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Evaluation of an innovative sheep cheese with antioxidant activity enriched with different thyme essential oil lecithin liposomes

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ABSTRACT

The aim of this study was to develop an innovative sheep cheese enriched with *Thymus capitatus* L. essential oil (EO) nanoincorporated into small homogeneous liposomes. The latter were prepared using two types of lecithin: one commonly used for liposome production, and the other used as dietary supplement. Both EO liposomes, which showed similar physico-chemical characteristics (i.e., size, homogeneity, surface charge), were incorporated into fresh sheep cheese. These enriched sheep cheeses were produced in Sardinia (Italy) and analysed 20, 60 and 180 days after preparation. HS-SPME at 40 and 80 °C coupled with GC-MS/FID method was used to evaluate the volatile fraction and identify the main compounds of both EO and cheese. A validated HPLC-DAD analysis allowed the identification and quantification of thymol and carvacrol, and thymol amount dosed at 20 days was the highest (9.51–10.10 mg/kg). The amount of monoterpenoid phenols and the antiradical and total antioxidant capacity evaluated by FRAP and DPPH$^{\bullet}$ assays, decreased linearly ($r \geq 0.93$, $p \leq 0.05$) as the cheese maturation increased. Overall results suggested that sheep cheese enriched with *T. capitatus* EO nanoformulations had an enhanced antioxidant activity compared to cheese without liposomal EO, up to 180 days.

1. Introduction

Nowadays food is no longer meant to provide nutrients and satisfy hunger, but also to prevent nutrition-related diseases and to enhance physical and mental well-being. This demand led to the need for food producers to apply innovations, adjust technological processes and introduce new types of products (Bigliardi & Galati, 2013). Cheese, as one of the most popular and frequently consumed products, might be a good base from which to create an enriched food that can positively affect the health of consumers. Italy is one of the largest cheese producers in the world (Barone et al., 2018) and the island of Sardinia is well known for sheep cheese. Pecorino Sardo, Fiore Sardo and Pecorino Romano are three Protected Designation of Origin (PDO) products with highly appreciated sensory characteristics that may be eaten as a table cheese, but also have many culinary applications and can be added to many dishes (Coda et al., 2006). In any case, cheese producers in traditional areas are now looking for new products that can help to open new markets and refresh their image. Therefore, improving the nutritional properties of cheese with natural additives that could be perceived by the consumer as beneficial for their health is a key strategy. For this purpose, dietary antioxidants might be of great importance given the inverse relation between the dietary intake of food rich in antioxidants and frequency of diseases (Gülçin, 2012; Lobo, Patil, Phatak, & Chandra, 2010). Optimal daily intake of antioxidants leads to enhanced quality of life and health, protecting from ageing, cancer, cardiovascular and neurological disorders, and many other diseases (Carocho & Ferreira, 2013). Apart from fruits and vegetables, which are rich in antioxidants, herbs and spices like rosemary, thyme, oregano, sage, basil, clove, and cinnamon can all contribute to the taste of the meal, but also to its beneficial properties (Sindhi et al., 2013). The idea of adding herbs and spices or their extracts, like essential oils (EOs), to cheese is not new (El-Sayed & Youssef, 2019; dos Santos Gouvea, Rosenthal, & da Rocha Ferreira, 2017) and a lot of products can be found on the market worldwide. EOs contribute to give specific flavour and to inhibit specific pathogenic and spoilage microorganisms. However, the use of EOs still presents some challenges, due to their volatility and the

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difficulty of achieving a homogeneous distribution either in the cheese paste or in the rind. An innovative and promising approach aimed at maximizing the beneficial properties of EOs is their incorporation into nanocarriers. The microencapsulation strategy has been proposed for preparing novel edible coatings with antioxidant and antimicrobial activities (Costa, Maciel, Teixeira, Vicente, & Cerqueira, 2018; Shukla et al., 2017; Zambrano-Zaragoza et al., 2018). de Barros Fernandes et al. (2017) proposed a microencapsulation of rosemary essential oil in whey protein isolate and inulin matrix as biopreservative for cheese. Cui, Wu, and Lin (2016) incorporated lemongrass oil into liposomes aiming at both protecting the EO and preserving its antimicrobial activity, especially against *Listeria monocytogenes*. To the best of our knowledge, the use of liposomes as a nanotechnological system for the incorporation of EOs and the production of enriched cheese has not been evaluated so far, and neither has the positive effect of the vesicular systems on the enhancement of the antioxidant and beneficial properties of these EOs.

The choice of liposomes is mainly connected to their high versatility and possibility to incorporate both hydrophilic and hydrophobic bioactives. In particular, these vesicles can be obtained by using different phospholipids, which in turn may determine the final structure and physico-chemical properties. Among others, soy lecithin such as Lecinova® and Lipoid S75 are largely used for the formulation of vesicular systems, as they are easily available and less expensive than the pure phospholipids. In particular, Lipoid S75 (S75), which is a commercial mixture of soybean phospholipids mainly composed of phosphatidylcholine (70%), phosphatidylethanolamine (9%), lysophosphatidylcholine (3%), triglycerides and fatty acids, has been approved for oral, topical and parenteral administration. Both Lecinova® and Lipoid S75 led the formation of small and stable vesicles, capable of protecting the incorporated bioactives (even the volatile ones) from degradation and promoting their beneficial properties.

Given that, the goal of this study was to prepare an innovative cheese with antioxidant activity through enrichment with thyme (*Thymus capitatus* L.) essential oil (EO) nanoincorporated into liposomes. Two formulations of liposomes were developed using two types of lecithin: one commonly used for liposome production (S75), and the other used as dietary supplement (Lecinova®). The enriched cheeses were tested for volatiles and phenolics content, alongside for the antioxidant capacity.

2. Material and methods

2.1. Chemicals and reagents

All chemicals used were of analytical grade. Methanol, acetonitrile, phosphoric acid 85% w/w, Folin-Ciocalteu's phenol reagent, Na_2CO_3, ferrous sulphate heptahydrate, thymol, carvacrol, gallic acid, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH$^{\bullet}$), (±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ), ammonium acetate, iron (III) chloride hexahydrate, ferrous sulphate heptahydrate, $CuSO_4 \bullet 5H_2O$, were obtained from Sigma-Aldrich (Milan, Italy). The standards of volatile compounds were obtained from Merck KGaA (Darmstadt, Germany). Lecinova® (Céréal, Nutrition & Santé Italia S.p.A., Origgio, Italy) granular soy lecithin, was purchased in a local pharmacy. A commercial mixture of soybean phospholipids (Lipoid S75, S75) with 70% phosphatidylcholine, 9% phosphatidylethanolamine and 3% lysophosphatidylcholine, triglycerides and fatty acids, was supplied by Lipoid GmbH (Ludwigshafen, Germany). Ultrapure water (18 MΩ cm) was obtained with a Milli-Q Advantage A10 System apparatus (Millipore, Milan, Italy).

2.2. Plant material and essential oil distillation

Aerial parts of wild *Thymus capitatus* (L.) Hoffmanns. & Link were randomly collected in Capo Sant'Elia (Cagliari, Sardinia) in June 2019 during the blooming period. The plant was identified by Prof. Gianluigi Bacchetta and a voucher sample (number DISVA.ALI.03.2019) was deposited at the Department of Life and Environmental Sciences of the University of Cagliari (Italy). Fresh plant material was hydrodistilled for 2 h by using a semi-industrial stainless-steel distilling apparatus with recirculation of the condensed water following Cosentino et al. (1999). EO was dried with anhydrous sodium sulphate, and stored in full, dark vials at +4 °C.

2.3. Liposomes preparation and physico-chemical analysis

T. capitatus EO was incorporated in two types of liposomes (A and B) prepared following Manconi et al. (2018). In short, either Lecinova® or S75 (60 mg/mL) and *T. capitatus* EO (5 mg/mL) were weighed in a glass vial and dispersed in water. The dispersions were sonicated (25 consecutive cycles of 5-s sonication alternated with 2-s pause) by using a Soniprep 150 ultrasonic disintegrator (MSE Crowley, London, UK) to obtain small liposomes. The composition of the samples is reported in Table 1. The liposomes were stored at 4 °C until their addition to sheep cheeses (within 2 weeks). For the preparation of 5 kg of enriched cheese, 60 mL of each vesicular dispersion were used.

Size, PI (polydispersity index: a measure of the size distribution width) and Z-potential (surface charge) were analysed to evaluate the main physico-chemical properties of the vesicular systems. The average diameter and PI were determined via Photon Correlation Spectroscopy by means of a Zetasizer nano-ZS (Malvern Instruments, Worcestershire, UK), which uses a helium-neon laser (633 nm) with a 173° scattering angle and backscatter technology for high sensitivity. The zeta potential was estimated using the Zetasizer Nano-ZS, which employs the M3-PALS (Mixed Mode Measurement-Phase Analysis Light Scattering) technique to measure the electrophoretic mobility of particles in dispersion. The samples ($n \geq 6$, 100 μL) were diluted with water (10 mL) and analysed at 25 °C.

