used only once in this experiment. Standard pellet diet and water were provided to all groups. The experimental protocol was approved by the Local Animal Care Committee at Sfax University, Tunisia. The manipulation procedure applied in this study was in conformity with the International Guidelines for Animal Care (The European Parliament and the Council of the European union, 2010).

2.5.2. Determination of the median lethal dose (LD₅₀)

Serial doses of 25, 50, 75, 100, 150 and 350 mg/mL of PSEO diluted in Tween 80 (1% v/v), as described by Hilan et al. (2009), were administrated by intraperitoneal injection to 6 groups in a proportion of 0.01 mL/g of mice body weight. One group, served as vehicle control, received Tween 80 (1% v/v) and one untreated group served as normal control. All groups were placed under observation, symptoms of toxicity were noted and the mortality rate was checked for mice during the next 15 days. By the end of this period, dead mice were counted to determine the LD₅₀ by the logarithmic regression analysis of the dose-response curve. The mortality rate is calculated using the following formula:

$$\text{Mortality rate (\%)} = \frac{\text{Number of dead mice in a group}}{\text{Total number of mice in a group (N=8)}} \times 100$$

2.6. Cheese coating with alginate containing *P. saxifraga* essential oil

2.6.1. Preparation of cheese samples

Sodium alginate (SA: High molecular weight sodium alginate Protanal[®] RF6650; FMC, Belgium) coating solutions (2%, w/v) were prepared by dissolving 2 g in 100 mL distilled water with constant stirring at 50 °C until complete dissolution. Glycerol (15%, w/w, based on SA content) was then added and the mixture was stirred for 2 h at room temperature. The PSEO at different proportions 1%, 2% and 3% (w/w, based on SA content) was then added to each SA coating solution respectively. The mixtures were maintained under continuous stirring (DLS Stirrer, Velp Scientifica Germany, 500 rpm) to obtain homogeneous solutions. Thereafter, “Béja Sicilian cheese” (fresh cheese) was cut into 24 uniform cubes (80 g), sizing 25 mm³, divided in six groups according to the treatment: untreated control samples, samples coated with SA solution only, samples coated with SA solutions containing 1%, 2% and 3% PSEO (w/w, based on SA content)

and samples coated with SA solution containing 1% of vitamin C (w/w, based on SA content) used as standard. Coating was applied by dipping Sicilian cheese cubes into the different SA solutions during two minutes at room temperature. Cheese samples were air-dried and stored at 4 °C in transparent polypropylene plastic boxes with 95% relative humidity until analyses.

2.6.2. Evaluation of weight loss

The weight loss of the cheese samples on days 4, 7 and 10 days was calculated by the following equation:

$$\text{Weight loss (\%)} = (W_0 - W_t) / W_0 \times 100$$

where W_0 is the initial weight of sample and W_t is the weight of the same sample after 4, 7 and 10 days of storage at 4 °C.

2.6.1. Evaluation of water activity, pH and color

The pH and a_w were measured by a Metrohm 744 pH meter (Metrohm, Switzerland) and a NOVASINA a_w Sprint TH-500 apparatus (Novasina, Switzerland), respectively. The measurements were performed at 25 °C. CIELAB coordinates ((lightness (L^*), redness (a^*) and yellowness (b^*)) of the different samples were recorded using a MS/Y-2500 spectrophotometer (Hunterlab, USA).

2.6.1. Microbiological analysis

The microbiological analysis was determined according to the approach of Berghe and Vlietinck (2011). Cheese (1 g) from the different groups was homogenized with 9 mL of 1% NaCl solution. Ten-fold serial dilutions of these homogenates were performed and used in bacterial enumeration. Total mesophilic bacteria counts were conducted by the pour plate approach using plate count agar (PCA; Sigma-Aldrich, USA) and incubated for 24 h at 37 ± 1 °C. Lactic acid bacteria counts were performed by surface seeding method using Man, Rogosa and Sharpe agar plates (MRS; Microexpress, India). After incubation for 24 h at 37 ± 1 °C, colonies counts were

recorded. Bacterial counts were presented as logarithms of colony-forming units per gram of cheese (log CFU/g).

2.6.2. Lipid peroxidation

Lipid peroxidation of samples throughout the storage period was evaluated according to the method reported by Buege and Aust (1978). The formation of thiobarbituric acid reactive substances (TBARS) such as malondialdehydes (MDA) was estimated on the basis of their reactivity with TBA in an acidic condition to generate pink colored chromosphere. Sicilian cheese sample (0.5 g) was homogenized with 525 μL of TBS (150 mM NaCl and 50 mM Tris, pH 7.4) and 375 μL of TCA-BHT (Butylated hydroxytoluene) (TCA 20%, BHT 1%) to precipitate proteins, and then centrifuged (1000 g, 15 min, 4 °C). A volume of 400 μL of the supernatant was mixed with 80 μL of HCl (0.6 M) and 320 μL of Tris-TBA (26 mM Tris and 120 mM TBA). The mixture was then incubated for 10 min at 90 °C. After cooling, the absorbance was recorded on a UV-Vis spectrophotometer (Agilent, USA) at 530 nm. The TBARS values were calculated based on a standard curve of MDA (expressed as μg MDA/100 g cheese).

2.6.3. Sensory analysis

The consumer acceptance of coated cheese was conducted by sensory evaluation inspired from Abid, Yaich, Hidouri, Attia, & Ayadi (2018) works. Cheese samples were served at room temperature (26 °C), within 24 h after preparation, under normal light conditions in paper plates marked with two-digit randomized codes. Color, flavor, odor, aspect, and texture were evaluated by a panel of 21 untrained random subjects of both genders using the five-point hedonic scale; 1 (Not acceptable), 2 (fair acceptable), 3 (acceptable), 4 (very acceptable), 5 (highly acceptable). Each subject tested approximately the same amount of each cheese sample.

2.6.4. Statistical analyses

Statistical analyses were performed by IBM SPSS ver.22.0. The mean differences between groups were estimated by Duncan test and compared using the one way analysis of variance (ANOVA). Differences were considered significant at p -value < 0.05 .

