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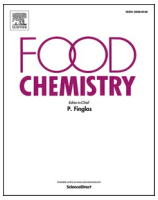

Synthesis and characterisation of Mānuka and rosemary oil-based nano-entities and their application in meat

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ABSTRACT

Mānuka (MO) and rosemary oils (RO) -containing nanoemulsions and nanocapsules made of sodium alginate and whey protein, were designed and compared for their antioxidant effect. Mānuka oil-nanoemulsions and nanocapsules had smaller particle sizes (343 and 330 nm), less negative zeta potential (−12 mV and −10 mV), higher phenolic content, and antiradical characteristics than RO-nano-entities. However, nano-entities of both oils showed more thermostability and sustained release than free oils. Further, the antioxidant effect of essential oils and their nano-entities was compared against sodium nitrite (SN)-added and without antioxidants-added (controls) and Wagyu and crossbred beef pastes (14 days refrigerated storage). No significant difference among MO, RO and their nano-entities was noticed in crossbred pastes, while in Wagyu, nanoemulsions showed the lowest oxidation values than controls and SN-added pastes. Hence, nano-entities can be alternatives to chemical preservatives as natural antioxidants in meat preservation, along with improved thermal stability and release than free oils.

1. Introduction

Due to the overexposure of meat surfaces to oxygen and catalysts (metals) and the distribution of microbes during the grinding process, ground meat products are more prone to spoilage lipid oxidation reactions and microbial growth (Amiri et al., 2019). Other than the loss of colour, flavour, texture and nutritional values, these deteriorating reactions can produce free radicals and pathogenic microbes, which may be harmful and pathogenic to human health (Devatkal et al., 2010). The use of antioxidant agents is one of the most promising and practical approaches to reduce oxidative reactions and prolong the shelf of meat products. In recent years, natural antioxidants from plant sources have gained broad interest due to safety approvals, less regulatory requirements and consumer preferences over chemical preservatives like sodium nitrite (Pereira et al., 2017).

Several researchers have reported that the addition of plant essential oils to raw and processed meat products can be an additional hurdle to alleviate oxidative reactions (lipid and proteins) (Fratianni et al., 2010; Ghaderi-Ghahfarokhi et al., 2016). *Leptospermum scoparium* belonging to the Myrtaceae family is an essential plant in Māori culture and grows in New Zealand and some parts of Australia. This plant, also known as mānuka, has been utilised in beverages and medicinal purposes since ancient times (Chen et al., 2016; Porter & Wilkins, 1999). Mānuka oil has been listed as a food flavouring ingredient in the Australia New Zealand Food Standards code; however, safety assessments are required at the proposed levels and patterns before its use (Australia New Zealand Food Standards, 2020). *Rosmarinus officinalis* is an aromatic plant, recognised as rosemary, belongs to the *Lamiaceae* family and comes from the Mediterranean region (Kaur et al., 2021). In the literature, rosemary is widely studied as a natural preservative to retard oxidative reactions and increase the shelf life of meat commodities (Kaur et al., 2021; Vital et al., 2016).

Nevertheless, facile degradation, low water solubility and undesirable taste and flavour, especially in the case of essential oils, pose a great challenge for their use in food preservation. The application of nanotechnology is a promising and innovative approach to overcoming these challenges by improving stability and providing targeted delivery and controlled release characteristics to bioactives (Kaur & Kaur, 2020).

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Extensive research has been conducted to devise various nano-entities, such as nanoemulsions, nanocapsules, nanospheres and nanoparticles, by employing several materials and techniques (Abbasi et al., 2019). Emulsion-based systems are mainly fabricated for this purpose because their composition and structures can be designed to alter the solubility and transformation of lipophilic bioactives. However, nanocapsules can be synthesised to provide targeted delivery, controlled release and protection against pH, light, oxygen and other environmental factors (Ghaderi-Ghahfarokhi et al., 2016).

Alginate is a biopolymer commonly utilised to encapsulate different bioactive compounds due to its easy availability, low cost, non-toxicity and ease of gelation (Rahnemoon et al., 2021). The use of alginate as an encapsulating material for delivery and protection of bioactive compounds, either in emulsions or capsules form, showed good efficiency and has the function of prolonging the shelf life of food products (Salvia-Trujillo et al., 2013; Zhang et al., 2022). However, in these encapsulating systems, the partitioning and release of bioactive compounds from carrier material into food systems can be influenced by food constituents like fat and water. Thus the efficacy of bioactive compounds may differ according to the food composition. Wang, Heising, et al. (2020) studied the ability of polylactic acid films loaded with carvacrol to prolong the shelf-life of beef with different fat contents (5 and 12%). The mass transfer release of carvacrol into headspace, food, and packaging film in two kinds of beef having different fat content (5 and 12%) was also studied. The results exhibited that the partitioning of bioactive compounds from films was influenced by the fat content of foods and storage temperature. The food's fat content or composition can also influence the release of essential oils from different forms, such as emulsions or capsules, but have never been studied. This study, for the first time, explores the antioxidant effects imparted by mānuka and rosemary oils that are encapsulated in two different nanoforms, i.e., nanoemulsions and nanocapsules, when added to Wagyu and normal beef. Meat products produced from Wagyu and other beef breeds are considerably different in composition due to the higher intramuscular fat content and high amounts of unsaturated fatty acids. Thus, Wagyu beef shows more vulnerability to lipid oxidation than other kinds of beef (Bermingham et al., 2021; Boylston et al., 1996). This research hypothesised that nanoencapsulation of mānuka and rosemary oils in nanoemulsions and nanocapsules form can improve their stability, *in vitro* antioxidant activity, release characteristics and antioxidant effects when added to meat.

Hence, the objectives of this study were the synthesis and characterisation of mānuka and rosemary oils -containing nanoemulsions and nanocapsules and comparing their antioxidant effects against free oils. Encapsulated forms were characterised by laser diffraction, thermogravimetry, microscopy, Fourier-transform spectroscopy, and *in vitro* antioxidant assays. Further, the antioxidant effect of nano-entities was determined in meat pastes prepared from New Zealand Wagyu and crossbred (Angus/Hereford) beef tenderloins during 14 days of storage at 4 °C. The antioxidant efficacy of prepared nano-entities was compared against free oils, chemical preservatives (sodium nitrite)- added meat pastes, and control meat pastes (prepared without any added preservatives).

2. Materials and methods

2.1. Materials

The steam-distilled mānuka essential oil (triketone content around 25%) was kindly supplied by Tairawhiti Pharmaceuticals Ltd. (Te Araroa, New Zealand). The rosemary oil was purchased from "Now Foods" (Auckland, New Zealand). The oil samples were stored in a freezer at −20 °C to avoid the effect of the light and temperature. Oils samples were removed from the freezer and kept at room temperature for 30–45 min just before the analysis. The vacuum-packed grain-fed Wagyu beef tenderloins were supplied by the Black origin (New Zealand Wagyu, Christchurch). The tenderloins from crossbred Angus/ Hereford were obtained from Gourmet Butchery, Napier (New Zealand). Meat samples were stored in the freezer at −20 °C and thawed overnight before the analysis.

Chemical reagents used in this study such as Folin-Ciocalteu reagent, sodium bicarbonate, gallic acid, ethanol, 1, 1-diphenyl-2, picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate, sodium alginate, whey protein, calcium chloride, sodium acetate buffer, Iron (III) chloride hexahydrate FeCl3.6H2O and tripyridyl triazine, hydrochloric acid, ferrous sulfate (FeSO4), and sodium nitrite were of analytical grade.

2.2. Methods

2.2.1. Preparation of essential oil containing nanoemulsions

In this study, nanoemulsions and nanocapsules were synthesised using sodium alginate solution as an aqueous phase, mānuka/rosemary oil as the oil phase and whey protein as an emulsifier. The sodium alginate (1%) was dissolved in distilled water under constant stirring for 24 h to allow complete hydration. Separately, whey protein concentrate (WPC) was hydrated in distilled water under constant magnetic stirring for 12–24 h. To prepare denatured WPC, the overnight hydrated protein solution was heated at 80 °C, and pH 8 was adjusted after cooling at room temperature. Both solutions were filtered through 0.4 μm membrane filters to remove any- aggregate materials. Finally, mānuka and rosemary oils were added to the whey protein solution, and then both solutions were mixed and homogenised at 12,000 rpm using an ultra-turrax homogeniser (Ultra-turrax, IKA) for 2–3 min. This coarse emulsion was prepared according to the method of Volić et al. (2022). The emulsion was subjected to a high-pressure homogenisation system (microfluidiser, Massachusetts, USA) to obtain nanoemulsions. The emulsions were passed through a microfluidiser working at 150 MPa for three cycles (Salvia-Trujillo et al., 2013).