2.4. Cheese preparation and sampling

Cheese samples were produced in triplicate by F.lli Maoddi srl cheese factory (Laconi, OR, Italy) by using a modified version of their standard preparation of traditional "Pecorino Romano" cheese. In brief, sheep's milk was pasteurized for 30 s at 72.1 °C. Afterwards lamb rennet paste and mesophilic starter culture (*Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus delbrueckii* subsp. *lactis*, *Lactobacillus helveticus*) were added to the milk. Once coagulation took place, the curd was broken, placed in 22 cm diameter moulds and manually pressed. Moulds were transferred to the steam chamber (kept at 37–38 °C with minimum 95% humidity) for 2 h until a pH value of 5.80 was reached in the cheese. At this point, the cheese was removed from the steam chamber and liposomes with 5 mg/mL of *T. capitatus* EO were added by syringing 60 mL of the vesicular dispersion per 5 kg of cheese. Six injections (10 mL each) were performed in different parts of the cheese to obtain a homogenous distribution of the nanoformulation. Finally, the cheese was hand salted and left to mature in a thermostated room at 8–12 °C. Two sets of experimental enriched cheeses were analysed (TA with Lecinova® liposomes A, and TB with S75 liposomes B) along with two blank samples (BA, BB), obtained from the corresponding batches, but without *T. capitatus* EO (empty liposomes). Indeed, the preparation of sheep cheese with pure EO was difficult mainly because of the small volume to be used and its irregular distribution in the cheese paste. On the contrary, liposomes, regardless of the type of lecithin used (Lecinova® or S75), were homogeneously and easily distributed in the cheese, as shown by the light-amber coloured lecithin dispersion.

The produced cheeses were analysed at three different time points: after 20 days (fresh cheese), 60 days (semi-mature cheese), and 180 days (mature cheese) from production. For the analyses, cheese samples were delivered by the producer in vacuum-packed pieces corresponding to a quarter of a wheel (ca. 1 kg of cheese).

Table 1
Composition and physico-chemical properties of the *T. capitatus* EO liposomes.

	Composition			Physico-chemical properties		
	T. capitatus EO (mg/mL)	Phospholipid (mg/mL)	Water (mL)	Size [a] (nm)	PI	Z-potential[a] (mV)
Liposomes A (Lecinova®)	5.0	60.0	1.0	81.1 ± 9.9[a]	0.32 ± 0.07[a]	−59.0 ± 3.2[a]
Liposomes B (S75)	5.0	60.0	1.0	90.6 ± 4.0[a]	0.28 ± 0.02[a]	−64.0 ± 3.0[a]

[a] Mean value ± SD, $n \geq 6$.PI: polydispersity index. Means in each column not sharing a superscript letter are significantly different ($p \leq 0.05$).

2.5. Extraction of polar compounds for antiradical and total antioxidant activity and HPLC-DAD analysis

Each block of cheese was cleaned of the rind and appropriately cut into 5 slices of similar size (1-5): the two external parts that were in contact with the plastic packaging (1 and 5), and the central part (3) were discharged (Fig. S1). The remaining two wedge-shaped parts (2 and 4) were finely grated and mixed together. 10 g of the obtained grated cheese sample were put into a 50 mL Falcon® conical tube together with 10 mL of methanol solution (MeOH: H_2O, 80:20, v/v). Afterwards, the conical tube content was homogenized with an IKA T18 digital Ultra Turrax (14,000 r/min for 20 s). Then, the homogenized mixture was placed in an ultrasonic bath for 10 min at ca. 10 ± 2 °C. Immediately after this step, the sample was centrifuged at a speed of 4000 r/min and a temperature of 7 ± 1 °C for 15 min by using a 5810R Eppendorf Centrifuge. After the centrifugation process, the sample mixture was separated into 3 phases: precipitate, supernatant (liquid phase) and a layer of fat on the surface. The supernatant was removed by using a syringe with a needle, filtered through 0.2 μm cellulose acetate filter (Ø = 13 mm) and transferred to a 15 mL Falcon® conical tube. Tubes containing extracts were closed, sealed with Parafilm®M and stored at −20 °C until analysis (within 1 month). This extraction method was validated in agreement with the International Conference on Harmoniation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidance note which describes the validation of analytical methods (ICH Topic Q2, 2006) (see paragraph 2.9.).

2.6. Evaluation of total polyphenol content (Folin-Ciocalteu's assay), total reducing power (FRAP assay) and free radical scavenging activity (DPPH• assay)

All assays were performed on the extracts obtained from cheeses as described above (paragraph 2.5.) by using a Cary 50 spectrophotometer (Varian, Leinì, TO, Italy) according to the spectrophotometric procedures described previously by Tuberoso, Boban, Bifulco, Budimir, and Pirisi (2013). Total phenol (TP) content of the extracts was determined with a modified Folin-Ciocalteu's method by adding 100 μL of sample to 0.5 mL of Folin-Ciocalteu's phenol reagent. After 5 min, 3 mL of 10% Na_2CO_3 (w/v) was added and the mixture was shaken and diluted with H_2O to the final volume of 10 mL. After 90 min of incubation at room temperature, the absorbance was read at 725 nm in 10 mm polystyrene cuvette (Kartell 01937) against blank. The total phenolics content was expressed as mg/kg of gallic acid equivalent (GAE) using the calibration curve made of freshly prepared gallic acid standard solutions (10–200 mg/L). The FRAP and DPPH• assays were performed in order to assess the antiradical and the antioxidant activities of the cheese extracts. The FRAP assay was evaluated preparing a ferric complex of 2,4,6-tris(pyridin-2-yl)-1,3,5-triazine (TPTZ) and Fe^{3+} (0.3123 g TPTZ, 0.5406 g $FeCl_3 \cdot 6H_2O$ in 100 mL acetate buffer pH 3.6). 20 μL of the extract were dissolved in 2 mL of ferric complex and, after an incubation period of 4 min in the dark, absorbance at 593 nm was measured. The results were expressed as millimoles of Fe^{2+} per kg of cheese. Finally, the DPPH• assay was performed to measure the ability of the antioxidant to scavenge the radical cation 1,1-diphenyl-2-picrylhydrazyl radical. 50 μL of the extract were dissolved in 2 mL of 0.06 mmol/L DPPH• in methanol. Then, spectrophotometric readings were carried out at 517 nm after an incubation period of 60 min in the dark. A calibration curve in the range of 0.02–1.0 mmol/L was prepared for Trolox, and the data were expressed as Trolox equivalent antioxidant capacity (TEAC mmol/kg).

2.7. Headspace solid-phase microextraction (HS-SPME)

An automated PAL RSI SPME holder was used, equipped separately with two different types of fibres covered with DVB/CAR/PDMS (Divinylbenzene/Carboxene/Polydimethylsiloxane) and CARB/PDMS (Carboxene/Polydimethylsiloxane). The fibres were obtained from Supelco Co. (Bellefonte, PA, USA). As described in section 2.5., the two wedge-shaped parts 2 and 4 without rind were finely grated and each sample (2 g) was placed in a 20 mL clear screw top vial and hermetically sealed with a cap containing PTFE/silicone septum. The automated PAL RSI experimental procedure involved the following steps: conditioning SPME fibre according to the instructions provided by Supelco Co., sample agitation (agitation speed: 250 r/min; agitator on time: 5 s; agitator off time: 2 s) and equilibration of the sample for 30 min at 40 °C, the extraction of volatiles for 40 min without agitation, and the injection of SPME fibre in the GC injector for desorption (7 min). The procedure was modified by changing the temperature of the agitator module to 80 °C during the equilibration and extraction of volatiles in the second set of experiments.

2.8. GC-FID/MS analyses of T. capitatus EO and cheese headspace volatile

GC-FID/MS analyses were performed according to the method described previously by Jerković et al. (2016). The GC-FID analyses were carried out by using an Agilent Technologies (Palo Alto, CA, USA) gas chromatograph model 7890A equipped with Flame Ionization Detector (FID) and PAL RSI 85 Autosampler System (CTC Analytics AG, Zwingen, Switzerland). The chromatographic separations were performed on a 30 m capillary column HP-5MS (5%-phenyl--methylpolysiloxane, Agilent J & W GC column) with 0.25 mm internal diameter and 0.25 μm coating thickness. The GC conditions were: split ratio 1:50; oven programmed at 70 °C for 2 min, then the temperature was increased at the rate of 3 °C/min to 200 °C and held isothermal for 15 min; injector temp. 250 °C; detector temp. 300 °C; carrier gas was helium (velocity: 1 mL/min). For EO analysis PAL RSI was not used, but 1 μL of diluted EO (10 μL of the oil in 1 mL of pentane) was manually inserted with a syringe into the GC injector. The GC-FID analysis was performed under the same conditions as with PAL RSI.

The GC-MS analyses were performed using an Agilent Technologies (Palo Alto, CA, USA) gas chromatograph model 7820A equipped with Mass Selective Detector (MSD) model 5977E (Agilent Technologies) and PAL RSI 85 Autosampler System (CTC Analytics AG, Zwingen, Switzerland). An HP-5MS capillary column was used for GC-MS analysis under the same conditions as for the GC-FID analysis. The MSD (EI mode) was operated at 70 eV, and the mass range was 30–300 average mass unit (amu) as described by Jerković, Kranjac, Marijanović, Roje, and Jokić (2019). For EO analysis PAL RSI was not used, but 1 μL of diluted essential oil (10 μL of the oil in 1 mL of pentane) was manually inserted with a syringe into the GC injector. The GC-MS analysis was performed under the same conditions as with PAL RSI. The identification of the volatile constituents was based on the comparison of their retention indices (RI) determined relative to the retention times of *n*-alkanes (C_9–C_{25}), with those reported in the literature (NIST, 2018)

and their mass spectra with the spectra from Wiley 9 (Wiley, New York, NY, USA) and NIST 17 (D-Gaithersburg) mass spectral libraries. The percentage composition of the samples was computed from the GC peak areas using the normalization method (without correction factors). The average component percentages were calculated from GC-FID and GC-MS analyses. All the experiments were performed in triplicate and the results are presented as mean values ± standard deviation (Tables 2 and 3).