3. Results and discussion

3.1. Chemical composition of *Pimpinella saxifraga* essential oil

The extraction yield of the essential oil from *P. saxifraga* ground seeds was 1.12% (g essential oil/100 g dry weight). As observed in Table 1, the analysis of PSEO by GC-HRMS Orbitrap revealed 15 components representing 93.5% of the total composition. PSEO was mainly composed of anethole (59.47%) and pseudoisoeugenol (20.15%) belonging to the phenylpropenoids family (Supplementary Fig. 1). The study of Grys et al. (2009) showed that the essential oil of *P. saxifraga* roots collected from Poland contained limonene, α -pinene and p -cymen. However, the essential oil extracted from the whole aerial part of *P. saxifraga* in Iran was rich in trans- α -bergamotene, β -bisabolene and β -sesquiphellandrene (Masoudi et al., 2009). The observed essential oils composition changes could be related to geographical and plant differences. Previous studies have shown that the *Pimpinella* genus namely *P. anisum* and *P. anisetum* contain high amounts of anethole (80-94% and 82.8%, respectively) (Arslan, Gürbüz, Sarihan, Bayrak, & Gümüşçü, 2004; Tepe et al., 2006). This phenolic derivate was also detected in the essential oils of many species of the Apiaceae family such as *Foeniculum vulgare* (70.1%) and *Anethum graveolens* (11%) (Gulfraz et al., 2008; Singh, Maurya, Lampasona, & Catalan, 2005). The second most abundant compound in PSEO was pseudoisoeugenol (20.15%) which is an isomer of eugenol found in the essential oil of *P. anisum* fruits and roots (Santos et al., 1998). In fact, it has been proposed that pseudoisoeugenol is synthesized by NIH shift of anethole which involved the migration of a hydrogen atom on an aromatic ring during a hydroxylation reaction (Reichling & Rainer, 1990). This could explain the

simultaneous abundance of anethole and pseudoisoeugenol in PSEO. *P*-anisaldehyde and anisketone occurred at different concentrations (7.53 and 1.71%, respectively). These aromatic compounds are mostly present in aniseed essential oil but they could be found in other species especially those of the Apiaceae family like fennel (Arslan et al., 2004; Gulfraz et al., 2008).

3.2. Evaluation of the antioxidant activity of *P. saxifraga* essential oil

The antioxidant capacity of PSEO was evaluated using five complementary tests, namely the scavenging ability of the DPPH radicals, the β -carotene bleaching assay, the iron (III) reducing power ability, the total antioxidant activity and the oxidative damage DNA protective assay. The results are presented in Fig. 1.

3.2.1. DPPH radical scavenging capacity

Free radical scavenging is thought to be one of the main mechanisms exhibited by antioxidants to delay oxidative processes. DPPH \cdot is a stable free radical which accepts an electron or hydrogen radical to become a stable diamagnetic molecule. As shown in Fig. 1A, the DPPH radical scavenging activity increased with PSEO concentration increasing. PSEO exhibited a very strong radical scavenging ability ($IC_{50} = 6.81 \mu\text{g/mL}$). This result is similar to the IC_{50} obtained by Tepe et al. (2006) for the essential oils of *P. anisetum* (5.62 $\mu\text{g/mL}$) and *P. flabellifolia* (8.49 $\mu\text{g/mL}$). It has been proved in a previous study that pure anethole showed a moderate radical scavenging capacity in comparison with BHT (Freire, Morais, Catunda, & Pinheiro, 2005). Consequently, anethole presenting 59.47% of the PSEO, could not be only responsible of the DPPH radical scavenging activity. Thus, it is important to point out the contribution of the other compounds of PSEO in its scavenging capacity mainly pseudoisoeugenol which contains a hydroxyl group on its ring that probably acts as a hydrogen donator to stabilize the DPPH radical (Supplementary Fig. 1B).

3.2.2. The inhibition of β -carotene bleaching

This test is based on the loss of the yellow color of β -carotene due to its reaction with conjugated diene hydroperoxides resulting from linoleic acid oxidation in the emulsion (Marco, 1968). This β -carotene bleaching is retarded in presence of antioxidants. As revealed in Fig. 1B, PSEO protected β -carotene from bleaching reaching 80% of inhibition at 700 $\mu\text{g/mL}$ with an IC_{50} of 206 $\mu\text{g/mL}$. In fact, it is known that nonpolar antioxidants display strong antioxidative properties in emulsions because they concentrate at the lipid/air surface allowing high protection of the emulsion itself (Frankel, Huang, Kanner, & German, 1994). This explains the strong antioxidant activity exhibited by PSEO in the β -carotene/linoleic acid system. Previous studies recorded the capacity of the essential oils from Apiaceae family to protect β -carotene from bleaching like those of anise, dill (60.91% and 35.54% at 1 mg/mL, respectively) and coriander ($\text{IC}_{50}=230 \mu\text{g/mL}$) (Duarte, Luís, Oleastro, & Domingues, 2016; Nanasombat & Wimuttigosol, 2011).

3.2.3. Reducing power assay

Fe (III) reduction can be used as an indicator of electron-donating activity which reflects an important mechanism of antioxidant action. As observed in Fig. 1C, the reducing power of PSEO and BHA increased in a concentration-dependent manner. PSEO displayed strong reducing potential ($\text{EC}_{50} = 35.20 \mu\text{g/mL}$) as previously reported for *P. anisum* by Singh et al. (2008). The components present in the *P. saxifraga* and *P. anisum* essential oil could act as good reductants due to their electron donating capacity to transform Fe^{3+} to the ferrous form (Fe^{2+}) (Singh et al., 2008).

3.2.4. Total antioxidant activity

The antioxidant ability could be also determined by the reduction of Mo(VI) to Mo(V) and the subsequent formation of a green phosphate/Mo(V) complex at acidic media (Prieto, Pineda, & Aguilar, 1999). As depicted in Fig. 1D, PSEO exhibited high total antioxidant activity that increased with concentration ($213.96 \pm 11.12 \mu\text{mol/mL}$ α -tocopherol equivalent at the concentration

of 500 $\mu\text{g/mL}$), while, the positive standard BHA did not exceed $160.23 \pm 0.06 \mu\text{mol/mL}$ α -tocopherol equivalent at the same concentration. Even at low concentrations, PSEO showed higher total antioxidant activity than BHA. The expression of the antioxidant capacity in terms of α -tocopherol equivalent is based on the reducing potential of the hydroxyl group of the 6-hydroxychroman ring involved in tocopherol structure (Prieto, Pineda, & Aguilar, 1999). Thus, the hydroxyl group on the pseudoisoeugenol ring is probably the reason of the very strong total antioxidant activity of PSEO (Supplementary Fig. 1B).