2.2.2. Preparation of essential oil containing nanocapsules

The calcium chloride (1%) solution was added dropwise while magnetic stirring (at room temperature) the nanoemulsions to produce crosslinks and obtain nanocapsules. The nanocapsules were suspended in the same solution overnight and then recovered by centrifugation and washing twice with distilled water. Finally, obtained nanocapsules were re-suspended in distilled water and used for further characterisation.

2.2.2.1. Particle size and zeta potential analysis of essential oil containing nanocapsules and nanoemulsions. The particle size and zeta potential of the nanoemulsion and nanocapsules were measured using a Malvern Zetasizer Nano ZS instrument (Horiba, Japan). The samples were diluted with milli-Q water, added to the electrophoretic mobility cell, and examined at a scattering angle of 173° utilising Malvern Zetasizer (Nano ZS).

2.2.2.2. Fourier-transform infrared spectroscopic (FTIR) analysis of essential oils and their nanoforms (nanoemulsions and nanocapsules). Fourier-transform infrared spectroscopy (FTIR) analysis of oil, nanoemulsion and nanocapsule samples was performed with an iDr 7 ATR-FTIR spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) to confirm the presence of chemical constituents. Each nanoemulsion and oil sample were put directly on the surface of platinum-diamond crystal using glass transfer pipettes, while the nanocapsule was dried in ambient air and then mixed with potassium bromide. The spectrum for each sample was obtained from 400 to 4000 cm^{-1}. The obtained data were examined using OMNIC^{TM} software (Thermo Scientific, Auckland, New Zealand). The obtained data were corrected with background data from the empty cell at 25 °C and the spectra were converted to % transmittance data. Peaks were also identified using the "peak identification" function.

2.2.2.3. Microscopic analysis of essential oil containing nanoemulsions and nanocapsules. Light microscopy was used to study the shape and distribution of nanoemulsions while scanning electron microscope image was conducted to evaluate the shape and size of nanocapsules. Before SEM analysis, nanocapsules were mounted on stubs with double adhesive tape and sputter coating with gold (Baltec SCD 050, Balzers, Liechtenstein). The coated samples were observed under SEM (FEI Quanta 200, FEI Electron Optics, Eindhoven, the Netherlands) at 20 kV accelerating voltage.

2.2.2.4. Thermogravimetric analysis of essential oils and their nano-entities (nanoemulsions and nanocapsules). To determine the thermal stability of mānuka oil, nanoemulsion and nanocapsules, thermal gravimetric analysis was carried out using a thermal analyser (TGA, model STA 449 F5 Jupiter). Each sample of around 10 mg was heated from 30 to 300 °C with a heating rate of 10 °C. The curves were analysed using the NETZSCH ASC software (NETZSCH, Selb, Germany).

2.2.2.5. Total phenolic content determination of essential oils and their nano-entities (nanoemulsions and nanocapsules). The total phenolic content of essential oil, nanoemulsion and nanocapsules was determined using the Folin-Ciocalteu method, according to Sridhar and Charles (2018). The 100 µL samples were mixed with 1000 µL Folin-Ciocalteu reagent (0.20 N), and after 6 min, 800 uL of sodium bicarbonate (7.5%) was added, followed by vigorously mixing. The contents were incubated for 30 min in the dark, and absorbance was read at 765 nm using a spectrophotometer. The TPC content was expressed as µg of gallic acid equivalents per µL of the sample.

2.2.2.6. In vitro release profile of essential oils from their nano-entities (nanoemulsions and nanocapsules). To determine the release profile of mānuka oil from nano-entities, model food systems were prepared to mimic real aqueous, alcohol-containing, and fatty foods using distilled water (with 0.02% tween 80), 10% and 50% ethanol-containing solutions (Amani et al., 2021). The exact quantities of free, emulsified, and encapsulated mānuka oil were added and sealed into a dialysis membrane cut off between 12 and 14 kDa (Snakeskin dialysis tubing, Thermo Fisher, USA) 25 mL of food simulants and stirred at 150 rpm at 4 °C. At preselected time intervals of 0, 1, 2, 4, 5, 7, 9, 24, 48 and 96 h, 2 mL of samples were removed from the food stimulants and replaced with fresh simulant to maintain a constant volume. The absorbance of samples was noted at 260 nm using a Multiskan GO microplate spectrophotometer equipped with Skanlt software version 3.2 (Thermo Scientific, USA). Different mathematical models were applied to the obtained data to understand the release mechanisms (supplmentary materials).

2.2.2.7. Determination of in vitro antioxidant activities of essential oils and their nano-entities (nanoemulsions and nanocapsules)

2.2.2.7.1. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. The electron-donating ability of mānuka-free oil, nanoemulsion and nanocapsules was measured with 1, 1-diphenyl-2, picrylhydrazyl (DPPH) as a free radical, according to the method of Li et al. (2022) with slight modifications. For this purpose, the nanoemulsion and nanocapsule samples were diluted in ethanol and vortexed, followed by filtration using 0.45 µm filter paper. To determine the antiradical activity, 100 µL of the prepared sample was mixed with 2 mL of DPPH solution (0.5 mmol/L). The mixture was shaken vigorously and left in the dark for 30 min for incubation at room temperature. The absorbance was measured at 515 nm using a UV–Vis spectrophotometer (Evolution 201) equipped with INSIGHT ™ software (Thermo Scientific™, United States). The following equation was used to calculate the free radical scavenging activity:

$$Radical\ scavenging\ activity = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

Here, A $_{sample}$ = absorbance of the DPPH + essential oil sample, and A $_{control}$ = absorbance of the DPPH methanol.

2.2.2.7.2. 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity. The ABTS radical scavenging activity of the oil, nanoemulsion and nanocapsule samples was determined according to the method of Sridhar and Charles (2018). Firstly, $ABTS^{\bullet+}$ radical was generated by dissolving equal amounts of 7 mM of ABTS aqueous solution and 2.45 mM aqueous potassium persulfate solution, allowing it to react at room temperature for 12–16 h in the dark. The stock solution of $ABTS^{\bullet+}$ radical was prepared by diluting it with ethanol and adjusting its absorbance value in the range of 0.90 ± 0.02 at 734 nm. Then, 0.2 mL of the sample/ standard was mixed with the 1.8 mL of the generated $ABTS^{\bullet+}$ radical. The control sample was prepared by adding the exact amounts of radicals with double distilled water. Lastly, the absorbance reading was noted at 734 nm, and the percentage inhibition was determined using Equation 1.

2.2.2.7.3. Ferric radical absorbing power (FRAP) assay. The free radical absorbing power (FRAP) assay of essential oil, nanoemulsions and nanocapsules samples was conducted using a method of Sridhar and Charles (2018) with few modifications. Firstly, FRAP reagent was produced through the mixing of sodium acetate buffer (300 mM, pH 3.6), $FeCl_3$·6H2O (20 mM) and tripyridyl triazine (10 mM in 40 mM HCl) in a ratio of 10:1:1 in volume. Samples of essential oil, nanoemulsions and nanocapsules were mixed with FRAP reagent and incubated for 30 min at 37 °C. The increase in absorbance of the ferrous tripyridyl triazine-coloured complex was noted at 593 nm against the blank containing all the reagents except the sample. In this assay, $FeSO_4$ was used as a standard to make a calibration curve at different concentrations. The antioxidant activity of samples was calculated from the obtained curve and expressed as µM of $FeSO_4$ / µL of the sample.

2.2.2.8. Meat paste preparation and storage conditions (at refrigerated storage of two weeks). To prepare meat paste, crossbred and Wagyu beef tenderloins were chopped into small cubes using a knife and then minced through a meat mincer (Mainca, PM-98, Barcelona, Spain) with a plate of 8 mm diameter holes. The minced samples were transferred into Hobart meat bowl Chopper (Ohio, USA), attached with a knife, and mixed for about 15 min to obtain a uniform paste. The prepared paste was further used for the different treatments of essential oils, nanoemulsions and nanocapsules.

2.2.2.9. Preparation of meat-essential oils systems. The prepared pastes were divided into eight different lots for treatments with 2.5% mānuka oil (MO), 2.5% rosemary oil (RO), 2.5% mānuka oil nanoemulsion (ME), 2.5% mānuka oil nanocapsules (MC), 2.5% rosemary oil nanoemulsions (RE), 2.5% rosemary oil nanocapsules (RC), and 150 mg/kg sodium nitrite (SN), followed by mixing in a bench mixer (Kogan, 1600 W, New Zealand) for about 15 min at room temperatures. All meat samples and nanoentities (nanoemulsions and nanocapsules) were prepared in a sterile environment to prevent any microbial contamination, which may influence the safety of meat systems. The ingredients and containers (container of the mixer) were sterilized to ensure the safety of preservatives/treatments. A control sample without any preservative/ treatment was also prepared from crossbred, and Wagyu meat pastes. All prepared samples were packed in zip-lock bags (100 mm × 155 mm) and stored at 4 °C in a dark room. Samples were removed at different time intervals of 0, 7 and 14 days and checked for colour and lipid oxidation.