2.9. HPLC-DAD analysis of monoterpenoid phenols

Thymol and carvacrol in the cheese extracts were identified and dosed by using an Agilent 1260 Infinity II HPLC system (Varian, Leinì, TO, Italy) fitted with a pump module G7111A, an autosampler module G7129A, and an Agilent G4212B photodiode array detector (Agilent Technologies, Cernusco sul Naviglio, MI, Italy). The separation was obtained with a Kinetex PFP C18 column (150 × 4.60 mm, 5 μm, Phenomenex, Casalecchio di Reno, Bologna, Italy) using 0.22 mol/L phosphoric acid (solvent A) and acetonitrile (solvent B) as mobile phase, at a constant flow rate of 1.0 mL/min. A gradient was generated by decreasing solvent A from 60% to 50% for 20 min and reaching 60% again at 30 min. The chromatograms and spectra were elaborated with OpenLab V. 2.51 data system (Agilent Technologies, Cernusco sul Naviglio, MI, Italy), and monoterpenoid phenols were detected and quantified at 220 nm (Fig. S2). The samples were diluted with acetonitrile (1:1, v/v) and injected (10 μL) into the HPLC without any further purification. The established analytical method was validated in agreement with the ICH guidance note (ICH Topic Q2, 2006) by determining linearity, limits of detection (LOD), limits of quantification (LOQ), precision and accuracy. Stock standard solutions were prepared in methanol, and the working standard solutions were prepared in ultrapure water. The linearity was evaluated by preparing standard mixtures at six different concentrations and analysing them by HPLC-DAD. The calibration curves for commercial standards were plotted with the method of the external standard, correlating the peak area with the concentration by means of the least-squares method, with a coefficient of determination (r^2) > 0.998 for both carvacrol and thymol in the range of 0.5–20 mg/L. The LODs and LOQs were calculated according to the equation LOD = 3.3 r/S and LOQ = 10 r/S, respectively (where r = standard deviation of the blank, and S = slope of the calibration curve) (ICH Topic Q2, 2006). The precision of this method was evaluated testing intra- and interday repeatability. Six injections of the same standard containing thymol and carvacrol were performed within one day and over three consecutive days. The relative standard deviation (RSD) for the area under the peak was determined as a measure of precision, and all RSDs were lower than 5%. The accuracy of the method was evaluated using recovery rates. Cheese samples BA were spiked with two concentrations of thymol (5 and 20 mg/kg) and each spiked sample was analysed in triplicate. Recovery rates were between 94.2% and 102.4%. The matrix effect was evaluated by comparing the response of a standard mix containing thymol at 5 and 20 mg/kg, prepared both in the sample BA and in water. No statistical differences were observed ($p \leq 0.05$). The specificity, intended as the lack of interference with other substances detected in the region of interest, was assessed by means of OpenLab V. 2.51 (total peak purity ≥0.99), and resulted in being specific without any other peak interfering at the retention times of the dosed compounds in the HPLC-DAD detection mode.

2.10. Statistical analyses

All measurements were performed in triplicate and results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's test was performed in order to ascertain possible significant differences between groups as affected by type of liposomes, time after cheese production, SPME temperature and fibre at the level of $p \leq 0.05$, using the Graph Pad Prism 7 software (GraphPad software, San Diego, CA, USA). Correlation analysis was performed and the evaluation of statistical significance of observed differences was performed by using Spearman coefficients of correlation.

3. Results and discussion

3.1. Nanoincorporation of T. capitatus EO into liposomes and addition into sheep cheese

The nanoincorporation of *T. capitatus* EO into liposomes was obtained by the direct sonication of Lecinova® or S75 in water, and turned out to be a simple, low-cost, and organic solvent-free procedure that allowed the production of homogeneous systems with small particles. The results showed that both liposomes were small in size (81 nm for liposomes A (Lecinova®) and 90 nm for liposomes B (S75); $p > 0.05$), slightly polydispersed (PI ~0.3, $p > 0.05$), and highly negatively charged (~−60 mV, $p > 0.05$) (Table 1). High zeta potential values are predictive of a good stability of samples over time.

Different approaches were carried out to find the best procedure to ensure the best and most uniform distribution of *T. capitatus* L. EO liposomes in the sheep cheeses. Of all of them, the approach based on the injection of the nanoformulations into fresh cheese immediately after the whey drainage, proved to be the most appropriate, as it prevented the loss of the formulations together with the whey. Moreover, traditionally whey is further processed into Ricotta cheese and thus it was important to eliminate the possibility of contaminating the whey with liposomal *T. capitatus* EO. Soy lecithin was used to produce liposomes as it is a low-cost product and easy to find worldwide. More specifically, two type of liposomes were developed with the aim of comparing two types of lecithin, S75 and Lecinova®. The former is a more expensive commercial mixture of phospholipids, besides triglycerides and fatty acids, commonly used for the preparation of liposomes, and the latter is a cheaper dietary supplement of gluten-free, non-GMO soy lecithin containing phospholipids, ω-3 and ω-6 fatty acids, vitamin E and B6, marketed as an aid to lowering cholesterol. It should be noted that soy lecithin can contain residual soy protein, which could provoke allergic reactions. Nevertheless, soy lecithin is present in several marketed pharmaceuticals, among which liposomal amphotericin B, and reports of allergic reactions in patients with soy allergy are rare and largely unsubstantiated, and in general, such patients need not to avoid these products (Kelso, 2014). Furthermore, it is noteworthy that, in addition to functional properties, including the improvement of the bioavailability of the incorporated compounds, liposomes have completely natural compositions, which can eliminate or reduce issues related to their inclusion in food systems. Indeed, several liposome-based products are already on the market as food supplements or food preservatives (Shukla et al., 2017). Therefore, it can be concluded that our liposomes can be safely used to enrich foods and, in our specific case, dairy products.

The addition of *T. capitatus* EO liposomes to sheep cheeses did not cause interference in growing and developing of the lactic flora with normal aging of the cheese. This confirmed what was observed by de Carvalho et al. (2015) who reported no inhibitory activity of EO on mesophilic flora with respect to pathogenic bacteria at level of 1.25 μL/g, an amount six times higher than that used in our experiment.

3.2. Composition of T. capitatus essential oil

T. capitatus was chosen because it is an indigenous plant of the Mediterranean area that grows spontaneously in Sardinia (Usai, Foddai, Sechi, Juliano, & Marchetti, 2010; Cosentino et al., 1999). GC-MS and GC-FID analysis of *T. capitatus* essential oil is reported in Table S1 of the supplementary file. Sixteen compounds were identified, accounting for 95.4% of the total peak area. Among all detected components, thymol (40.1%) and *p*-cymene (33.0%) were found in greatest abundance.

Table 2
Volatile compounds (%) contained in the cheese samples and determined by HS-SPME (Headspace Solid-Phase Microextraction) with DVB/CAR/PDMS and CARB/PDMS fibres at 40 °C followed by GC-MS and GC-FID at 20, 60, and 180 days after cheese preparation.