3.2.5. Oxidative DNA damage protective activity

Damage of DNA in plasmids results in a cleavage of one of the phosphodiester chains of the supercoiled DNA and produces a relaxed open circular form. Further, cleavage near the first breakage results in linear double-stranded DNA molecules. The formation of circular form of DNA is indicative of single-strand breaks and the formation of linear form of DNA is indicative of double-strand breaks (Burrows & Muller, 1998). In this study, oxidative DNA damage protective activity of PSEO was evaluated against OH^\bullet induced damage on pGAP plasmid DNA. As shown in lane 2 (Fig. 1E and F), incubation of DNA with Fenton's reagent resulted in a complete degradation of DNA, indicating that OH^\bullet generated by Fenton's reagent produced multiple double-strand DNA breaks. Addition of PSEO at 0.625 mg/mL (lane 3) showed partial retention of supercoiled DNA (46%) and mitigated the oxidative stress produced by Fenton's reagent. However, at 1.25 and 2.5 mg/mL (lanes 4 and 5), the PSEO induced a significant reduction in the formation of open circular and increased the retention of the supercoiled form of plasmid DNA (88% and 89%, respectively). As previously depicted in the DPPH, β -carotene bleaching, reducing power and antioxidant activity assays, PSEO exhibited great potential to quench reactive oxygen species by donating hydrogen atom or electron. This type of action might prevent the free radicals-mediated oxidation of DNA through directly scavenging OH^\bullet and therefore protecting the supercoiled plasmid DNA from OH radicals.

In this study, it has been shown that PSEO exhibited strong antioxidant activity through different mechanisms that could be used in various food and pharmaceutical applications to protect products from oxidation and enhance their health effects.

3.3. Evaluation of the antibacterial activity of *P. saxifraga* essential oil

Controlling bacterial infections has become a major problem due to the development of bacterial resistance against a large spectrum of commercial antibiotics. Thus, the research of new natural materials with antibacterial agents has recently expanded (Fair & Tor, 2014). In this study, antibacterial activity of PSEO was assessed against three Gram- and three Gram+ bacteria. *S. Typhimurium* and *B. cereus* were the most sensitive strains to the PSEO (Inhibition zone diameter ID = 14 and 14.5 mm, respectively; Table 2). Our results are in accordance with those of Tepe et al. (2006) reporting that *P. anisetum* and *P. flabellifoli* had a moderate antibacterial activity against *B. cereus* and *E. coli*. However, *B. cereus* was more sensitive to PSEO (MIC = 1.56 mg/mL) than to those of *P. anisetum* and *P. flabellifoli* (MIC = 72 mg/mL for both species). In fact, PSEO exhibited a bactericide effect against *S. Typhimurium*, *B. cereus* and *M. luteus* (MBC/MIC < 4), while it showed only a bacteriostatic effect against *E. coli*, *P. aeruginosa* and *L. monocytogenes* (MBC/MIC > 4). According to Diao et al. (2014), anethole and its isomers are mainly responsible for the antimicrobial activity of the essential oils which contain them. Thus, the antibacterial activity of PSEO could be attributed to the presence of anethole. In general, the large number of essential oil components makes it difficult to attribute the antibacterial activity to one specific mechanism. Nevertheless, it has been demonstrated in several studies that one of the main antibacterial action mechanisms is related to the dissolution of essential oil components in the lipids of the bacterial cell membrane and mitochondria due to their lipophilic character. This disturbs cell membrane and induces ions leakage leading to cell death (Sikkema, de Bont, & Poolman, 1994).

3.4. Acute toxicity study of *P. saxifraga* essential oil

In the present study, acute toxicity is conducted to evaluate the safety of PSEO for a further food application. The acute toxicity was assessed in mice model by the observation of the animal behaviour after PSEO administration. All the observations are noted in Table 3. When PSEO was administered to mice by intraperitoneal injection, doses of 250 and 500 mg/kg had no harmful effects on mice. In fact, the plants belonging to *Pimpinella* genus are known for their therapeutic properties and they are widely used in folk medicine. Superior doses induced abnormal behaviour in mice starting few minutes (5 to 10 min) after the injection like fast breathing and hypoactivity for groups administrated with 750 and 1000 mg/kg. Death was lately recorded for some mice but the survived ones were completely recovered and gradually resumed normal activity. In addition to fast breathing and hypoactivity, high doses of PSEO such as 1500 and 3500 mg/kg lead to loss of consciousness followed by death (85 and 100% dead animals, respectively). The estimated LD₅₀ by linear regression of the dose-response curve was 976.2 mg/kg (Supplementary Fig. 2). The LD₅₀ is crucial for the prediction of the symptomatology of poisoning after acute overdosing in humans (Hilan et al., 2009). It was demonstrated that intraperitoneal LD₅₀ values for anethole, major compound in PSEO, were ranged between 650 and 1410 mg/kg in mice. In fact, it has been previously showed that the essential oil of *P. anisum*, mainly composed of anethole exhibited anticonvulsant and neuroprotective effects. However, its excessive administration could induce disturbance in motor coordination (Pourgholami et al., 1999). Although the toxicity of essential oils is influenced by several factors like species, strain, environment and processing, it is important to assess toxicological tests including the LD₅₀ determination before any application of essential oils.

3.5. Cheese preservation by alginate coating containing *P. saxifraga* essential oil

It is known that uncoated fresh cheese including “Béja Sicilian cheese” is very sensitive to microbial contamination and lipid peroxidation leading to quality deterioration. In this case, cheese

edible coating could be a solution to preserve its nutritional and organoleptic properties during storage. In this work, the effect of sodium alginate coating enriched with PSEO on “Béja Sicilian cheese” preservation was studied by the evaluation of weight loss, pH, water activity, color and oxidative and microbial stability. Furthermore, a sensorial analysis was conducted to evaluate the acceptance of the coated cheese by consumers.