2.2.2.9.1. Lipid oxidation analysis. The 2-Thiobarbituric acid (TBA) values were evaluated with modifications to the method of Botsoglou et al. (1994). To plot the calibration curve, 1, 1, 3, 3- tetraethoxypropane (TEP) was used as a standard. The results were noted as mg of malonaldehyde per kg of meat sample.

2.2.2.9.2. Colour. The colour of the meat pastes was evaluated by using the Minolta colourimeter (Chroma meter, CR 400, Hong Kong, China). Firstly, equipment was calibrated using a white tile, and each

meat paste was scanned for colour values (L^*, a^* and b^*) in a petri dish. All readings were taken in triplicates.

2.2.2.10. Statistical analysis. To determine the effects of different treatments on meat, a one-way analysis of variance (ANOVA), followed by the *Tukey* method analysis (at a 95% confidence interval), was conducted using Minitab Version 19.2020.2.0 (Minitab Inc., State College, PA, USA). A general linear model in Minitab was used to compare different meats (between crossbred and Wagyu), storage periods (between the 0th, 7th and 14th day of storage), and treatments (MO*RO = comparison between mānuka oil and rosemary oil, MO*ME = comparison between mānuka nanoemulsion and mānuka oil, MO*MC = comparison between mānuka oil and mānuka nanocapsules, and ME*MC = comparison between mānuka nanoemulsion and mānuka nanocapsules). The analyses were performed for each meat and storage day separately.

3. Results and discussions

3.1. Characterisation of nanoemulsions and nanocapsules

The results of particle size and zeta potential (PDI) of nanoemulsions and nanocapsules are presented in Fig. 1 (a and b) and Table 1. The average particle size analysis showed that rosemary nanoemulsion (369 ± 18.38 nm) and nanocapsules (550 ± 65.06 nm) had significantly larger particle sizes than the mānuka nano-entities. The nanocapsules and nanoemulsions containing mānuka oil had particle sizes around 343.8 ± 15.12 nm and 330.6 ± 17.61 nm, respectively. The dynamic laser scattering showed that all nanoemulsions and nanoparticles were uniformly scattered between 220 and 800 nm (Fig. 1a). The size of essential oils containing nanoemulsions was within the size range reported by other studies. Abbasi et al. (2019) also reported that particle size of ultrasound assisted-water in oil nanoemulsion stabilised by alginate and whey protein and designed as carrier delivery of α-linolenic acid of flaxseed oil was uniformly distributed in <1000 nm. Similarly, Yilmaz et al. (2019) reported the particle size of oregano oil nanoparticles between 290 and 483 nm. A schematic representation of nanoemulsions and nanocapsules is presented in supplementary data (Fig. S1). Salvia-Trujillo et al. (2013) reported that the coarse emulsion of lemongrass essential and sodium alginate was 1410 ± 366 nm, while it reduced to 23 ± 2 nm after passing it through one cycle of microfluidizer at 150 MPa.

The essential oil stability and retention inside a core material depend on various factors such as the chemical nature of essential oil (active groups and chemical functionality), molecular weight, polarity and wall material characteristics (Assadpour & Mahdi Jafari, 2019; Ju et al., 2019). The hydrophobicity-hydrophilicity of an essential oil affects the encapsulation process, and surfactants/emulsifiers allow it to balance this character and ameliorate the encapsulation efficiency (Pavoni et al., 2020).

Zeta potential represents the surface charge of nano-entities in solutions and is an essential factor influencing these nanodispersions' stability. In this study, the mānuka nanoemulsions and nanocapsules were moderately negatively charged, varying from –12 mV to −10 mV. The zeta potential of rosemary nanoemulsions was −14 mV, while rosemary nanocapsules were highly negative (−25.31 mV). The negative zeta potential values of nanoemulsions and nanocapsules indicate the adsorption of sodium alginate onto whey protein to form a bilayer around the droplets. The results agree with previous reports describing the negative zeta potential of essential oil-loaded nanoforms. Abbasi et al. (2019) also reported the formation of negatively charged nanoemulsions using sodium alginate and whey protein. However, Yilmaz et al. (2019) showed positively charged nanoparticles, and their zeta potential values increased (from +25.2 to 47.7 mV) with an increase in essential oil. Zeta potential values usually vary from −100 to + 100 mV. Higher values (positive to negative) of zeta potential result in a stable nanosystem (Abbasi et al., 2019; Jafari, 2017). These values depend on the charge of actual particles and cationic and anionic ions, which move with particles in the electric field. Thereby, the negative charge seems to be attributed to the anionic constituents in whey protein and sodium alginate nanodispersions (Surh et al., 2006; Yilmaz et al., 2019). Food hydrocolloids such as sodium alginate have been reported as emulsion stabilizers, as these can be absorbed into the interfacial layer, while their

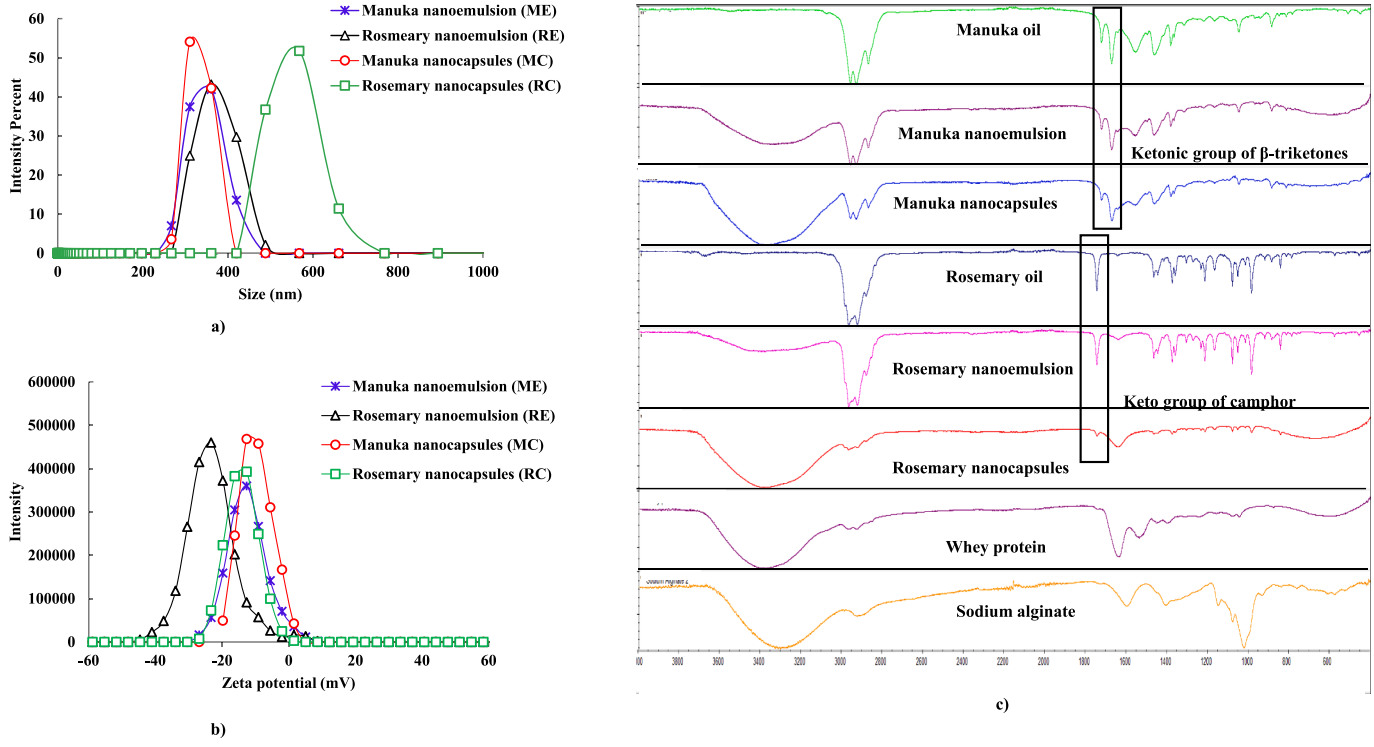

Fig. 1. Particle size distribution (a), zeta potential (b), and Fourier transform infrared spectroscopic (c) analysis of nanoentities.

Table 1
Physico-chemical and *in vitro* antioxidant properties of nanoemulsions, nanocapsules and free essential oils (mānuka and rosemary oils).