No.	Compound*	RI	HS-SPME (DVB/CAR/PDMS) 40 °C Area percentages (%)								HS-SPME (CARB/PDMS) 40 °C Area percentages (%)							
			TA			TB			BA	BB	TA			TB			BA	BB
			20	60	180	20	60	180			20	60	180	20	60	180		
1.	Acetaldehyde	<900	–	–	–	–	–	1.2 ± 0.1^{a}	–	–	–	–	1.5 ± 0.1^{a}	1.0 ± 0.0^{b}	0.2 ± 0.0^{c}	1.3 ± 0.0^{d}	2.3 ± 0.1^{e}	0.7 ± 0.0^{f}
2.	Ethanol	<900	5.1 ± 0.4^{a}	7.0 ± 0.3^{b}	5.3 ± 0.3^{a}	5.4 ± 0.4^{a}	7.5 ± 0.2^{b}	2.3 ± 0.2^{c}	5.6 ± 0.2^{a}	2.0 ± 0.1^{c}	15.4 ± 1.1^{a}	17.4 ± 1.3^{a}	11.1 ± 0.8^{b}	9.7 ± 0.7^{b}	13.4 ± 0.9^{c}	4.0 ± 0.2^{d}	3.7 ± 0.2^{d}	1.7 ± 0.1^{e}
3.	Acetone	<900			–				4.6 ± 0.3^{a}	6.5 ± 0.53^{b}	–	–	–	–	–	–	4.5 ± 0.2^{a}	3.3 ± 0.3^{b}
4.	Carbon disulfide	<900	1.0 ± 0.0^{a}	2.3 ± 0.1^{b}	2.5 ± 0.1^{b}	0.8 ± 0.0^{c}	1.5 ± 0.1^{d}	0.9 ± 0.1^{ac}	0.1 ± 0.0^{e}	8.3 ± 0.3^{f}	3.0 ± 0.2^{a}	2.5 ± 0.1^{b}	2.4 ± 0.1^{b}	1.9 ± 0.1^{c}	2.5 ± 0.2^{b}	1.7 ± 0.1^{d}	1.0 ± 0.0^{e}	3.0 ± 0.2^{a}
5.	Acetic acid	<900	–	–	–	–	–	–	–	–	3.8 ± 0.2^{a}	2.5 ± 0.1^{b}	18.2 ± 1.4^{c}	8.2 ± 0.6^{d}	9.2 ± 0.4^{e}	7.4 ± 0.4^{d}	2.2 ± 0.1^{f}	14.6 ± 1.1^{g}
6.	3-Hydroxybutan-2-one (Acetoin)	<900	1.8 ± 0.2^{a}	1.8 ± 0.1^{a}	2.3 ± 0.1^{b}	1.7 ± 0.1^{a}	1.2 ± 0.0^{c}	1.9 ± 0.1^{a}	29.1 ± 1.8^{d}	21.0 ± 1.2^{e}	4.3 ± 0.1^{a}	2.5 ± 0.1^{b}	4.1 ± 0.2^{ad}	4.9 ± 0.3^{c}	3.9 ± 0.1^{d}	4.0 ± 0.2^{ad}	53.4 ± 4.1^{e}	38.0 ± 2.7^{f}
7.	Methyl butanoate	<900	0.4 ± 0.0^{a}	1.1 ± 0.0^{b}	–	0.4 ± 0.0^{a}	0.5 ± 0.0^{c}	–	–	–	–	–	–	–	–	–	–	–
8.	Butanoic acid	<900	8.6 ± 0.6^{a}	14.9 ± 1.2^{bd}	28.5 ± 2.4^{c}	8.5 ± 0.5^{a}	13.7 ± 0.9^{b}	16.7 ± 1.4^{d}	25.6 ± 1.9^{c}	26.5 ± 1.8^{c}	11.3 ± 0.8^{a}	27.5 ± 1.5^{b}	22.6 ± 1.9^{c}	18.9 ± 1.3^{d}	19.3 ± 1.2^{d}	23.8 ± 1.7^{c}	17.5 ± 1.3^{d}	19.3 ± 1.6^{d}
9.	Ethyl butanoate	<900	1.1 ± 0.0^{a}	1.3 ± 0.1^{bd}	1.8 ± 0.1^{c}	1.2 ± 0.0^{b}	1.5 ± 0.1^{d}	1.1 ± 0.0^{a}	1.4 ± 0.0^{d}	1.8 ± 0.1^{c}	–	–	–	–	–	–	–	1.7 ± 0.1^{a}
10.	Heptan-2-one	<900	0.2 ± 0.0^{a}	0.1 ± 0.0^{b}	–	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}		1.2 ± 0.0^{c}	1.0 ± 0.1^{b}	–	–	–	0.1 ± 0.0^{a}	–	–	–	0.3 ± 0.0^{b}
11.	Methyl hexanoate (methyl caproate)	928	0.4 ± 0.0^{a}	0.3 ± 0.0^{b}	0.1 ± 0.0^{c}	0.3 ± 0.0^{b}	0.4 ± 0.0^{a}	0.1 ± 0.0^{c}	1.7 ± 0.1^{d}	1.0 ± 0.0^{e}	0.2 ± 0.0^{a}	–	–	0.2 ± 0.0^{a}	–	–	–	0.2 ± 0.0^{a}
12.	α-Pinene	942	1.5 ± 0.1^{a}	1.4 ± 0.1^{a}	0.8 ± 0.0^{b}	1.8 ± 0.1^{c}	1.5 ± 0.0^{a}	1.6 ± 0.1^{a}	–	–	5.4 ± 0.2^{a}	3.8 ± 0.2^{b}	1.5 ± 0.1^{c}	3.4 ± 0.2^{b}	4.1 ± 0.3^{d}	4.3 ± 0.2^{d}	–	–
13.	Camphene	959	0.6 ± 0.0^{a}	0.5 ± 0.0^{b}	–	0.7 ± 0.1^{a}	0.5 ± 0.0^{b}	0.5 ± 0.0^{b}	–	–	1.8 ± 0.1^{a}	1.0 ± 0.0^{b}	0.3 ± 0.0^{c}	0.8 ± 0.0^{d}	1.0 ± 0.0^{b}	1.1 ± 0.1^{b}	–	–
14.	β-Pinene	985	–	–	–	–	–	–	–	–	0.2 ± 0.0^{a}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}	0.2 ± 0.0^{a}	0.1 ± 0.0^{b}	0.2 ± 0.0^{a}	–	–
15.	Hexanoic acid	992	7.3 ± 0.6^{a}	7.5 ± 0.7^{a}	18.7 ± 1.1^{b}	5.5 ± 0.2^{c}	7.4 ± 0.4^{a}	6.7 ± 0.5^{a}	20.8 ± 1.5^{bd}	22.9 ± 1.8^{d}	4.4 ± 0.2^{a}	4.8 ± 0.1^{b}	6.0 ± 0.2^{c}	7.3 ± 0.2^{d}	8.5 ± 0.3^{e}	9.5 ± 0.6^{f}	6.8 ± 0.3^{d}	11.1 ± 0.6^{g}
16.	β-Myrcene	994	2.3 ± 0.1^{a}	3.0 ± 0.1^{b}	–	2.3 ± 0.0^{a}	3.3 ± 0.2^{b}	3.2 ± 0.1^{b}	–	–	1.5 ± 0.1^{a}	1.7 ± 0.1^{a}	1.1 ± 0.0^{b}	1.1 ± 0.0^{b}	1.0 ± 0.0^{b}	1.7 ± 0.1^{a}	–	–
17.	Ethyl hexanoate (Ethyl caproate)	1000	0.9 ± 0.0^{a}	0.5 ± 0.0^{b}	0.9 ± 0.1^{a}	1.1 ± 0.0^{c}	0.8 ± 0.0^{d}	0.3 ± 0.0^{e}	–	–	–	–	–	–	–	–	–	–
18.	α-Terpinene	1023	0.2 ± 0.0^{a}	–	–	0.2 ± 0.0^{a}	–	–	–	–	–	–	–	–	–	–	–	–
19.	*p*-Cymene	1031	51.0 ± 3.4^{ad}	43.0 ± 2.5^{b}	30.5 ± 1.8^{c}	56.0 ± 2.7^{a}	46.6 ± 3.5^{bd}	51.4 ± 2.9^{a}	–	–	40.4 ± 3.1^{a}	27.9 ± 1.8^{b}	30.0 ± 2.7^{cb}	32.9 ± 2.1^{c}	28.1 ± 1.9^{b}	41.9 ± 3.4^{a}		–
20.	Limonene	1035	1.7 ± 0.1^{a}	1.6 ± 0.1^{a}	0.8 ± 0.03^{a}	1.8 ± 0.2^{a}	1.6 ± 0.1^{a}	1.7 ± 0.13^{a}	–	–	–	–	–	–	–	–	–	–
21.	1,8-Cineole	1039	1.0 ± 0.0^{a}	0.6 ± 0.0^{b}	–	0.9 ± 0.0^{c}	0.7 ± 0.0^{d}	0.8 ± 0.0^{e}	–	–	–	–	–	–	–	–	–	–
22.	γ-Terpinene	1065	0.6 ± 0.0^{a}	0.4 ± 0.0^{b}	–	0.6 ± 0.0^{a}	0.5 ± 0.0^{c}	0.6 ± 0.0^{a}	–	–	–	–	–	–	–	–	–	–
23.	Linalool	1102	3.6 ± 0.2^{a}	1.9 ± 0.1^{b}	1.2 ± 0.0^{c}	3.5 ± 0.1^{a}	1.7 ± 0.0^{d}	1.6 ± 0.0^{e}	–	–	1.7 ± 0.1^{a}	0.8 ± 0.0^{b}	0.1 ± 0.0^{c}	1.3 ± 0.1^{d}	0.7 ± 0.0^{e}	0.7 ± 0.0^{e}	–	–
24.	Methyl octanoate	1127	0.2 ± 0.0^{a}	–	–		–	–	–	–		–	–	–	–	–	–	–
25.	Camphor	1150	0.2 ± 0.0^{a}	–	–	0.3 ± 0.0^{a}	–	–	–	–	0.4 ± 0.0^{a}	–	–	–	–	–	–	–
26.	Borneol	1172	0.3 ± 0.0^{a}	–	–	0.3 ± 0.0^{a}	–	–	–	–	0.2 ± 0.0^{a}	–	–	0.2 ± 0.0^{a}	–	–	–	–
27.	Octanoic acid	1185	0.5 ± 0.0^{a}	–	0.9 ± 0.0^{a}	–	–	–	–	–		–	–	–	–	–	–	–
28.	Thymol	1299	2.1 ± 0.0^{a}	1.0 ± 0.0^{b}	–	1.5 ± 0.0^{c}	0.7 ± 0.0^{d}	0.7 ± 0.0^{d}	–	–	1.0 ± 0.0^{a}	0.4 ± 0.0^{b}	0.3 ± 0.0^{c}	0.8 ± 0.1^{d}	0.5 ± 0.0^{e}	0.4 ± 0.0^{b}	–	–

*The identity of all compounds was confirmed by comparison of mass spectrum and RI with those of authentic standard compounds. RI: retention index; Means in each row for each fibre not sharing a superscript letter are significantly different ($p \leq 0.05$). TA: *T. capitatus* EO Lecinova® liposomes; TB: *T. capitatus* EO S75 liposomes; BA and BB: blank cheese samples (without *T. capitatus* EO).

Table 3
Volatile compounds (%) detected in the cheese samples by using HS-SPME (Headspace Solid-Phase Microextraction) with DVB/CAR/PDMS and CARB/PDMS fibres at 80 °C (after HS-SPME at 40 °C) followed GC-MS and GC-FID at 20, 60, and 180 days after cheese preparation.