3.5.1. Evaluation of weight loss

For all coated and uncoated samples, the loss of weight was measured at 4, 7 and 10 days of storage at 4 °C. As observed in Fig. 2A, the weight loss increased during chilled storage time in all cheese samples. This is possibly due to the phenomena of whey exudation during storage leading to continuous moisture migration from cheese to surrounding environment (Samal, Pearce, Bennett, & Dunlop, 1993). In fact, the uncoated control sample weight loss reached 9.7%, 17.3% and 23.4% after 4, 7 and 10 days of storage, respectively. However, SA coating allowed the reduction of the weight loss to of 6.7%, 9.9% and 14.9% at 4, 7 and 10 days, respectively. The study of Cerqueira et al. (2010) showed that galactomannans coatings reduced the weight loss (about 20%) of the cheese by 3.8% during storage at 4 °C. The effect of *k*-carrageenan and alginate coating on the decrease in weight loss of semi-soft cheese was also shown by Kampf & Nussinovitch (2000). It is known that sodium alginate, a marine linear polysaccharide, has a good moisture-retention ability especially in cheese where guluronic residues bind to Ca^{2+} ions to form an egg-box structure which act like a barrier (Costa et al., 2018). The addition of PSEO to the SA coating showed an important decrease of weight loss. A 3% of PSEO reduced the weight loss to 4.3%, 7.1% and 10% at 4, 7 and 10 days, respectively. In general, the incorporation of hydrophobic essential oils into hydrophilic polymer matrices could enhance their barrier properties against water transfer (Ju et al., 2018).

3.5.1. Evaluation of pH, water activity and color

The pH, water activity and color were evaluated at the beginning (day 1) and the end of the storage period (day 1 and day 10) (Table 4A). During chilled storage, the pH decreased in all samples and this is probably due to the presence of lactic bacteria involved in lactose fermentation leading to the cheese acidification. Free fatty acids generated by lipid peroxidation of cheese during storage could also contribute to its acidification (Di Pierro, Sorrentino, Mariniello, Giosafatto, & Porta, 2011). However, the SA coating reduced significantly ($p < 0.05$) the cheese acidification especially in the presence of 3% PSEO where pH varied only from 6.17 at day 1 to 5.92 at day 10. This is probably due to antibacterial activity of PSEO which slowed down the development of lactic bacteria, and consequently reduced the acidification.

Water activity (a_w) is defined as the amount of free water present in a product and available for bacterial growth. After 10 days of storage, the a_w increased in all samples due to whey exudation. Since the coating represents a barrier against moisture loss, the a_w of the coated cheese was significantly ($p < 0.05$) lower than that of the uncoated cheese. The presence of PSEO in the sodium alginate didn't have an effect on a_w ($p > 0.05$).

Moreover, the SA coating had obvious impact on color changes of the cheese as it described in Table 4A. The coating slightly increased the lightness of cheese (84.64-86.78) in comparison to the uncoated cheese (83.14) (Table 4A). During the storage, a decrease of L^* to 80.99 in uncoated cheese was observed. It was less important in coated cheese especially in presence of PSEO (86.55 in presence of 3% PSEO). The decrease of L^* was attributed to microbial growth on the cheese surface (Bermúdez-Aguirre & Barbosa-Cánovas, 2010). Since coating and PSEO reduced the microbial growth in cheese, they allowed to preserve its lightness. Unlike the uncoated cheese, the a^* and b^* values varied slightly within 10 days of chilled storage of coated cheese in presence of essential oil which prove that this active coating preserved the color of the cheese.

3.5.1. Microbial analysis of cheese

Evolution of microbial population in cheese samples during chilled storage period was investigated (Table 4B). On the first day of storage, the mesophilic bacteria count was 3.89 log CFU/g while lactic bacteria count was 3.27 log CFU/g probably due to the use of pasteurized milk and hygienic manipulation during cheese industrial production. During storage, the mesophilic bacteria of all samples increased reaching maximal levels at day 10 (5.66 CFU/g of uncoated cheese). The coated cheese showed lower mesophilic bacteria counts than those of uncoated cheese especially. The presence of 3% PSEO in SA coating reduced the proliferation of mesophilic bacteria from 5.44 to 4.03 log CFU/g at day 7 of chilled storage. These results are in agreement with the study of Cerqueira et al. (2010) showing that galactomannan coating limited growth of mesophilic bacteria in cheese (from 5.9 to 5.2 log CFU / g at day 14). In fact, the coating reduces the transfer of gases (O₂ and CO₂) to the cheese and the oxygen becomes less available for microbial growth (Cerqueira et al., 2010). Likewise, coating reduced the proliferation of the lactic bacteria, especially in presence of 3% PSEO, from 4.07 to 3.33 log CFU/g at day 7. These results are in agreement with those of Martins, Cerqueira, Souza, Carmo Avides, & Vicente (2010) showing that the addition of an antimicrobial agent to the galactomannan coating of ricotta cheese reduced the proliferation of *Listeria monocytogenes* by 2.2 log CFU/g in comparison with the uncoated cheese. Coatings enriched with essential oils of oregano and pepper have also been applied in the preservation of beef to improve its microbial stability (Oussalah et al., 2004). These results showed the efficiency of the addition of the essential oil in edible coating as a natural antibacterial agent.

3.5.2. Oxidative stability of cheese

Lipid peroxidation is a spontaneous phenomenon involved in nutritional and organoleptic quality deterioration and food shelf-life reduction. Lipid peroxidation was evaluated during cheese storage at 4 °C by measuring TBARS (µg of malondialdehyde (MDA) per 100 g of cheese) (Fig.