		Mānuka oil	Mānuka nanoemulsion	Mānuka nanocapsules	Rosemary oil	Rosemary nanoemulsion	Rosemary nanocapsules
Total phenolic compounds (μg GA / μL sample)		1104 ± 37.65^{a}	389 ± 52.14^{c}	316 ± 34.32 ^{cd}	704 ± 54.27^{b}	246 ± 18.60^{d}	268 ± 44.79^{d}
Radical scavenging activity	**DPPH•** (%)	85 ± 2.28^{a}	48 ± 2.66^{b}	47 ± 1.73^{bc}	39 ± 1.34^{bcd}	37 ± 4.66 ^{cd}	34 ± 6.11^{d}
	ABTS• (%)	65 ± 4.15^{a}	38 ± 0.23^{b}	24 ± 3.05^{c}	49 ± 2.37^{b}	41 ± 10.38^{b}	17 ± 0.46^{c}
	$Fe^{2+\bullet}$	2050 ± 147.2^{a}	691 ± 20.59^{c}	636 ± 13.01^{c}	1448 ± 155.6^{b}	574 ± 1.31^{c}	524 ± 17.09^{c}
Average Particle size (nm)		–	343.8 ± 15.12^{c}	330.6 ± 17.61^{c}	–	369 ± 18.38^{b}	550 ± 65.06^{a}
Zeta potential (mV)		–	−12.64 ± 3.58	−10.07 ± 0.28	–	−25.31 ± 0.68	−14.12 ± 1.138

FRAP units: (μg of $FesO_4$/μL of the sample), Total phenolic compounds (TPC) units: (μg GA / μL sample).
Different superscripts within a row represent a statistically significant difference ($p \leq 0.05$).

emulsion-stabilizing property is dependent on interfering interactions, and the presence of competitive adsorbed species (Dickinson, 2003, 2009). Salvia-Trujillo et al. (2013) documented that the strong negative-zeta potential of microfluidizer assisted-lemon-grass oil-alginate nano-emulsions was owing to the presence of anionic hydrocolloid i.e., sodium alginate, which was used as an emulsion stabilizer.

3.2. FTIR analysis

FT-IR spectroscopic analysis of essential oils, nanoemulsions, and nanocapsules was performed to determine the molecular interactions between the components in nanoemulsions and nanocapsules. The absorption peaks in alginate spectra near 1418 and 1616 cm^{-1} are due to a carboxyl group's symmetric and asymmetric vibration (COO–), respectively. These peaks were also observed in mānuka and rosemary nano-entities, either nanocapsules or nanoemulsions; however, the absorption was weakened. For the sodium alginate, whey protein, nano-emulsions and nanocapsules, it was observed that characteristics peak in the range between 3200 and 3600, which was due to the stretching vibration of O—H bonds of these molecules. Other peaks attributed to the stretching vibration of C—H aliphatic chains are observed near 2900 cm^{-1} in the spectrum of all compounds. In the whey protein, peaks between 3000 and 3500 cm-1 correspond to the stretching vibration of free hydroxyl and N—H bonds in the amino groups. These peaks in the mānuka and rosemary nano-entities spectrum indicate that essential oils were successfully encapsulated in the sodium alginate and whey protein because these peaks were absent in native oils. Absorption bands appearing near the 2750 and 3000 cm^{-1} could be due to the stretching vibration of the C—H bond in the CH_2 and CH_3 groups, respectively. Two absorption bands at 1403 and 1541 cm^{-1} associated with the O—H bending are less discernible in the nanoemulsions and nanocapsules containing rosemary oil than in the neat rosemary oil. It may be due to the hydrogen bonding between the O—H group of rosemary compounds and the N—H and O—H group of carrier materials such as whey protein and sodium alginate.

Observing Fig. 1c, an absorption peak between 1690 and 1720 cm^{-1} in the infrared spectrum of mānuka oil, ME and MC was due to strong C═O stretching and attributed to the presence of β-triketones, including leptospermone, isoleptospermone, flavesone and grandiflorone (Liu et al., 2021). These peaks were stronger in neat mānuka oil compared to the mānuka nanoemulsions and nanocapsules. Characteristics peak of the keto group of camphor in rosemary oil and its nano-entities displayed at 1746 cm^{-1}, whereas peaks around 1375 cm^{-1} and 1450 cm^{-1} could be due to the ether group from the epoxy region of 1,8 cineole. The major compounds in rosemary are 1, 8 cineole and camphor, which constitute more than 50% of its total content (confirmed by Gas Chromatography-Mass Spectroscopy (GCMS), data not shown). All these compounds contribute to C—H stretching bands between 1375 and 1442 cm^{-1} in the fingerprint region and between 2873 and 2967 cm^{-1} in the functional group region. In spectra of rosemary oil and rosemary oil containing nano-entities, an intense peak was observed at 885 cm due to the carbonyl (CH and CH_2) groups bending, which seems to be due to the presence of α-pinene. Mānuka oil and its nanoemulsions and nanocapsules also exhibited this peak but were sustainably lower than the rosemary oil because alpha-pinene was present in lower quantities in the mānuka oil (Kinninmonth et al., 2013). In studies by Liu et al. (2021) and Kim et al. (2022), the presence of aliphatic and conjugated triketones bands between 1724 and 1674 cm^{-1} in the FTIR spectrum of mānuka oil was also noticed. A recent study by Kaur et al., (2023a) confirmed the presence of α-pinene and alcoholic compounds in rosemary oil and β-triketones in the mānuka oil by exhibiting their peaks in the FTIR spectra of these oils. The intensity of ketonic peaks in the spectrum was increased as β-triketone content increased from 5 to 40% in mānuka oil (Kaur et al., 2023a).

The changes observed in the absorption of the FTIR spectrum indicate the molecular interactions between essential oils and carrier agents, i.e., sodium alginate and whey protein.

3.3. Thermogravimetric analysis

The thermograms of essential oil, nanoemulsions and nanocapsules are presented in Fig. 2 (a and b). This analysis was conducted to determine the effect of nano-emulsification and nanoencapsulation on the thermal stability of essential oils. The mānuka and rosemary oil presented a single thermal event associated with its evaporation, beginning at 50 °C and having maximum evaporation at 160 °C. When the maximum temperature was reached, mānuka oil was completely degraded, while rosemary oil showed some remaining ashes. Essential oils are usually made up of volatile constituents, which sometimes account for more than 95% of the compounds of the total oil constituents.

In the TGA thermogram of mānuka oil nanoemulsions and nanocapsules, the first weight reduction was under 100 °C, which may be due to the evaporation of internal water. The second weight loss was observed between 100 and 175 °C related to the mānuka oil decomposition. For rosemary oil thermograms, instant weight loss was observed up to 130 °C, and then 28% of the rosemary oil compounds were maintained at 250 °C. Nanocapsules loaded with rosemary oil lost a very small amount of weight below 100 °C (internal water evaporation) and then between 100 and 150 °C (rosemary oil compounds evaporation). Rosemary nanoemulsions and nanocapsules had around 42 and 65% of the remaining compounds, which were not decomposed even at 275 °C.