No.	Compound*	RI	HS-SPME (DVB/CAR/PDMS) 80 °C Area percentages (%)								HS-SPME (CARB/PDMS) 80 °C Area percentages (%)							
			TA			TB			BA	BB	TA			TB			BA	BB
			20	60	180	20	60	180			20	60	180	20	60	180		
1.	Acetaldehyde	<900	–	–	–	–	–	–	–	–	0.5 ± 0.0^{a}	–	–	–	–	–	–	–
2.	Ethanol	<900	4.3 ± 0.3^{a}	6.5 ± 0.3^{b}	3.0 ± 0.1^{c}	3.8 ± 0.2^{a}	5.7 ± 0.2^{d}	4.1 ± 0.1^{a}	1.8 ± 0.1^{e}	0.9 ± 0.0^{f}	2.3 ± 0.1^{a}	2.8 ± 0.2^{b}	2.4 ± 0.1^{a}	1.0 ± 0.0^{c}	3.0 ± 0.2^{b}	1.5 ± 0.1^{d}	0.8 ± 0.0^{e}	0.7 ± 0.1^{e}
3.	Acetic acid	<900	0.6 ± 0.0^{a}	3.3 ± 0.2^{b}	3.5 ± 0.1^{b}	0.8 ± 0.0^{c}	1.4 ± 0.1^{d}	1.2 ± 0.0^{e}	3.0 ± 0.2^{b}	4.3 ± 0.2^{f}	2.0 ± 0.1^{a}	1.6 ± 0.1^{b}	5.3 ± 0.3^{c}	4.3 ± 0.3^{d}	1.9 ± 0.1^{a}	2.3 ± 0.1^{e}	5.7 ± 0.2^{c}	5.7 ± 0.3^{c}
4.	3-Hydroxybutan-2-one (Acetoin)	<900	1.6 ± 0.1^{a}	1.5 ± 0.1^{a}	1.0 ± 0.0^{b}	1.9 ± 0.1^{c}	0.6 ± 0.0^{d}	1.0 ± 0.1^{b}	5.3 ± 0.3^{e}	10.5 ± 0.7^{f}	1.8 ± 0.1^{a}	0.3 ± 0.0^{b}	0.7 ± 0.0^{c}	14.8 ± 1.2^{d}	0.3 ± 0.0^{b}	0.5 ± 0.0^{e}	12.3 ± 0.9^{f}	11.7 ± 0.7^{f}
5.	Butanoic acid	<900	5.6 ± 0.4^{a}	10.6 ± 0.7^{b}	10.7 ± 0.9^{b}	6.0 ± 0.4^{a}	9.4 ± 0.6^{b}	9.9 ± 0.8^{b}	6.2 ± 0.3^{ac}	6.9 ± 0.4^{c}	12.9 ± 1.1^{a}	22.6 ± 1.6^{b}	23.9 ± 1.9^{b}	7.5 ± 0.4^{c}	16.5 ± 1.4^{d}	16.1 ± 0.9^{d}	16.1 ± 1.3^{d}	6.8 ± 0.5^{c}
6.	α-Pinene	942	0.5 ± 0.0^{a}	0.5 ± 0.0^{a}	0.1 ± 0.0^{b}	0.6 ± 0.0^{c}	0.4 ± 0.0^{d}	0.5 ± 0.0^{a}	–	–	0.3 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	–	–
9.	Camphene	959	–	–	–	0.2 ± 0.0^{a}	–	–	–	–	–	–	–	–	–	–	–	–
10.	Hexanoic acid	992	14.3 ± 1.0^{ac}	17.3 ± 1.5^{bc}	19.3 ± 1.6^{b}	14.6 ± 0.9^{ac}	18.8 ± 1.4^{bc}	17.9 ± 1.6^{bc}	18.2 ± 1.5^{b}	16.3 ± 1.1^{c}	16.0 ± 1.2^{a}	18.8 ± 1.4^{b}	31.4 ± 2.6^{c}	12.3 ± 0.8^{d}	21.1 ± 1.7^{b}	21.2 ± 1.5^{b}	30.2 ± 2.5^{c}	18.5 ± 1.4^{ab}
11.	*p*-Cymene	1031	15.0 ± 0.9^{a}	13.4 ± 1.0^{ac}	5.8 ± 0.3^{b}	14.9 ± 1.1^{a}	12.0 ± 0.7^{c}	14.4 ± 1.2^{a}	–	–	31.8 ± 2.4^{a}	26.4 ± 2.1^{b}	9.1 ± 0.6^{c}	24.2 ± 1.7^{bd}	22.6 ± 1.9^{b}	23.2 ± 2.0^{b}	–	–
12.	Limonene	1035	0.4 ± 0.0^{a}	–	–	0.4 ± 0.0^{a}	–	0.2 ± 0.0^{b}	–	–	–	–	–	–	–	–	–	–
13.	Linalool	1102	3.3 ± 0.2^{a}	2.3 ± 0.1^{b}	0.8 ± 0.0^{c}	3.8 ± 0.3^{a}	1.9 ± 0.1^{d}	2.6 ± 0.1^{e}	–	–	2.6 ± 0.1^{a}	1.5 ± 0.1^{b}	0.5 ± 0.0^{c}	2.2 ± 0.1^{d}	1.6 ± 0.1^{b}	1.4 ± 0.1^{b}	–	–
14.	Camphor	1150	–	–	–	0.3 ± 0.0^{a}	–	–	–	–	–	–	–	–	–	–	–	–
15.	Octanoic acid	1185	14.0 ± 0.8^{a}	12.1 ± 0.9^{a}	13.2 ± 0.7^{a}	14.0 ± 1.3^{a}	16.4 ± 1.1^{a}	14.4 ± 1.5^{a}	33.3 ± 2.5^{a}	24.7 ± 1.6^{a}	8.6 ± 0.7^{a}	8.0 ± 0.6^{a}	15.8 ± 1.3^{b}	8.3 ± 0.6^{a}	14.4 ± 1.3^{bc}	13.4 ± 1.0^{c}	20.1 ± 1.6^{d}	30.3 ± 2.7^{e}
16.	Thymol	1299	19.9 ± 1.5^{a}	14.4 ± 1.0^{b}	14.9 ± 1.1^{b}	22.5 ± 1.8^{a}	13.2 ± 0.9^{b}	13.1 ± 1.1^{b}	–	–	7.5 ± 0.5^{a}	4.4 ± 0.2^{b}	1.2 ± 0.1^{c}	12.1 ± 0.8^{d}	8.0 ± 0.5^{a}	8.6 ± 0.6^{a}	–	–
17.	Methyl decanoate	1326	–	–	–	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	–	0.5 ± 0.0^{b}	–	–	–	–	–	–	–	–	0.2 ± 0.0^{a}
18.	Decanoic acid	1384	14.7 ± 1.2^{a}	10.2 ± 0.9^{b}	22.8 ± 1.8^{c}	10.9 ± 0.9^{b}	13.4 ± 0.8^{a}	13.7 ± 1.0^{a}	22.2 ± 2.0^{c}	25.8 ± 1.9^{c}	3.5 ± 0.2^{a}	4.5 ± 0.3^{b}	7.6 ± 0.6^{c}	3.7 ± 0.1^{a}	8.3 ± 0.6^{c}	8.9 ± 0.9^{cd}	10.3 ± 0.7^{d}	20.5 ± 1.4^{e}
19.	Ethyl decanoate	1396	–	–	0.5 ± 0.0^{a}	1.1 ± 0.1^{b}	0.8 ± 0.0^{c}	–	–	–	–	–	–	0.2 ± 0.0^{a}	–	–	–	–

*The identity of all compounds was confirmed by comparison of mass spectrum and RI with those of authentic standard compounds. RI: retention index; Means in each row for each fibre not sharing a superscript letter are significantly different ($p \leq 0.05$). TA: *T. capitatus* EO Lecinova® liposomes; TB: *T. capitatus* EO S75 liposomes; BA and BB: blank cheese samples (without *T. capitatus* EO).

Linalool (6.5%) was the most abundant compound among oxygenated monoterpenes followed by 1,8-cineole, borneol and camphor, which were detected in lower amounts. Monoterpene hydrocarbons found in minor percentages were γ-terpinene, β-myrcene, limonene and α-pinene. This chemical composition dominated by thymol aligns with data previously reported by Cosentino et al. (1999), confirming that *T. capitatus* growing in south Sardinia is a thymol chemotype with a small amount of carvacrol. Indeed, three chemotypes were described in *T. capitatus:* thymol, thymol-carvacrol, and carvacrol (Karousou, Koureas, & Kokkini, 2005; Miceli, Negro, & Tommasi, 2006). Several studies have associated the biological activities of *T. capitatus* EO with these two monoterpenoid phenols present in the essential oil. Cosentino et al. (1999) tested the susceptibility of many microorganisms (*i.e.* food-derived strains: *E. coli, S. aureus, B. cereus*) to the compounds contained in the EO of *T. capitatus* and noticed that thymol and carvacrol express the most potent antimicrobial activity. Other studies on *T. capitatus* EO confirmed antifungal and antimicrobial properties, besides antioxidant, cytotoxic, and antitumor activities (Bounatirou et al., 2007; Goudjil et al., 2020; Salehi et al., 2019).

3.3. Headspace volatile composition of the cheese samples

The typical volatile substances of *T. capitatus* EO were detected in the enriched cheeses (TA and TB) by comparison with those identified in blank cheeses (BA and BB). Indeed, the substances not detected in blank cheese samples were associated with the EO, as confirmed by the EO composition reported in Table S1 in the supplementary file. It was observed that the presence of terpenes in the cheese headspace was connected only to the addition of *T. capitatus* EO, as they were not detected in blank cheese samples. This can be also supported by the positive correlation among all the terpenes detected in cheese extracts (Tables 2 and 3), and by the statistically significant ($p \leq 0.05$, Table S2) Pearson correlations for most of them, suggesting a common origin for all terpenes from the *T. capitatus* EO. This aspect is important as it has been reported that small amounts of terpenes can be found in cheese as a result of pasture feeding (Coda et al., 2006; Pinho, Pérès & Ferreira, 2003). In order to obtain a complete headspace volatile profile, two fibres of different polarities were used: DVB/CAR/PDMS of higher polarity and lower polarity CARB/PDMS. In fact, as reported by Milosavljević, Blagojević, Savić, and Radulović (2012), the polarity of fibres has a distinct impact on the adsorption of the volatile compounds. Additionally, two temperatures (40 °C and 80 °C) were applied to investigate if this parameter can influence the distribution of the sample compounds in the headspace. Depending on the different types of fibre used and temperatures applied for the HS-SPME, different results were obtained for each cheese maturation stage (Tables 2 and 3).