2B). The MDA amount increased during the storage time from 19.5 μg MDA/100 g at day 4 to 43.7 μg MDA/100 g at day 10 in the uncoated cheese while it didn't exceed 34 μg MDA/100 g in the SA coated cheese. It has been demonstrated that SA is endowed with antioxidant activity and it could act as barrier to oxygen, which is one of the most important factors of peroxidation (Sellimi et al., 2015). The addition of essential oil in the SA coating contributed significantly to the decrease of the lipid oxidation ($p < 0.05$) (Fig. 2B). A concentration of 2% of PSEO had similar effect on the decrease of lipid peroxidation to the vitamin C added to SA coating (15.96 and 16.24 μg MDA/100 g, respectively at 10 days of storage). The cheese coated with SA enriched with 3% of PSEO presented the lowest MDA values (8.5, 10.5 and 11.7 μg MDA/100 g at 4, 7 and 10 days of storage). In fact, in all cases, the addition of PSEO allowed to slow down the peroxidation since it was no significant increase of MDA amounts ($p > 0.05$) between 7 and 10 days of storage (Fig. 2B). The efficiency of the PSEO against cheese peroxidation confirms the results found above in the *in-vitro* tests of antioxidant activity that demonstrated its strong capacity in quenching and stabilizing free radicals, generated by lipid peroxidation in this case. The effect of essential oils incorporated in edible coatings on lipid peroxidation reduction of many products containing a high amount of fats (such as meat) has been previously described. For instance, Vital et al. (2016) showed that alginate coatings enriched with essential oils of oregano and rosemary reduced the lipid peroxidation of beef by 47% and 39%, respectively.

3.5.3. Sensory evaluation of cheese

The sensory evaluation is very important when testing new active edible coatings in order to predict the acceptability of the coated product by the consumer. In our study, sensory analysis was conducted by a panel of 21 subjects to evaluate the effect of SA coating enriched with PSEO on the cheese. Overall acceptability average scores ranged from 2.57 to 4 in the 5-point Hedonic scale (Fig. 2C). In general the coated cheese was more appreciated than the uncoated cheese. The coated cheese with SA enriched with 3% of PSEO was the most appreciated in terms of odor and flavor

where it scored the highest average acceptability (3.71 and 3.42, respectively). This is probably due to the important presence of anethole, which is widely used as sweet flavoring agent and scent in food like candy, chewing gum and cigarettes (Marinov & Valcheva-Kuzmanova, 2015). The coated samples with SA enriched with PSEO were the most appreciated in terms of color and aspect whatever the PSEO concentration was. This is in accordance with color analysis showing the high lightness of these samples. The panel subjects noted that texture was slightly enhanced with an average score of 3.57 for coated cheese and 3.42 in control. However, they did not detect any texture difference between coated cheese with SA and cheese coated with SA enriched with PSEO.

4. Conclusion

The essential oil of *P. saxifraga* (PSEO), as a novel natural bioactive compound, was successfully incorporated in sodium alginate coating for cheese preservation. PSEO, mainly composed of anethole, exhibited potent antioxidant and antibacterial abilities. The addition of PSEO (1-3%) in sodium alginate coating (2% sodium alginate+15% glycerol) was effective in reducing the weight loss, preserving pH and color and improving the oxidative and bacterial stability of the coated cheese. The sensory analysis showed high appreciation of coated cheese in presence of PSEO. For the prediction of the symptomatology of poisoning after acute overdosing in humans, the acute toxicity of PSEO was determined using a mice model ($LD_{50} = 976.2$ mg/kg).

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Declarations of interest: none

ACCEPTED MANUSCRIPT

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Figure captions:

Figure 1. Antioxidant activity of PSEO: **(A)** DPPH radical scavenging capacity, **(B)** Inhibition of β -carotene bleaching assay, **(C)** Reducing power assay, **(D)** Total antioxidant activity, **BHA** : synthetic commercial antioxidant used as positive control **(E)** Gel electrophoresis pattern of pGap DNA incubated with Fenton's reagent in the presence and absence of PSEO; Lane 1: native DNA, lane 2: DNA incubated with Fenton's reagent; lanes 3, 4 and 5 represent DNA + Fenton's reagent + essential oil of *P. saxifraga* at different concentrations (0.625, 1.25 and 2.5 mg/mL, respectively) a: Nicked circular form; b: Supercoiled form, **(F)** Retention of supercoiled DNA (%).

Figure 2. Evaluation of the weight loss, pH, water activity, oxidative and bacterial stability and consumer acceptability of cheese **(A)** Weight loss of cheese samples (%) at 4, 7 and 10 days of storage at 4 °C, **(B)** Lipid peroxidation ($\mu\text{g MDA}/100 \text{ g cheese}$) of cheese samples evaluated at 1, 4, 7 and 10 days of storage at 4 °C, **(C)** Radar chart representing mean scores of the sensory evaluation of cheese samples after 24 h of storage at 4 °C. Control: uncoated cheese; SA: Cheese coated with only sodium alginate solution, SA + 1% PSEO: Cheese coated with sodium alginate solution containing 1% PSEO; SA + 2% PSEO: Cheese coated with sodium alginate solution containing 2% PSEO; SA + 3% PSEO: Cheese coated with sodium alginate solution containing 3% PSEO; SA + Vit C: Cheese coated with sodium alginate solution containing 1% of vitamin C. (a-c) Different subscripts indicate significant differences for the same sample within different days of storage ($p < 0.05$). (A-D) Different letters indicate significant differences between samples in the same storage day ($p < 0.05$).