Nonetheless, nanoemulsions and nanocapsules loaded with essential

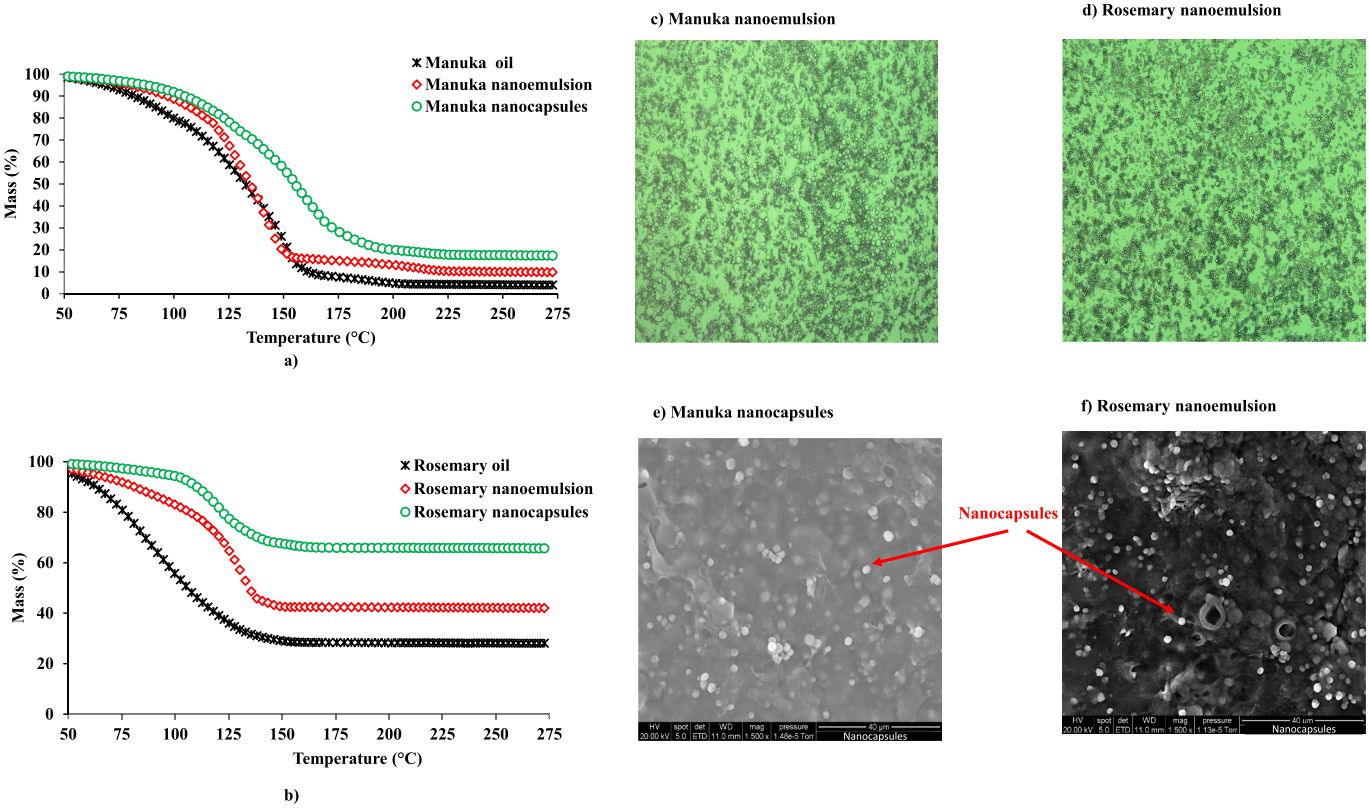

Fig. 2. Thermogravimetric analysis of mānuka (a) and rosemary oil (b) and their nanoentities. Optical microscopic images of mānuka (c) and rosemary nanoemulsions (d). Scanning electron microscope images of mānuka (e) and rosemary nanocapsules (f).

oils presented a similar degradation profile to their respective essential oils; however, a slighter shift towards higher temperatures may be due to alginate coating and crosslinking reaction. Among all forms (free oil, nanoemulsions and nanocapsules), nanocapsules of both oils showed the highest thermal stability. This thermal behaviour confirms the high volatility of free essential oils and justifies the ameliorated thermal stability of nanocapsules. Amani et al. (2021) also reported improvement in the thermal stability of rosemary essential oil after encapsulation in high amylose corn starch nanoparticles through inclusion complexation. Karimi-Khorrami et al. (2022) reported the comparable heat stability of calcium alginate films containing thymol-loaded nanostructured lipid carriers (NLC) than the control films. The authors also confirmed the evaporation of water and volatile constituents such as thymol occurs below 120 °C (Bagheri et al., 2019).

3.4. Microscopic analysis

The structure and dispersion of nanoemulsions were observed through an optical microscope (Fig. 2c and d). These images confirm the presence of essential oils as dispersed phases in an aqueous phase of sodium alginate and whey protein. It suggests that oil droplets have consistent shapes and were uniformly distributed in the aqueous phase, thus confirming the oil in water-type nanoemulsion formation. In the microscope images, since the freshly prepared nanoemulsions were observed, it can be seen that a significantly less degree of aggregation occurred. A scanning electron microscope was used to observe the structure of nanocapsules after coating them with gold. The nanocapsule's core–shell type structure can be clearly observed from the markings in Fig. 2 (f and e). The synthesised nanocapsules showed a spherical structure and, up to some extent, aggregation. This aggregation could be due to the higher concentration of alginate and emulsifier (Yang et al., 2021). A difference in the droplet size of mānuka and rosemary nanoemulsions was observed, also confirmed by the Zetasizer analysis, which might be due to the difference in viscosities of both oils (Wooster et al., 2008). In general, particle disruption during homogenisation could be hindered when the viscosity of the lipid phase increases, thereby leading to a larger particle size of droplets (Wooster et al., 2008). In addition, studies by Shamekhi et al. (2018) and Sarmento et al. (2007) confirmed the aggregation of alginate nanocapsules, which might be attributed to the higher calcium ions concentrations resulting in intermolecular crosslinking of alginates (Sarmento et al., 2007: Shamekhi et al., 2018).

3.5. Total phenolic content and in vitro antioxidant activity

The mānuka essential oil had a higher total phenolic content (1104 μg GA / μL sample) than the rosemary oil (704 μg GA/ μL sample), as shown in Table 1. Among the free mānuka oil, nanoemulsions and nanocapsules containing mānuka oil, free mānuka oil showed higher total phenolics than the nanoemulsions and nanocapsules (Table 1). However, the total phenolic content of rosemary nanocapsules and nanoemulsions were not significantly different. Rosemary oil possessed significantly lower phenolic content than mānuka oil. The phenolic content and composition of rosemary oil have been widely studied (Kaur et al., 2021). In a previous study, the total phenolic content of rosemary oil was 225 ± 6 mg/L, which was significantly lower than the clove, thyme and oregano (Viuda-Martos et al., 2010). The higher total phenolic content of mānuka oil than rosemary oil has been reported by our recent study of Kaur et al., (2023a). A recent study also confirmed that rosemary oil possessed polyphenolic constituents and differences in the polyphenolic composition of essential oils could be due to their extraction method (solvent, temperature, and pressure conditions) and different solubility of phenols in the solvents (Miljanović et al., 2023; Park et al., 2019).

The antiradical activities of free oils, nanoemulsions, and nanocapsules were evaluated by the DPPH, ABTS and FRAP assay. Both

essential oils exhibited concentration-dependent antioxidant effects. As shown in Supplementary Fig. S2 (a, b, c, and d), mānuka and rosemary oils exhibited strong antioxidant activities against DPPH, ABTS and Fe^{2+} radicals. The highest DPPH•, ABTS• and $Fe^{2+\bullet}$ scavenging activity was shown by mānuka oil (85%, 64% and 2050 μg of $FesO_4$/μL of the sample, respectively)) followed by rosemary oil (39%, 49% and 1448 μg of $FesO_4$/μL of the sample). Nanoemulsions and nanocapsules of essential oils showed lower free radical (DPPH•, Fe^{2+}•, and ABTS•) scavenging activities than the free form of both oils. The reason may be the limited release of essential oils/bioactive compounds from the nanocapsules and nanoemulsions into the antioxidant measuring medium. It has been reported that adding bioactive droplets to the whey protein matrix reduces their mobility and diminishes the release from the matrix and antioxidant efficacy. In addition, the low solubility of whey protein in organic solvents like ethanol, which was used to prepare antioxidant reagent solution DPPH and ABTS, has been noticed (Daniel et al., 2004). Nikolaidis and Moschakis (2018) reported that ethanol induces reversible denaturation-related structural changes in the structure of whey proteins (Nikolaidis & Moschakis, 2018), which may interfere with the essential oil retention or release in nanoemulsions and nanocapsules. The antioxidant activity of rosemary oil is due to the presence of carnosol and carnosic acid, while alpha-terpinene and terpineol are responsible for the antioxidant characteristics of mānuka oil. Previous studies have reported the strong antioxidant efficacy of rosemary oil (Kaur et al., 2021). The antioxidant activity of mānuka oil against tea tree oil (*Melaleuca alterifolia*) and kanuka oil has been reported by Lis-Balchin et al. (2000) and Lis-Balchin (2006) by showing its more consistent antioxidant effect on mice skin than the other two oils. Like this, our recent study has shown a higher antioxidant activity of mānuka oil than kanuka and rosemary oil (Kaur et al., 2023a). The antioxidant activity of mānuka oil could be attributed to the presence of sesquiterpene compounds in this oil (Kwon et al., 2013; Lis-Balchin et al., 2000, Lis-Balchin, 2006; Kaur et al., 2023a).

3.6. *In vitro release profile of mānuka oil from nanoemulsions and nanocapsules*

The effect of different incubation times on the release profile of mānuka oil in three food-simulating materials is presented in Fig. 3. Incubation time significantly influenced the release of essential oils from nanocapsules and nanoemulsions.