The main volatile compounds detected in the enriched cheese by means of HS-SPME method using high polarity fibre DVB/CAR/PDMS at 40 °C on day 20 were *p*-cymene (51.0%–56.0%), butanoic (8.5%–8.6%) and hexanoic acids (5.5%–7.3%) (Table 2). *p*-Cymene was also the main compound identified with the low polarity fibre CARB/PDMS at 40 °C on day 20, and it was still the main substance identified by both fibres at 60 and 120 days after cheese preparation. Not all substances identified by using fibre DVB/CAR/PDMS were detected with the fibre CARB/PDMS and *vice versa*. For example, among monoterpenes 1,8-cineole and γ-terpinene were detected only with the fibre DVB/CAR/PDMS and β-pinene was identified only in the sample analysed with CARB/PDMS fibre. Among the other compounds, acetic acid was detected only with CARB/PDMS fibre. It is interesting to note that the relative abundance of acetoin in enriched cheese was around ten times lower than in the corresponding blanks. A statistically significant ($p \leq 0.05$) negative correlation between acetoin and *p*-cymene amounts was observed at 40 °C (Pearson correlation: $r_{\text{DVB/CAR/PDMS}} = -0.9290$, and $r_{\text{CARB/PDMS}} = -0.9219$), suggesting that this compound can be responsible for the inhibition of acetoin production (Table S2). Fortified cheese samples in the headspace exhibited elevated concentration of *p*-cymene (30.5%–56.0%; 28.1%–41.9%) indicating that the partition coefficient of this compound in the headspace was significant and, according to the obtained results, influenced the most acetoin abundance in the total headspace composition.

At 80 °C and 20 days after cheese preparation, the main volatile compounds detected by DVB/CAR/PDMS fibre in the headspace of the cheese samples were thymol (19.9%–22.5%) and *p*-cymene (14.9%–15.0%) (Table 3). These monoterpenes were followed by lower aliphatic carboxylic acids such as hexanoic (14.3%–18.8%), octanoic (14.0%), and decanoic (10.9%–14.7%) acids. *p*-Cymene, thymol and lower aliphatic acids were also detected with CARB/PDMS fibre in both samples. Additionally, by comparing the results of BA with two different fibres, it was noticed that butanoic acid was detected in the sample with CARB/PDMS fibre in a percentage almost 3 times higher (16.1%). In contrast, with CARB/PDMS fibre applied on day 20 *p*-cymene was the main compound in both TA and TB samples and it contributed to 31.8% (TA) and to 24.2% (TB) of total area. It is worth noting that carvacrol, which was present in pure EO (1.7%) (Table S1), was not identified in the headspace of the cheese samples (Tables 2 and 3).

Evolution during cheese maturation showed interesting trends at 60 and 180 days after preparation. A gradual increase in the amount of butanoic and hexanoic acids was detectable in TA cheese enriched with Lecinova® liposomes throughout maturation (in the results obtained with both DVB/CAR/PDMS and CARB/PDMS fibres). Additionally, in TA samples, regardless of the fibre used, relative abundance of hexanoic acid increased gradually over time. In TB cheese enriched with S75 liposomes, the values of this acid detected with DVB/CAR/PDMS fibre fluctuated, while using CARB/PDMS fibre the difference between day 60 (21.1%) and day 180 (21.2%) was negligible. An increase in the presence of volatile free fatty acids (FFA) was observed, which is a tendency that has been previously noted (Jerković et al., 2019). According to Khattab, Guirguis, Tawfik, and Farag (2019) this is related to the progressive lipolysis taking place in the cheese matrix during maturation. Short chain fatty acids contribute to the aroma and flavour of the cheese. The presence of FFAs also determines the production of esters, which are formed in the reaction of FFA with alcohols. The main alcohol detected by HS-SPME-GC-FID/MS was ethanol, which explains why ethyl esters were present. The volatile compounds formed during maturation are responsible for unique sensory characteristics of the cheese. On the contrary, the relative amounts of volatile terpenes, e.g. *p*-cymene, thymol and linalool, showed a general decrease throughout maturation. Some fluctuating data during maturation were observed, especially in TB samples, which can be related to minor uneven distribution of the nanoformulation within the cheese replicates or the samples prepared for the headspace extraction.

A decrease in the abundance of thymol was observed by using both fibres. Interestingly, the difference between 60 and 180 days was less evident than that detected between 20 and 60 days, where statistically significant differences ($p \leq 0.05$) were observed. Similarly, the percentage of linalool decreased gradually during maturation in the results obtained by CARB/PDMS fibre, whereas fluctuating values were found by using the other fibre during the same maturation period. The abundance of thymol detected at 80 °C was higher than that measured at 40 °C ($p \leq 0.05$), which means that it is not highly volatile and needs higher temperatures to be released. This finding confirms that, in the case of less volatile terpenes from matrix such as cheese, high temperature for the equilibration and extraction for HS-SPME are preferable. Additionally, DVB/CAR/PDMS fibre is more useful in identifying thymol than CARB/PDMS fibre at the same temperature ($p \leq 0.05$). As explained by Milosavljević et al. (2012), this was probably related to the fibre-coating polarity and sensitivity for the extraction of certain compounds, such as thymol. The evolution and differences in volatiles throughout the ripening process are the outcome of chemical and microbiological transitions of milk compounds and loss of the most volatile compounds, like terpenes, due to their evaporation (Milosavljević et al., 2012).

3.4. HPLC-DAD analysis of targeted monoterpenoid phenols

Although HS-SPME followed by GC-MS/FID is a very powerful method for evaluating compounds contained from *T. capitatus* EO, it has hard limits from a quantitative point of view. For this reason, an HPLC-DAD method was developed and validated for dosing thymol and carvacrol in the cheese extracts. The analysis was targeted to these two monoterpenoid phenols as they are considered responsible for the antioxidant properties of *Thymus* EO (Jukić & Miloš, 2005; Goudjil et a.l, 2020; Salehi et al., 2019). The HPLC-DAD method turned out to be very sensitive, with values of LOD ≥0.13 mg/kg and LOQ ≥0.40 mg/kg for both compounds.

The chromatograms reported in Fig. S3 show that in the blank cheese samples (panel A) there were no signs of the presence of thymol and carvacrol. Panel B shows the two peaks of carvacrol and thymol in the enriched cheeses, where standards of substances of interest were added at the same concentration (2.00 mg/kg). In chromatograms representing cheese samples on day 60 of ripening (panel C and D), the two monoterpenoid phenols are clearly detectable. Table 4 reports the quantitative data, and it was confirmed that both thymol and carvacrol were obviously not present in the blank cheese samples (BA, BB). As expected from *T. capitatus* EO composition, thymol was the most abundant monoterpenoid phenol. In TA and TB on day 20 of maturation, the amount detected was of 9.51 and 10.10 mg/kg respectively (Table 4). Moreover, it was observed that the amount of thymol significantly decreased with time ($p \leq 0.05$, Table S3). On day 60, it reached 5.85 mg/kg in TA and 7.14 mg/kg in TB, whereas on day 180 the values in TA and TB were 2.53 and 3.53 mg/kg, respectively. In contrast to the HS-SPME-GC-MS/FID method, carvacrol was detectable on day 20 at ca. 1 mg/kg. However, on day 60 only traces were detected, while on day 180 the amount of carvacrol was < LOD. Fig. S4 shows the evolution of antioxidant compounds during maturation: after 60 days the decrease percentage was ca. 1/3 of the initial amount (29% and 38% for TB and TA, respectively), and after 180 days, the decrease was around 2/3 (precisely 65% and 73% for TB and TA, respectively). This means that the decrease rate was higher during the first 60 days after preparation, but it was reduced over time (180 days). That said, the results obtained by HPLC-DAD analysis performed during the maturation process, are in agreement with those obtained by HS-SPME-GC-MS/FID. In any case, the HPLC-DAD method led to a more sensitive and specific evaluation of the quantitative decrease of monoterpenoid phenols in the enriched cheeses over time.

3.5. Determination of total phenolic content

The estimated total amount of phenolic compounds (TP) in the cheese samples produced with and without the addition of liposomal *T. capitatus* EO, is reported in Table 4. It should be noted that blank cheese samples showed an amount of 211.46 and 203.03 mg GAE/kg for BA and BB, respectively. The addition of *T. capitatus* EO liposomes increased the amount of phenolic compounds in the cheeses, more than twofold in the cheese samples analysed 20 days after preparation, ranging from 423.44 to 434.05 mg GAE/kg of cheese for TA and TB cheeses, respectively. During maturation, the amount of TP gradually decreased, since lower values were reached for both TA and TB samples 180 days after preparation (278.21 and to 298.39 mg GAE/kg for TA and TB, respectively). However, even at 180 days TP values of both TA and TB were significantly different from the corresponding blanks ($p \leq 0.05$, Table S3). The fact that blank sheep cheese presents an amount of TP detectable by means of the Folin-Ciocalteu assay, is probably connected to the mechanism of reaction of this assay and the cheese composition. Indeed, Folin-Ciocalteu's reagent measures a sample's reducing capacity, thus it can react with other compounds in the cheese which have antioxidant capacity, mainly peptides with aromatic or sulphur containing amino acids, vitamins, carotenoids, metals, enzyme systems and lipids (Khan et al., 2019; Lamuela-Raventós, 2018).