Fig. 1

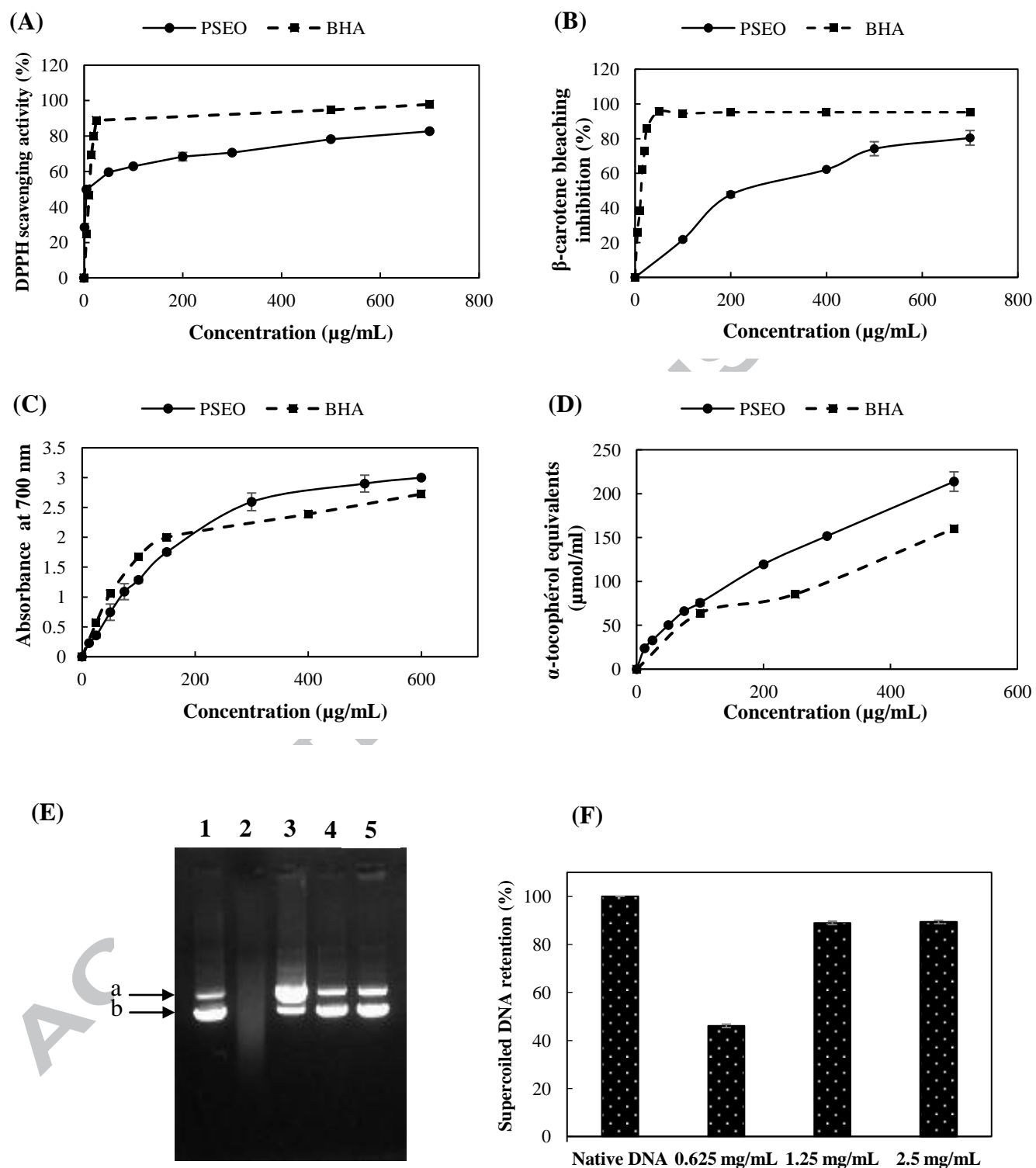
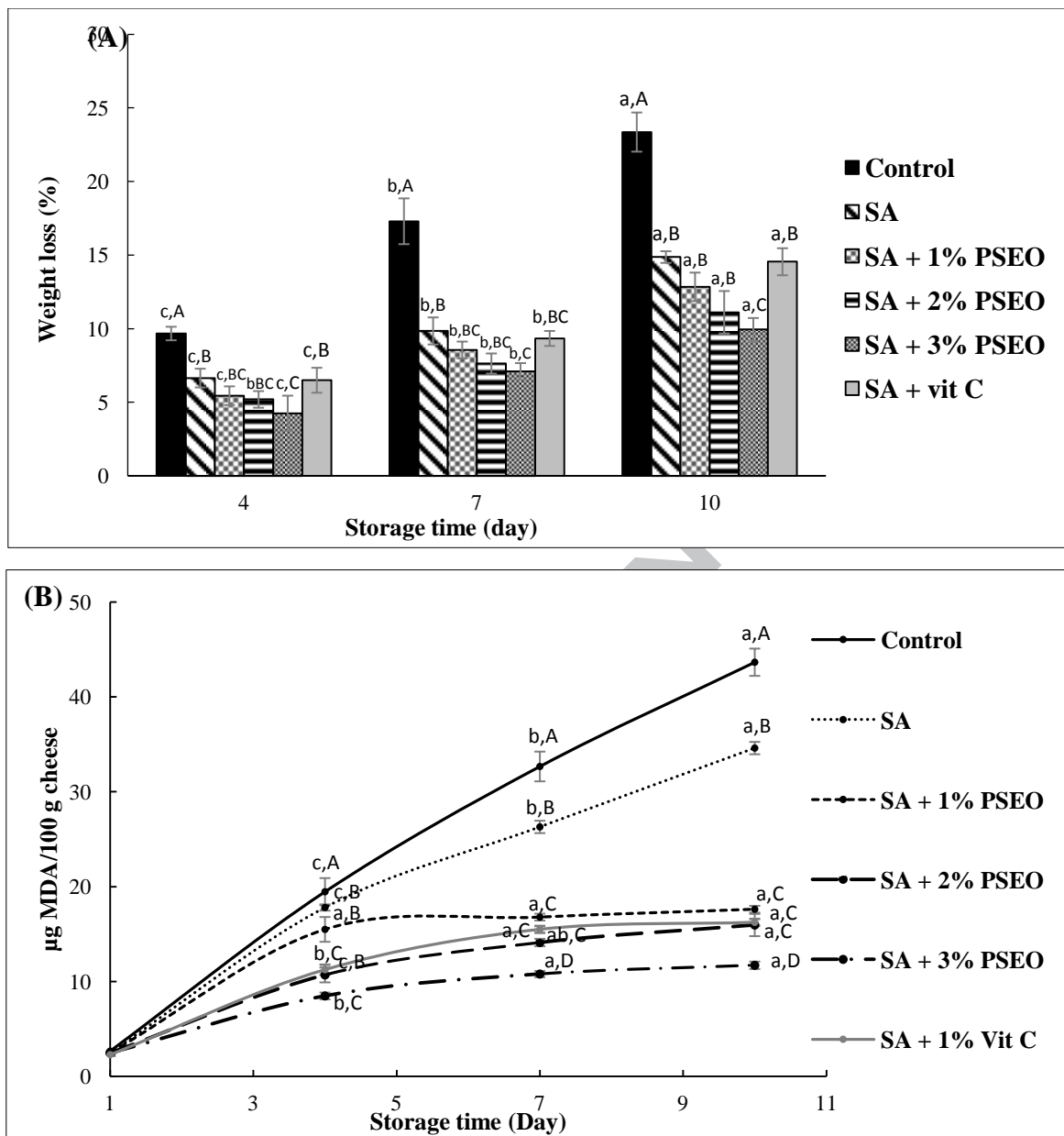