In general, a rapid release of mānuka oil was observed, followed by a slowed release at longer intervals until a plateau value was achieved. In distilled water, 45% of mānuka oil was released from the free form before reaching its plateau value of full release, while nanoemulsions and nanocapsules released 72 and 85% of oil, respectively. After 24 h, nanoemulsions and nanocapsules continuously released the oil, indicating their continuous release than the free oil, which showed its burst release for 24 h. A similar pattern was observed for the mānuka oil suspended in 10% ethanol either in free, emulsified or encapsulated form. However, all forms released their maximum amount during the first 24 h in 50% ethanol. The difference in release profile of encapsulated and emulsified mānuka oil in different food-simulating media could be related to their solubility in the aqueous medium, as well as the stability of the essential oil compounds. For example, free mānuka oil suspended in distilled water and 10% ethanol solution showed a slower

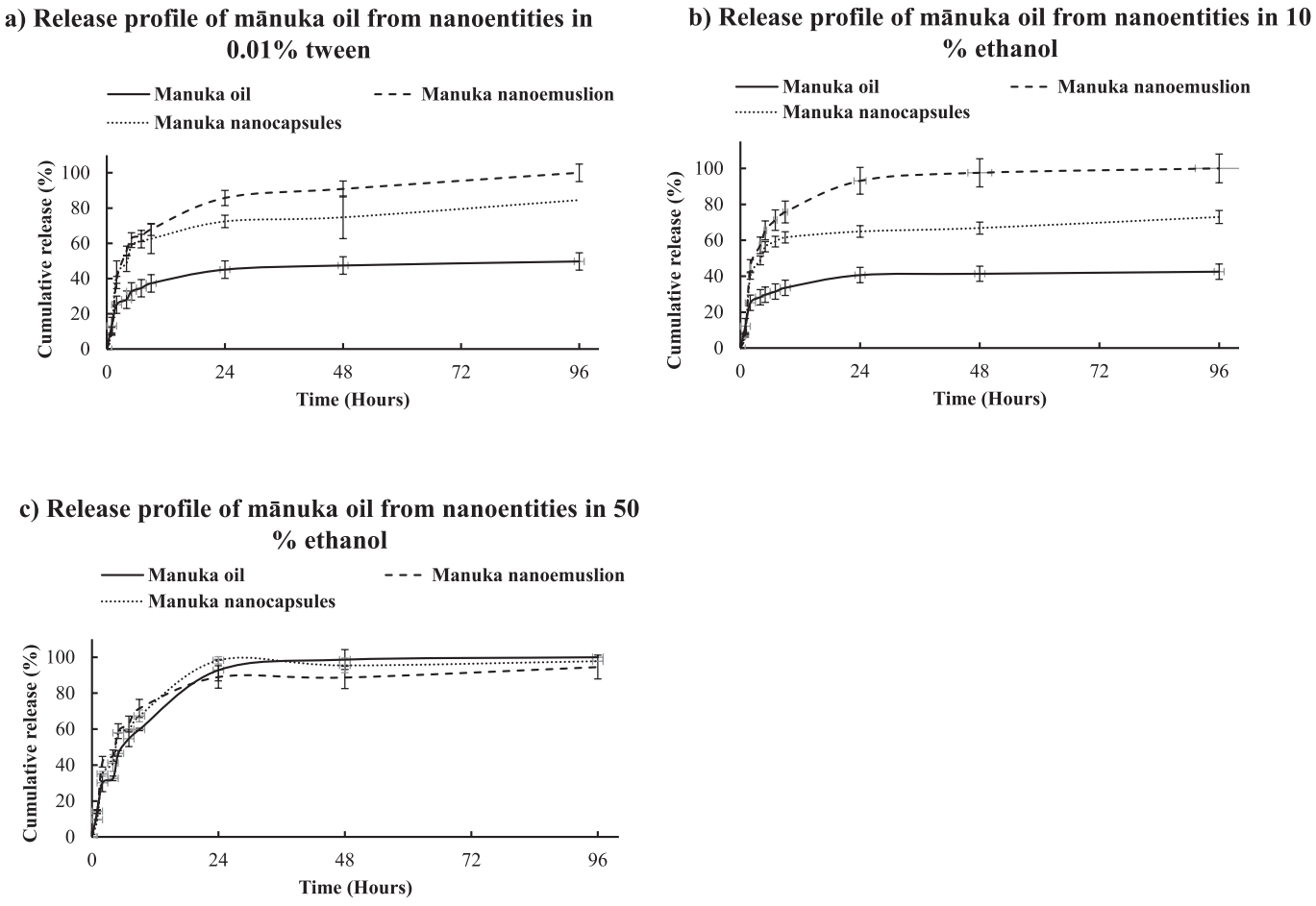

Fig. 3. Release profile of mānuka oil from nanocapsules and nanoemulsions in a) distilled water containing 0.01% tween 80, b) 10% ethanol solution, and c) 50% ethanol solution.

release than its nanoforms. However, the higher release of mānuka oil in a 50% ethanol solution could be linked to its increased solubility in ethanol. The increased release of mānuka oil from nano-entities could be attributed to the smaller droplet size and increased surface areas for oils to come in contact with a simulating solvent. A similar pattern of the release profile of rosemary oil from nanocapsules and nanoemulsions was observed (data not shown). The mathematical modelling to understand the release mechanisms of mānuka oil from nanoemulsions and nanocapsules was shown in supplementary data (Figs. S3, S4, and S5).

The release rate and extent of bioactive compounds depend on the polarity of the model food systems (Karimi-Khorrami et al., 2022). In the case of encapsulated systems, the mixture of solid and liquid can enable the hydrophobic bioactive compounds to be solubilized more readily, which may increase the dissolution of essential oil compounds in the simulating food systems (Bondì et al., 2007; Karimi-Khorrami et al., 2022). However, the higher release rate of oil from the nanoemulsions than nanocapsules could be attributed to the absence of crosslinks in the former. We hypothesise that the reduced release of essential oils from the nanocapsules was due to crosslinks, which served as a barrier to the mobility and partitioning of essential oils through the why protein particles. A similar study by Karimi-Khorrami et al. (2022) reported a lower release of thymol from the calcium alginate films containing thymol-loaded nanostructured lipid carriers (NLC) than the films containing nanoemulsions due to the presence of triglycerides with crystalline domains acting as a barrier to release of thymol. Similar to our results, Amani et al. (2021) also reported a slow release of rosemary essential oil in distilled water and 10% ethanol from high amylose corn starch nanoparticles prepared through inclusion complexation than the 50% ethanol solution (Amani et al., 2021).

3.7. Effects of nanoemulsions and nanocapsules in meat systems

3.7.1. Lipid oxidation

The changes in lipid oxidation values during refrigerated storage of meat pastes, either with or without antioxidants, are presented in Fig. 4. Both storage time and treatment of preservatives significantly influenced the TBARS values of meat pastes.

On day 0th of storage, TBARS values were significantly higher in control Wagyu paste than in essential oil, nanoemulsion, and nanocapsules-treated pastes. During the storage period, TBARS values increased in all control and treated Wagyu pastes, whilst the rate of lipid oxidation was higher in control, sodium nitrite, and control nanocapsules-treated Wagyu pastes. Comparing the free, nanoemulsions and nanocapsules-form of mānuka and rosemary oil, nanoemulsions showed the best antioxidant effect in Wagyu beef and resulted in the lowest TBARS values. This could be due to the reduced particle size and increased surface area in the case of nanoemulsions. The improved antioxidant activity and stability of nanoemulsions than free oils would allow reducing the concentration to be added in food formulations.

For TBARS analysis of crossbred beef pastes, no significant difference was observed in treatments with free oil, nanoemulsified, and nanoencapsulated or not, at the beginning of the storage period (on the 0th day). Like Wagyu beef pastes, TBARS values of crossbred continuously increased during storage due to the continuous production of lipid oxidation products. In free, nanoemulsion, and nanocapsules-treated crossbred beef pastes, the formation of lipid oxidation products was lower, thereby exhibiting significantly lower TBARS values than the sodium nitrite, meat-only, control nanoemulsions, and nanocapsules-treated samples. Unlike the Wagyu beef pastes, no significant difference in essential oil treatment of crossbred pastes, either mānuka or rosemary oil, with free, nanoemulsified and nanoencapsulated forms was noticed.