3.6. Antioxidant activity assays

In order to examine the antioxidant activity of the cheeses enriched with *T. capitatus* EO liposomes, FRAP and DPPH$^{\bullet}$ assays were performed. Both tests are based on electron transfer reactions, but FRAP is used to gather information on total antioxidant potential and DPPH$^{\bullet}$ on the radical scavenging capacity. Blank cheese samples produced without the addition of nanoincorporated *T. capitatus* EO presented a detectable antioxidant activity (0.41 and 0.39 mmol Fe^{2+}/kg and 0.02 mmol TEAC/kg, in BA and BB for FRAP and DPPH$^{\bullet}$, respectively) (Table 4). This activity increased in all cheese samples enriched with liposomal *T. capitatus* EO at three different maturation stages. For both FRAP and DPPH$^{\bullet}$ assays, the strongest activity was observed on day 20 and 60 (ca. 0.55 mmol Fe^{2+}/kg and 0.09 mmol TEAC/kg, for FRAP and DPPH$^{\bullet}$, respectively) followed by day 180 (0.46–0.47 mmol Fe^{2+}/kg and 0.04–0.05 mmol TEAC/kg, in TA and TB for FRAP and DPPH$^{\bullet}$, respectively). This means that the antioxidant activity decreased throughout cheese maturation, in a similar manner to that observed for monoterpenoid phenols and TP. A statistically significant ($p \leq 0.05$) positive correlation among TP, FRAP and DPPH$^{\bullet}$ (Pearson correlation: $r_{TP/FRAP} = 0.9440$, $r_{TP/DPPH\bullet} = 0.9275$ and $r_{FRAP/DPPH\bullet} = 0.9842$) results was observed, and this is most likely due to the mechanisms of reaction behind these assays (Huang, Ou, & Prior, 2005). More interesting was the statistically significant ($p \leq 0.05$) positive correlation among TP, FRAP and DPPH$^{\bullet}$ with the amount of monoterpenoid phenols (sum of thymol and carvacrol) detected by HPLC (Pearson correlation: $r_{T+C/FRAP} = 0.9296$, $r_{T+C/DPPH\bullet} = 0.9289$ and $r_{T+C/TP} = 0.9936$) (Table S4). These results support the findings that the antioxidant properties of sheep cheese enriched with nanoincorporated *T. capitatus* EO arise from the presence of thymol and carvacrol in the EO. Finally, one-way ANOVA followed Tukey's multiple comparison test on FRAP, DPPH$^{\bullet}$, TP and T + C means for all eight samples (BA, BB, TA 20, TB 20, TA 60, TB 60, TA 180, and TB 180) showed no statistically significant

Table 4
Quantitative amounts of carvacrol, thymol, total phenols and antioxidant capacity of the cheese samples at 20, 60, and 180 days after cheese production (mean ± SD, $n = 3$).

Parameter	TA			TB			BA	BB
	20	60	180	20	60	180		
Carvacrol (mg/kg)	0.92 ± 0.08^{a}	tr	nd	0.97 ± 0.10^{a}	tr	nd	nd	nd
Thymol (mg/kg)	9.51 ± 0.57^{a}	5.85 ± 0.39^{c}	2.53 ± 0.11^{e}	10.10 ± 0.79^{a}	7.14 ± 0.56^{b}	3.53 ± 0.19^{d}	nd	nd
Total phenols (mg GAE*/kg)	423.44 ± 26.83^{a}	342.43 ± 9.44^{b}	278.21 ± 5.68^{c}	434.05 ± 14.19^{a}	348.59 ± 9.83^{b}	298.39 ± 17.27^{c}	211.46 ± 9.64^{d}	203.03 ± 5.66^{d}
FRAP[§] (mmol Fe^{2+}/kg)	0.55 ± 0.04^{a}	0.55 ± 0.04^{a}	0.46 ± 0.02^{b}	0.56 ± 0.04^{a}	0.54 ± 0.03^{a}	0.47 ± 0.02^{b}	0.41 ± 0.01^{d}	0.39 ± 0.02^{d}
DPPH$^{\bullet}$[#] (mmol TEAC/kg)	0.09 ± 0.02^{a}	0.08 ± 0.02^{a}	0.04 ± 0.01^{bc}	0.09 ± 0.01^{a}	0.09 ± 0.02^{a}	0.05 ± 0.01^{b}	0.02 ± 0.01^{c}	0.02 ± 0.02^{c}

* GAE: gallic acid equivalent; [§] FRAP value is expressed as Fe^{2+} millimolar concentration, obtained from a $FeSO_4$ solution having an antioxidant capacity equivalent to that of the dilution of the cheese extract; [#]DPPH$^{\bullet}$value is expressed as TEAC millimolar concentration, obtained from a Trolox solution having an antiradical capacity equivalent to that of the dilution of the cheese extract. tr: traces (<LOQ). nd: not detected (<LOD). Means in each row not sharing a superscript letter are significantly different ($p \leq 0.05$). TA: *T. capitatus* EO Lecinova® liposomes; TB: *T. capitatus* EO S75 liposomes; BA and BB: blank cheese samples (without *T. capitatus* EO).

difference ($p = 0.3772$) between A and B formulations. This finding confirm that two liposome formulations, despite the different lecithins composition, are interchangeable. Thus, the choice between one of the two can depend on the cost or the local availability of such lecithins. The outcomes obtained in this study are in agreement with the literature data on the antioxidant properties of *Thymus* EO, especially on the content of its monoterpenoid phenols (Aeschbach et al., 1994; Bounatirou et al., 2007; Goudjil et al., 2020; Jukić & Miloš, 2005; Salehi et al., 2019). The mechanism of the antioxidant function of thymol and carvacrol has been extensively investigated. Kinetic studies demonstrated that thymol is a better antioxidant in lipids than carvacrol due to the greater steric hindrance of the phenolic group in the former (Yanishlieva, Marinova, Gordon, & Raneva, 1999). According to Nagoor Meeran, Javed, Al Taee, Azimullah, and Ojha (2017) after ingestion, higher concentrations of thymol were detected in the mucosa and other inner contents of the intestines, and it was partially absorbed and distributed in the liver, lungs, kidneys, and muscles. The effects of absorbed thymol include the scavenging of free radicals by increasing the activities of several endogenous antioxidant enzymes levels (Nagoor Meeran et al., 2017). Moreover, the activity of thymol and its mixture with carvacrol was evaluated in the intestinal Caco-2 cell line and at low concentrations, and a protective effect against induced oxidative stress was observed (Llana-Ruiz-Cabello et al., 2015). Given that, consumption of foods enriched with EOs rich in monoterpenoid phenols can protect the human body from oxidative stress damages. However, although EOs are GRAS (generally recognized as safe) substances, to avoid potential pro-oxidant effects the amount of these polyphenols in enriched foods should be examined and modulated to ensure their safety and efficacy.

Overall results suggest that, due to the reduction of both TP content and antioxidant activity, our enriched sheep cheeses are better used after a short maturation. Recent studies based on marketing analyses reported that consumers do not eat sheep cheese due to its peculiar sensory properties (namely strong taste and hardness). Thus, a shorter maturation of the sheep cheese associated with new natural flavouring might attract the most unconvinced sheep cheese consumers. Besides, taking into account that aging has great economic impact due to the high costs of storage, the possibility to commercialize cheese in shorter times is considered as a profitable strategy. Given that, our enriched cheeses fully meet these needs and could boost the growth of the sheep cheese sector.

4. Conclusions

The study showed that enriched sheep cheese with nanoincorporated *T. capitatus* EO can be obtained through a reproducible and scalable technological process. Two liposome formulations were tested, both proving to be effective, being capable of stabilizing and protect the bioactives contained in the *T. capitatus* EO, and prolonging their beneficial effect, irrespective of the phospholipid used. Indeed, these new dairy product possess enhanced antioxidant properties compared to blank chesses ($p \leq 0.05$), especially ripened for a relatively short period of time (20 and 60 days). The choice of which formulation to use may be dictated by the greater availability, affordability, and accessibility of Lecinova®, which can be purchased at grocery stores. In this way, the tradition and the properties of well-known and widely recognized sheep cheese were the base to develop a new product by the use of local *T. capitatus* EO nanoincorporated into liposomes. Given that, this experimentation might be helpful for local dairy producers who may have the chance to enter new markets mainly characterized by a high demand for innovative and beneficial products.

CRediT authorship contribution statement

Katarzyna Angelika Gil: Investigation, Data curation, Writing – review & editing. **Igor Jerković:** Investigation, Data curation, Writing – review & editing. **Zvonimir Marijanović:** Methodology, Investigation, Data curation. **Maria Letizia Manca:** Methodology, Investigation, Data curation, Writing – review & editing. **Carla Caddeo:** Methodology, Investigation, Data curation, Writing – review & editing. **Carlo Ignazio Giovanni Tuberoso:** Conceptualization, Investigation, Methodology, Resources, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

Aeschbach, R., Löliger, J., Scott, B. C., Murcia, A., Butler, J., Halliwell, B., et al. (1994). Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. *Food and Chemical Toxicology, 32*(1), 31–36. https://doi.org/10.1016/0278-6915(84)90033-4

Barone, G., Dambrosio, A., Storelli, A., Busco, A., Ioanna, F., Quaglia, N. C., et al. (2018). Traditional Italian cheeses: Trace element levels and estimation of dietary intake. *Journal of Food Composition and Analysis, 66*, 205–211. https://doi.org/10.1016/j.jfca.2017.12.025

de Barros Fernandes, R. V., Costa Guimarães, I., Rodrigues Ferreira, C. L., Alvarenga Botrel, D., Vilela Borges, S., & Umbelina de Souza, A. (2017). Microencapsulated rosemary (*Rosmarinus officinalis*) essential oil as a biopreservative in Minas Frescal cheese. *Journal of Food Processing and Preservation, 41*(1), Article e12759. https://doi.org/10.1111/jfpp.12759

Bigliardi, B., & Galati, F. (2013). Innovation trends in the food industry: The case of functional foods. *Trends in Food Science & Technology, 31*(2), 118–129. https://doi.org/10.1016/j.tifs.2013.03.006

Bounatirou, S., Smiti, S., Miguel, M. G., Faleiro, L., Rejeb, M. N., Neffati, M., et al. (2007). Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian *Thymus capitatus* Hoff. et Link. *Food Chemistry, 105*(1), 146–155. https://doi.org/10.1016/j.foodchem.2007.03.059

Carocho, M., & Ferreira, I. C. F. R. (2013). A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and Chemical Toxicology, 51*, 15–25. https://doi.org/10.1016/j.fct.2012.09.021

de Carvalho, R. J., de Souza, G. T., Honório, V. G., de Sousa, J. P., da Conceição, M. L., Maganani, M., et al. (2015). Comparative inhibitory effects of *Thymus vulgaris* L. essential oil against *Staphylococcus aureus, Listeria monocytogenes* and mesophilic starter co-culture in cheese-mimicking models. *Food Microbiology, 52*, 59–65. https://doi.org/10.1016/j.fm.2015.07.003