Fig. 2



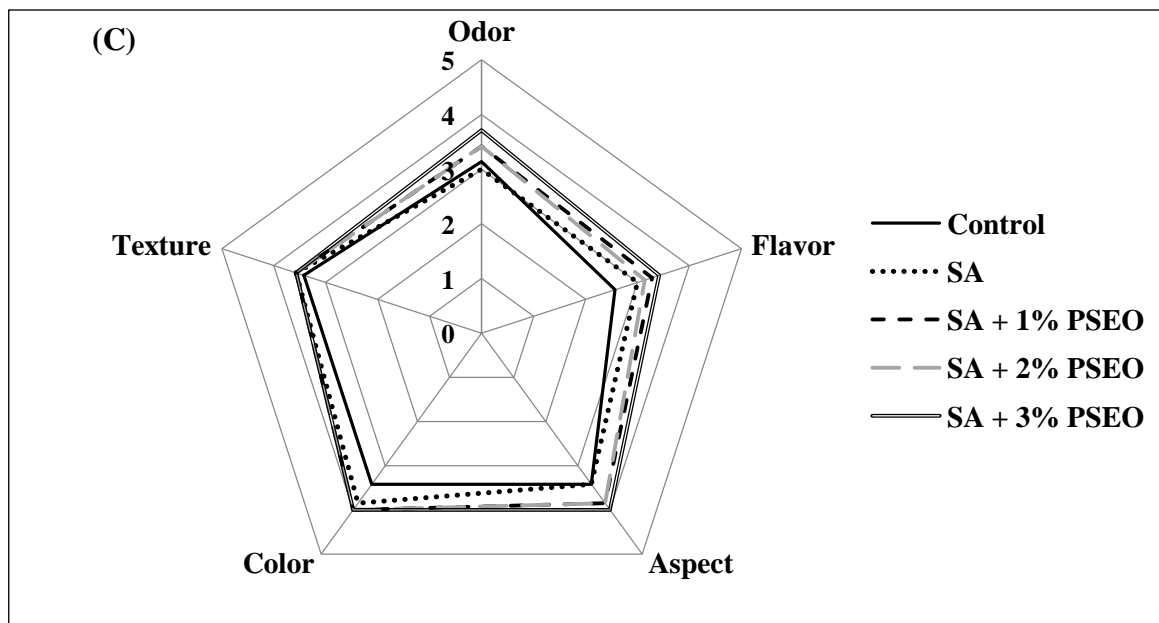


Table 1: Chemical composition of the essential oil of *P. saxifraga*.

Component	Retention index	% of total volatil compounds
<i>p</i> -Anisaldehyde	1245	7.53
Anethole	1282	59.47
2-Ethyl-4,5-dimethylphenol	1309	0.10
Acetanisole	1346	0.09
Anisketone	1377	1.71
Methyl <i>o</i> -coumarate	1429	0.25
Thujpsadiene	1469	0.22
4,11-selinadiene	1486	2.99
1-(2-Hydroxy-4-methoxyphenyl)propan-1-one	1542	0.49
4-methoxycinnamaldehyde	1556	0.09
Dillapiole	1605	0.51
l-Calamenene	1663	0.10
β -Selinene	1682	0.13
Pseudoisoeugenol	1833	20.15
Thellungianin G	1884	6.17
Total		100%
% of identified compounds		93.5
Number of identified compounds		15

Table 2: Evaluation of antibacterial activity of *P. saxifraga* essential oil.

Bacteria	Diameter of the inhibition zone ID (mm \pm SD)		MIC (mg/mL)	MBC (mg/mL)
	Gentamycin	<i>P. saxifraga</i> essential oil		
Gram-				
<i>E. coli</i>	15	7.5 \pm 0.2	0.78	3.125
<i>S. Typhimurium</i>	18	14.5 \pm 0.8	3.125	6.25
<i>P. aeruginosa</i>	18	11 \pm 0.3	1.56	6.25
Gram +				
<i>B. cereus</i>	22	14 \pm 0.7	1.56	3.125
<i>M. luteus</i>	18	11 \pm 0.3	3.125	6.25
<i>L. monocytogenes</i>	15	7.25 \pm 0.25	1.56	12.5

MIC and MBC were minimal inhibitory concentration and minimal bactericidal concentration, respectively. SD: Standard deviation

Table 3: Study of acute toxicity of *P. saxifraga* essential oil

Dose (mg/kg)	Number of dead mice	Mortality rate (%)	Symptoms
250	0	0	nd
500	0	0	nd
750	2	25	Fast breathing, hypoactivity, death after 14 hours and total recovery of the survived mice.
1000	6	75	Fast breathing, hypoactivity, death after 12 hours and total recovery of the survived mice.
1500	7	87.5	Fast breathing, hypoactivity, groaning, unconsciousness, death after 30 ± 9 min and total recovery of the survived mice.
3500	8	100	Fast breathing, hypoactivity, groaning, unconsciousness, death after 20 ± 5 min.

nd: not defined

Table 4A: pH and a_w measurements at 1, 4 and 10 days of storage and change of color between 1 and 10 days of storage at 4 °C