During the storage, control Wagyu pastes appeared more vulnerable to lipid oxidation than crossbred beef pastes, possibly due to their higher monounsaturated fatty acid content. Bermingham et al. have already reported higher unsaturated fatty acid and neutral lipid content in Wagyu beef tenderloins than Angus beef. Generally, lipid oxidation values increase with an increase in storage period due to the increased production of secondary oxidation products. It seems that all essential oil treatments were effective in controlling lipid oxidation in both beef

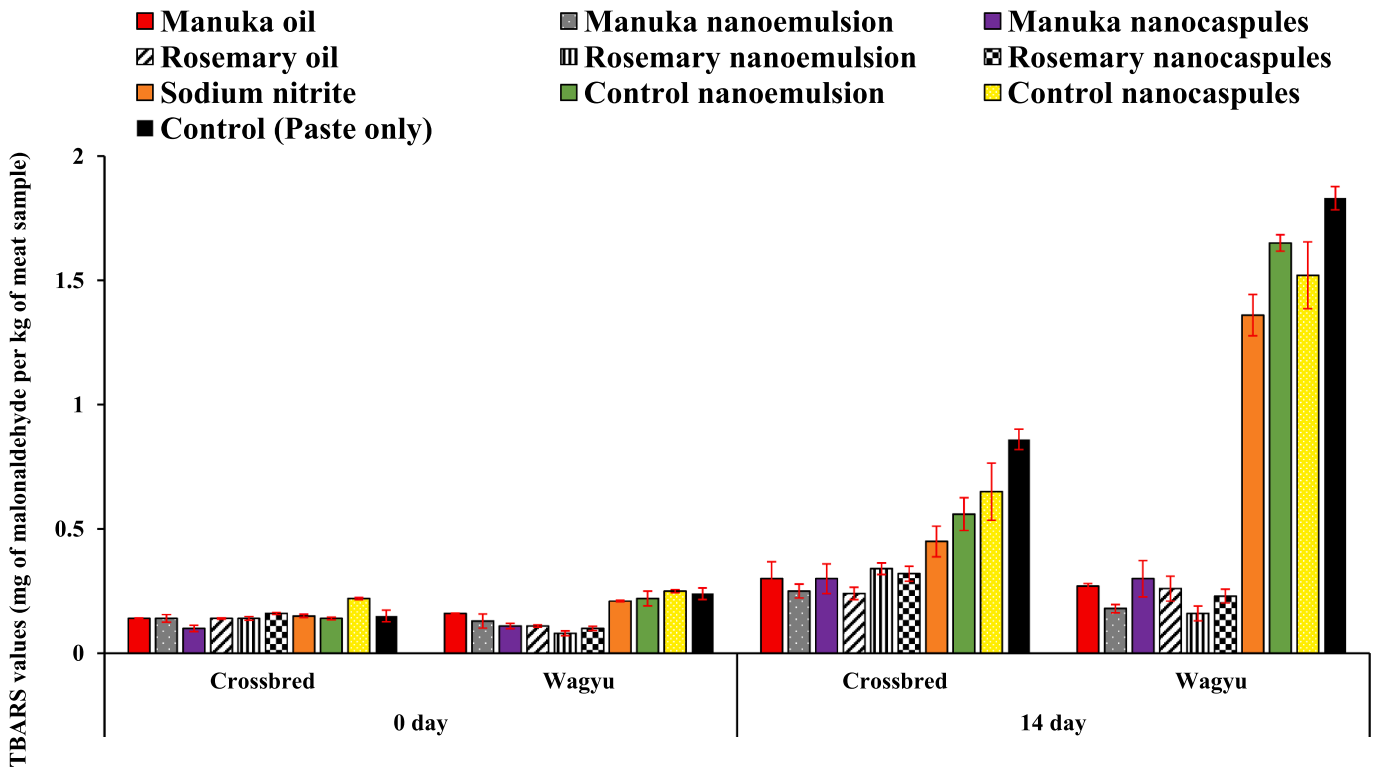

Fig. 4. The changes in TBARS values for wagyu and crossbred beef paste with or without any added antioxidant agent during storage at 4 °C for 14 days.

pastes, as observed from the lower TBARS values than the controls. The reason may be the presence of bioactive compounds, which are responsible for the antioxidant activity of essential oils, as documented in the literature (Kaur et al., 2021; Porter & Wilkins, 1999). The antioxidant mechanism of action of essential oils to inhibit or slow down lipid oxidation involves several mechanisms. The main mechanisms include the ability to donate H-atom, inhibit the chain initiation, bind transition metals ion catalysts, and scavenge free radicals and singlet oxygen formation (Tongnuanchan & Benjakul, 2014; Ruiz-Hernández et al., 2023).

Mainly, phenolic compounds such as thymol, eugenol, and carvacrol have been related to the antioxidant activity of essential, while some minor compounds like p-cymene and γ-terpinene also possess significant antioxidant characteristics (Ghaderi-Ghahfarokhi et al., 2016). The phenolic compounds act as typical chain-breaking antioxidants by preventing the initiation and propagation steps of the oxidation chain reaction through the donation of hydrogen atoms from the hydroxyl group to a peroxy radical (Baschieri et al., 2017; Amorati et al., 2013). The peroxy radical is responsible for the propagation of the oxidation chain and antioxidants make it unstable and unreactive until it catches the second peroxy radical (Amorati et al., 2016; Ruiz-Hernández et al., 2023).

Several research studies have reported that essential oils encapsulated in nanoemulsions and nanocapsules have strong antioxidant activity in beef and beef products. Similar to our findings, in a research study by Ghaderi-Ghahfarokhi et al. (2016), a significant reduction of TBARS values were observed for free and encapsulated thyme essential oil-treated beef burgers than the control burgers during 8 days of chilled storage. In another study, thyme essential oil-chitosan nanoemulsions, thymol-chitosan nanoemulsions and chitosan nanoemulsions exhibited reduced TBARS, pH and improved effect against colour degradation of refrigerated pork compared to the control (Wang et al., 2022).

3.7.2. Colour

The colour is an essential quality indicator used to determine the freshness of beef, thereby influencing the purchasing behaviour of consumers. The changes in colour values of Wagyu and crossbred beef paste with and without preservative treatments are presented in Table 2. Depending on the addition of the antioxidants, both meat pastes exhibited different colour characteristics during the storage period of 14 days at 4 °C.

At the beginning of storage, the highest L^* values of Wagyu beef were observed than those treated with essential oils and crossbred pastes. There was a significant reduction in L^* values of crossbred and Wagyu pastes during the storage period, either with or without antioxidants. This could be due to the fact that samples were decomposed through microbial growth, endogenous enzymes, and myoglobin oxidation into brown methemoglobin through long-term oxygen contact. Changes in protein structure and conformation during oxidation lead to a change in the brightness of meat and, thus, changes in the L^* values of pastes (Vital et al., 2016; Zhang et al., 2022).

As presented in Table 2, the b^* values of crossbred beef pastes containing essential oils were close to those obtained from the control nanoemulsions and nanocapsules-treated samples. Interestingly, different values of yellowness and blueness (b^*) for control crossbred and Wagyu pastes were noticed in the initial phase of the storage period. It could be related to the concentration of β-carotene deposited in the fat of some beef carcasses relative to other beef or cattle breeds (Jaborek et al., 2019). During the storage, a gradual decrease in b^* values of all treatments of crossbred beef was observed; however, this decrease was lower for encapsulated and nonencapsulated essential oils containing pastes than the untreated pastes (control). Wagyu paste showed a similar pattern to crossbred pastes in a decrease of b^* values. No significant difference in nanoemulsified, nanoencapsulated and free oil treatment was detected in the different forms of essential oils.

A significant and continuous a^* (redness) value reduction was seen in both Wagyu and crossbred pastes. During the storage, a sharp drop in a^* of control Wagyu and crossbred pastes were noticed by more than 90% and 50%, respectively. Even if this decrease was observed in free and encapsulated essential oil-treated samples, it was lower than in the controls, indicating that essential oils tended to stabilise the red colour due to their antioxidant effect against protein oxidation. A sudden decrease in redness values of pastes in the first week and then constant or higher a^* values of mānuka and rosemary-nanocapsules treated samples could be related to the gradual release (data not shown) of essential oils/bioactive compounds from the nanocarriers, thus retarding protein oxidation. Fresh meat colour is dependent on several factors such as lipid oxidation, microbial growth, and protein (especially myoglobin) oxidation. Essential oil contains bioactive compounds that inhibit or slow down discolouration by preventing changes in myoglobin, spoilage and pathogenic microbes' growth and thus improve the colour, quality, and shelf-life of meat. A recent study by Kaur et al., (2023a) and Kaur et al., (2023b) showed the antioxidant and antimicrobial action of mānuka oil against lipid oxidation and microbial in beef respectively. According to Shange et al. (2019), oregano essential oil positively affected the colour of the meat by increasing the yellowness and brownness values. On the other hand, coriander essential oil decreased the lightness values of the sausages, which may be due to the interaction between bioactive compounds of essential oil and myoglobin (Šojić et al., 2019).

The results of the change in colour values of meat after treatments with nanoencapsulated and nanoemulsified oil are consistent with those previously reported by Wang, Heising, et al. (2020), Wang, Zhang, et al. (2020) and Noori et al. (2018). Wang, Heising, et al. (2020) and Wang, Zhang, et al. (2020) documented that eugenol nanocapsules can reduce colour changes in L^*, a^* and b^* levels of chilled pork during refrigerated storage (16 days at 4 °C). Lowest colour changes (ΔE) in chicken breast fillets treated ginger essential oil nanoemulsions after 12 days storage at 4 °C was observed by Noori et al. (2018).