Coda, R., Brechany, E., De Angelis, M., De Candia, S., Di Cagno, R., & Gobbetti, M. (2006). Comparison of the compositional, microbiological, biochemical, and volatile profile characteristics of nine Italian Ewes' milk cheeses. *Journal of Dairy Science, 89*(11), 4126–4143. https://doi.org/10.3168/jds.S0022-0302(06)72458-4

Cosentino, S., Tuberoso, C. I. G., Pisano, B., Satta, M., Mascia, V., Arzedi, E., et al. (1999). *In-vitro* antimicrobial activity and chemical composition of Sardinian *Thymus* essential oils. *Letters in Applied Microbiology, 29*(2), 130–135. https://doi.org/10.1046/j.1472-765X.1999.00605.x

Costa, M. J., Maciel, L. C., Teixeira, J. A., Vicente, A. A., & Cerqueira, M. A. (2018). Use of edible films and coatings in cheese preservation: Opportunities and challenges. *Food Research International, 107*, 84–92. https://doi.org/10.1016/j.foodres.2018.02.013

Cui, H. Y., Wu, J., & Lin, L. (2016). Inhibitory effect of liposome-entrapped lemongrass oil on the growth of *Listeria monocytogenes* in cheese. *Journal of Dairy Science, 99*(8), 6097–6104. https://doi.org/10.3168/jds.2016-11133

El-Sayed, S. M., & Youssef, A. M. (2019). Potential application of herbs and spices and their effects in functional dairy products. *Heliyon, 5*(6), Article e01989. https://doi.org/10.1016/j.heliyon.2019.e01989

Goudjil, M. B., Zighmi, S., Hamada, D., Mahcene, Z., Bencheikh, S. E., & Ladjel, S. (2020). Biological activities of essential oils extracted from *Thymus capitatus* (Lamiaceae).

South African Journal of Botany, 128, 274–282. https://doi.org/10.1016/j.sajb.2019.11.020

Gülçin, I. (2012). Antioxidant activity of food constituents: An overview. *Archives of Toxicology, 86*, 345–391. https://doi.org/10.1007/s00204-011-0774-2

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry, 53*(6), 1841–1856. https://doi.org/10.1021/jf030723c

ICH Topic Q2 (R1). (2006). *Validation of analytical procedures: Text and methodology*. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-2-r1-validation-analytical-procedures-text-methodology-step-5_en.pdf, 13.05.19.

Jerković, I., Kranjac, M., Marijanović, Z., Roje, M., & Jokić, S. (2019). Chemical diversity of headspace and volatile oil composition of two brown algae (*Taonia atomaria* and *Padina pavonica*) from the Adriatic Sea. *Molecules, 24*(3), 495. https://doi.org/10.3390/molecules24030495

Jerković, I., Kranjac, M., Marijanović, Z., Zekić, M., Radonić, A., & Tuberoso, C. I. G. (2016). Screening of *Satureja subspicata* Vis. honey by HPLC-DAD, GC-FID/MS and UV/VIS: Prephenate derivatives as biomarkers. *Molecules, 21*(3), 377. https://doi.org/10.3390/molecules21030377

Jukić, M., & Miloš, M. (2005). Catalytic oxidation and antioxidant properties of thyme essential oils (*Thymus vulgarae* L.). *Croatica Chemica Acta, 78*, 105–110.

Karousou, R., Koureas, D. N., & Kokkini, S. (2005). Essential oil composition is related to the natural habitats: *Coridothymus capitatus* and *Satureja thymbra* in NATURA 2000 sites of crete. *Phytochemistry, 66*(22), 2668–2673. https://doi.org/10.1016/j.phytochem.2005.09.020

Kelso, J. M. (2014). Potential food allergens in medications. *The Journal of Allergy and Clinical Immunology, 133*(6), 1509–1518. https://doi.org/10.1016/j.jaci.2014.03.011

Khan, I. T., Nadeem, M., Imran, M., Ullah, R., Ajmal, M., & Jaspal, M. H. (2019). Antioxidant properties of milk and dairy products: A comprehensive review of the current knowledge. *Lipids in Health and Disease, 18*(1), 41. https://doi.org/10.1186/s12944-019-0969-8

Khattab, A. R., Guirguis, H. A., Tawfik, S. M., & Farag, M. A. (2019). Cheese ripening: A review on modern technologies towards flavor enhancement, process acceleration and improved quality assessment. *Trends in Food Science & Technology, 88*, 343–360. https://doi.org/10.1016/j.tifs.2019.03.009

Lamuela-Raventós, R. (2018). Folin-Ciocalteu method for the measurement of total phenolic content and antioxidant capacity. In R. Apak, E. Capanoglu, & F. Shahidi (Eds.), *Measurement of antioxidant activity and capacity: Recent trends and applications (Chapter 4)*. John Wiley & Sons Ltd. https://doi.org/10.1002/9781119135388.ch6. ISBN: 9781-119135388.

Llana-Ruiz-Cabello, M., Gutiérrez-Praena, D., Puerto, M., Pichardo, S., Jos, Á., & Cameán, A. M. (2015). *In vitro* pro-oxidant/antioxidant role of carvacrol, thymol and their mixture in the intestinal Caco-2 cell line. *Toxicology in Vitro, 29*(4), 647–656. https://doi.org/10.1016/j.tiv.2015.02.006

Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews, 4*(8), 118–126. https://doi.org/10.4103/0973-7847.70902

Manconi, M., Petretto, G., D'hallewin, G., Escribano, E., Milia, E., Pinna, R., et al. (2018). *Thymus* essential oil extraction, characterization and incorporation in T phospholipid vesicles for the antioxidant/antibacterial treatment of oral cavity diseases. *Colloids and Surfaces B: Biointerfaces, 171*, 115–122. https://doi.org/10.1016/j.colsurfb.2018.07.021

Miceli, A., Negro, C., & Tommasi, L. (2006). Essential oil variability in *Thymbra capitata* (L.) Cav. growing wild in Southern Apulia (Italy). *Biochemical Systematics and Ecology, 34*(6), 528–535. https://doi.org/10.1016/j.bse.2005.12.010

Milosavljević, N. P., Blagojević, P. D., Savić, D. S., & Radulović, N. S. (2012). Application of HS-SPME-GC-MS-derived variables for monitoring ripening-induced changes in cheese volatile compounds. *Dairy Science & Technology, 92*, 321–333. https://doi.org/10.1007/s13594-012-0070-9

Nagoor Meeran, M. F., Javed, H., Al Taee, H., Azimullah, S., & Ojha, S. K. (2017). Pharmacological properties and molecular mechanisms of thymol: Prospects for its therapeutic potential and pharmaceutical development. *Frontiers in Pharmacology, 8*, 380. https://doi.org/10.3389/fphar.2017.00380

NIST - National Institute of Standards and Technology. (2018). *Chemistry WebBook. NIST Standard Reference Database Number 69*. http://webbook.nist.gov/chemistry/, 13.09.19.

Pinho, O., Pérès, C., & Ferreira, I. M. P. L. V. O. (2003). Solid-phase microextraction of volatile compounds in "Terrincho" Ewe cheese: Comparison of different fibers. *Journal of Chromatography A, 1011*(1–2), 1–9. https://doi.org/10.1016/S0021-9673(03)01066-5

Salehi, B., Abu-Darwish, M. S., Tarawneh, A. H., Cabral, C., Gadetskaya, A. V., Salgueiro, L., et al. (2019). *Thymus* spp. plants - food applications and phytopharmacy properties. *Trends in Food Science & Technology, 85*, 287–306. https://doi.org/10.1016/j.tifs.2019.01.020

dos Santos Gouvea, F., Rosenthal, A., & da Rocha Ferreira, E. H. (2017). Plant extract and essential oils added as antimicrobials to cheeses: A review. *Ciência Rural, 47*(8), Article e20160908. https://doi.org/10.1590/0103-8478cr20160908

Shukla, S., Haldorai, Y., Hwang, S. K., Bajpai, V. K., Huh, Y. S., & Han, Y.-K. (2017). Current demands for food-approved liposome nanoparticles in food and safety sector. *Frontiers in Microbiology, 8*, 2398. https://doi.org/10.3389/fmicb.2017.02398

Sindhi, V., Gupta, V., Sharma, K., Bhatnagar, S., Kumari, R., & Dhaka, N. (2013). Potential applications of antioxidants - a review. *Journal of Pharmacy Research, 7*(9), 828–835. https://doi.org/10.1016/j.jopr.2013.10.001

Tuberoso, C. I. G., Boban, M., Bifulco, E., Budimir, D., & Pirisi, F. M. (2013). Antioxidant capacity and vasodilatory properties of Mediterranean food: The case of *Cannonau* wine, myrtle berries liqueur and strawberry-tree honey. *Food Chemistry, 140*(4), 686–691. https://doi.org/10.1016/j.foodchem.2012.09.071

Usai, M., Foddai, M., Sechi, B., Juliano, C., & Marchetti, M. (2010). Comparison of antibacterial activity of natural and hydroformylated essential oil of *Thymus capitatus* growing wild in North Sardinia with commercial *Thymus* essential oils. *Natural Product Communications, 5*(12), 1985–1989. https://doi.org/10.1177/1934578X1000501233

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*(1), 59–66. https://doi.org/10.1016/S0308-8146(98)00086-7

Zambrano-Zaragoza, M. L., González-Reza, R., Mendoza-Muñoz, N., Miranda-Linares, V., Bernal-Couoh, T. F., Mendoza-Elvira, S., et al. (2018). Nanosystems in edible coatings: A novel strategy for food preservation. *International Journal of Molecular Sciences, 19*(3), 705. https://doi.org/10.3390/ijms19030705