		Control	SA	SA + 1% PSEO	SA + 2% PSEO	SA + 3% PSEO	SA + vit C
pH	Day 1	6.31 ± 0.01 ^A	6.26 ± 0.00 ^A	6.17 ± 0.00 ^B	6.16 ± 0.00 ^B	6.17 ± 0.00 ^B	6.12 ± 0.02 ^C
	Day 10	5.78 ± 0.01 ^C	5.93 ± 0.00 ^A	5.88 ± 0.01 ^B	5.89 ± 0.00 ^B	5.92 ± 0.02 ^A	5.71 ± 0.01 ^D
a_w	Day 1	0.909 ± 0.00 ^A	0.909 ± 0.00 ^A	0.909 ± 0.00 ^A	0.909 ± 0.01 ^A	0.909 ± 0.01 ^A	0.908 ± 0.00 ^A
	Day 10	0.975 ± 0.00 ^A	0.953 ± 0.00 ^B	0.953 ± 0.00 ^B	0.951 ± 0.00 ^B	0.952 ± 0.00 ^B	0.955 ± 0.00 ^B
L*	Day 1	83.14 ± 1.22 ^C	84.64 ± 0.23 ^B	84.92 ± 0.10 ^B	85.51 ± 0.49 ^{AB}	86.78 ± 0.17 ^A	86.77 ± 0.30 ^A
	Day 10	80.99 ± 0.77 ^E	83.28 ± 3.11 ^D	84.81 ± 0.13 ^C	85.32 ± 0.38 ^{BC}	86.55 ± 0.20 ^A	85.92 ± 0.30 ^{AB}
a*	Day 1	0.37 ± 0.04 ^D	0.19 ± 0.01 ^E	0.96 ± 0.02 ^A	0.69 ± 0.01 ^B	0.53 ± 0.01 ^C	0.49 ± 0.02 ^C
	Day 10	1.26 ± 0.02 ^A	0.84 ± 0.01 ^C	1.03 ± 0.01 ^B	0.78 ± 0.01 ^D	0.67 ± 0.01 ^E	1.03 ± 0.02 ^B
b*	Day 1	15.68 ± 0.32 ^B	16.26 ± 0.34 ^B	16.97 ± 0.38 ^A	16.97 ± 0.33 ^A	17.33 ± 0.83 ^A	16.52 ± 0.54 ^{AB}
	Day 10	19.13 ± 0.73 ^A	18.41 ± 0.20 ^{AB}	18.25 ± 0.01 ^{ABC}	17.79 ± 0.30 ^{CD}	17.46 ± 0.08 ^{CD}	17.21 ± 0.37 ^D

L*: lightness, a*: redness, b*: yellowness

Table 4B: Evolution of mesophilic and lactic bacteria during storage at 4 °C expressed in log

		Control	SA	SA + 1% PSEO	SA + 2% PSEO	SA + 3% PSEO	SA + Vit C
Mesophilic bacteria	Day 1	3.89 ± 0.02 ^{d,A}	3.89 ± 0.02 ^{d,A}	3.89 ± 0.02 ^{c,A}	3.89 ± 0.02 ^{d,A}	3.89 ± 0.02 ^{a,A}	3.89 ± 0.02 ^{c,A}
	Day 4	4.30 ± 0.01 ^{c,A}	4.25 ± 0.01 ^{c,B}	4.26 ± 0.02 ^{b,B}	4.06 ± 0.02 ^{c,C}	3.97 ± 0.02 ^{b,D}	4.26 ± 0.01 ^{b,B}
	Day 7	5.44 ± 0.01 ^{b,A}	4.37 ± 0.01 ^{b,B}	4.29 ± 0.02 ^{ab,C}	4.14 ± 0.01 ^{b,D}	4.03 ± 0.01 ^{b,E}	4.39 ± 0.01 ^{a,B}
	Day 10	5.66 ± 0.03 ^{a,A}	4.42 ± 0.01 ^{a,B}	4.33 ± 0.01 ^{a,C}	4.20 ± 0.01 ^{a,D}	4.05 ± 0.02 ^{b,E}	4.41 ± 0.01 ^{a,B}
Lactic bacteria	Day 1	3.27 ± 0.01 ^{c,A}	3.27 ± 0.01 ^{c,A}	3.27 ± 0.01 ^{c,A}	3.27 ± 0.01 ^{c,A}	3.27 ± 0.01 ^{b,A}	3.27 ± 0.01 ^{d,A}
	Day 4	3.58 ± 0.01 ^{b,A}	3.54 ± 0.01 ^{b,B}	3.40 ± 0.01 ^{b,C}	3.36 ± 0.02 ^{b,C}	3.33 ± 0.01 ^{a,D}	3.50 ± 0.02 ^{c,B}
	Day 7	4.07 ± 0.03 ^{a,A}	3.89 ± 0.03 ^{a,B}	3.74 ± 0.05 ^{a,C}	3.52 ± 0.03 ^{a,D}	3.33 ± 0.08 ^{a,E}	3.91 ± 0.01 ^{b,B}
	Day 10	4.08 ± 0.03 ^{a,A}	3.92 ± 0.07 ^{a,B}	3.78 ± 0.02 ^{a,C}	3.53 ± 0.05 ^{a,D}	3.35 ± 0.05 ^{a,D}	3.96 ± 0.01 ^{a,B}

CFU/g cheese

Control: uncoated cheese; SA: Cheese coated with sodium alginate solution only, SA + 1% PSEO: Cheese coated with sodium alginate solution containing 1% PSEO; SA + 2% PSEO: Cheese coated with sodium alginate solution containing 2% PSEO; SA + 3% PSEO: Cheese coated with sodium alginate solution containing 3% PSEO; SA + Vit C: Cheese coated with sodium alginate solution containing 1% of vitamin C.

(a-d) Different subscripts indicate significant differences for the same sample within different days of storage ($p < 0.05$). (A-E) Different letters indicate significant differences between samples in the same storage day ($p < 0.05$).

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Highlights

- The main compound of *Pimpinella saxifraga* essential oil (PSEO) was anethole
- PSEO showed potential antioxidant and antibacterial activities
- The study of acute toxicity of PSEO showed that estimated LD₅₀ was 939 mg/kg
- Addition of PSEO in sodium alginate coating improved cheese preservation
- Sensory analysis showed consumer appreciation of coated cheese using PSEO

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