4. Conclusions

This study successfully fabricated essential oil-loaded nanoemulsions and nanocapsules using sodium alginate as an aqueous phase and whey protein as an emulsifier. Nanoemulsions and nanocapsules exhibited more thermostability and sustained release characteristics; however, their antioxidant activity was lower than the free oils. Molecular interactions between essential oil constituents and carrier materials in FTIR spectrums confirmed the successful loading of essential oils in these carriers. Further, in Wagyu and crossbred meat pastes, no significant differences between mānuka oil, rosemary oil, and their nanoforms were noticed in crossbred pastes, while in Wagyu, nanoemulsions of both oils showed significantly lower oxidation values than their free oil and nanocapsules forms. In both types of beef pastes, essential oils and their nanoforms-added pastes had lowered TBARS values than their respective controls and sodium nitrite-added samples. The findings of this showed that mānuka or rosemary oils containing nanoemulsions and nanocapsules could be used as a natural antioxidant agent for meat preservation, along with improved stability. Improved thermal stability of nanoemulsions and nanocapsules than free oils would allow using these bioactives in cooked food products. Encapsulation of volatile and unstabilised bioactive in food-grade carriers like sodium alginate and whey protein confer thermostable systems than their free forms. In addition, the present method may be used for encapsulating other food-grade essential oils. Future studies focusing on the safety and toxicity of these encapsulated oils can provide solid proof of their acceptance from a consumer viewpoint and facilitate a wide range of their applications in food industries.

CRediT authorship contribution statement

Ramandeep Kaur: Conceptualization, Methodology, Software,

Table 2
The changes in colour values for wagyu and crossbred beef pastes with or without any added antioxidant agent during storage at 4 °C for 14 days.

Days	Meat	Treatments										*p-values*				
		MO	**ME**	**MC**	**RO**	**RE**	**RC**	**SN**	**CC**	**CE**	**C**	**SEM**	**MO*ME**	**MO*MC**	**MC*ME**	**MO*RO**
L*																
0	Crossbred	51.9^{aA}	52.4^{aB}	52.5^{aB}	51.9^{aA}	52.4^{aA}	51.5^{abA}	46.6^{dB}	51.0^{bB}	49.9^{cB}	51.5^{aB}	0.21	ns	ns	ns	ns
	Wagyu	51.9^{bA}	54^{bA}	51.8^{bA}	51.3^{bA}	52.8^{bA}	51.5^{bcA}	50.5^{cA}	53.7^{bA}	51.9^{bA}	56.0^{aA}	0.56	ns	ns	ns	ns
7	Crossbred	45.9^{dA}	48.5^{aA}	47.7^{aA}	47.4^{aA}	47.3^{aA}	47.6^{abA}	43.3^{eB}	45.8^{dB}	45.4^{dA}	46.9^{dB}	0.28	*	*	ns	**
	Wagyu	45.1^{bA}	46.9^{bB}	46.0^{bB}	45.8^{bB}	46.8^{bB}	46.6^{bB}	45.8^{bA}	46.9^{bA}	46.6^{bA}	49.2^{aA}	0.39	ns	ns	ns	ns
14	Crossbred	44.2^{bB}	47.8^{aB}	47.2^{aA}	46.3^{bB}	47.7^{aB}	47.5^{aB}	42.3^{eB}	44.9^{cB}	45.4^{bB}	43.8^{dB}	0.35	**	**	ns	ns
	Wagyu	46.1^{cA}	48.5^{aA}	46.6^{bB}	47.4^{bA}	48.9^{bA}	48.4^{abA}	46.3^{bA}	48.8^{bA}	49.3^{aA}	46.9^{cA}	0.48	**	ns	ns	ns
Storage effect																
	Crossbred	***	***	***	***	***	***	***	***	***	***	***				
	Wagyu	***	***	**	***	***	***	*	***	***	***	***				
a*																
0	Crossbred	14.0^{dB}	14.7^{cB}	14.9^{cB}	15.7^{aB}	15.8^{aB}	14.7^{cdB}	12.5^{eB}	14.1^{cB}	14.5^{cB}	15.0^{bB}	0.16	*	*	ns	**
	Wagyu	17.4^{dA}	17.8^{dA}	17.8^{dA}	18.9^{bA}	18.6^{bA}	19.2^{aA}	16.6^{eA}	19.1^{aA}	19.5^{aA}	18.5^{bA}	0.24	ns	ns	ns	**
7	Crossbred	10.6^{aA}	7.2^{cdB}	7.1^{cdB}	8.0^{cdA}	7.5^{cdA}	7.5^{cdA}	7.0^{dA}	9.8^{bcA}	6.9^{dB}	5.9^{eB}	0.52	**	**	ns	**
	Wagyu	7.9^{bB}	8.3^{bA}	8.1^{bA}	7.7^{bA}	7.9^{bA}	7.7^{bA}	7.6^{bA}	8.5^{bB}	7.4^{bA}	10.4^{aA}	0.33	ns	ns	ns	ns
14	Crossbred	5.9^{aB}	6.0^{aB}	7.7^{aB}	5.6^{aB}	8.2^{aB}	8.5^{aB}	5.7^{aB}	6.8^{aB}	6.8^{aA}	1.8^{bB}	0.89	ns	ns	ns	ns
	Wagyu	10.9^{aA}	10.1^{aA}	12.7^{aA}	12.3^{aA}	12.5^{aA}	11.3^{aA}	8.6^{abA}	7.9^{abA}	4.8^{bB}	7.8^{abB}	1.18	ns	ns	ns	ns
Storage effect																
	Crossbred	**	***	**	**	***	***	***	***	***	***					
	Wagyu	***	**	***	***	***	***	***	***	***	***					
b*																
0	Crossbred	12.9^{aB}	13.5^{aB}	13.8^{aB}	13.2^{aA}	13.4^{aB}	12.2^{bB}	10.2^{dB}	11.2^{cB}	11.5^{cB}	11.9^{cB}	0.14	ns	ns	ns	ns
	Wagyu	14.2^{bA}	15^{aA}	14.3^{bA}	13.6^{cA}	14.3^{bA}	14.4^{bA}	14.4^{bA}	15.2^{aA}	14.8^{bA}	15.4^{aA}	0.19	*	ns	*	**
7	Crossbred	12.1^{aA}	12.6^{aA}	12.6^{aA}	11.6^{bA}	11.8^{bB}	11.8^{bA}	10.1^{cB}	10.9^{cB}	10.6^{cB}	11.1^{bB}	0.19	ns	ns	ns	ns
	Wagyu	11.9^{aA}	12.6^{aA}	12.6^{aA}	11.3^{Ab}	12.6^{aA}	11.7^{bA}	11.4^{bA}	12^{aA}	11.7^{bA}	12.9^{aA}	0.18	ns	ns	ns	**
14	Crossbred	12.4^{aA}	12.5^{aA}	12.4^{aA}	11.5^{bA}	11.7^{bB}	11.6^{bB}	10.2^{cB}	10.1^{cB}	9.5^{dB}	10.7^{cB}	0.17	*	*	ns	**
	Wagyu	12.2^{aA}	12.3^{aA}	12.0^{aA}	11.9^{Aa}	12.6^{aA}	12.5^{Aa}	11.9^{aA}	11.9^{aA}	11.9^{aA}	11.4^{aA}	0.21	ns	ns	ns	ns
Storage effect																
	Crossbred	*	***	***	***	***	*	ns	*	**	ns					
	Wagyu	***	***	***	***	***	***	***	***	***	**					

Treatments- **MO**-Mānuka oil, **RO**- Rosemary oil, **ME**- Mānuka nanoemulsion, **MC**- Mānuka nanocapsules, **RN**- Rosemary nanoemulsion, **RC**- Rosemary nanocapsules, **C**- Control, **CE**- control nanoemulsion, **CC**– control nanocapsules, **MO*RO** = comparison between mānuka oil and rosemary oil, **MO*ME** = comparison between mānuka nanoemulsion and mānuka oil, **MC*ME** = comparison between mānuka nanoemulsion and mānuka nanocapsules**, MO*MC** = comparison between mānuka oil and mānuka nanocapsules, **Storage effect (0th × 7th × 14th day)** = comparison between 0th,7th and 14th day. **ns** = $p > 0.05$, * = $P \leq 0.05$, **= $p \leq 0.01$, *** = $p \leq 0.001$, **SEM**- Standard error mean.

[a-e] Means within a row with the same superscript letters are not significantly different ($p < 0.05$) between the treatments on the same storage day.

[AB] Means within a column with the same superscript letters are not significantly different ($p < 0.05$) between the meat systems.

Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Tanushree B. Gupta:** Methodology, Validation, Supervision, Writing – review & editing, Visualization. **John Bronlund:** Methodology, Supervision, Writing – review & editing, Visualization. **Jaspreet Singh:** Conceptualization, Methodology, Writing – review & editing, Visualization. **Lovedeep Kaur:** Conceptualization, Methodology, Validation, Resources, Data curation, Supervision, Writing – review & editing, Visualization, Project administration, 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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.137600.

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