

## Original article

**Effectiveness of mānuka and rosemary oils as natural and green antioxidants in wagyu and normal beef**Ramandeep Kaur,<sup>1,2</sup> Lovdeep Kaur,<sup>1,2\*</sup>  Tanushree B. Gupta<sup>3</sup> & John Bronlund<sup>1,2</sup>

1 School of Food and Advanced Technology, Massey University, Palmerston North 4442, New Zealand

2 Riddet Institute, Massey University 4442, Palmerston North, New Zealand

3 Hopkirk Research Institute, AgResearch Ltd., Palmerston North 4472, New Zealand

(Received 13 October 2022; Accepted in revised form 27 February 2023)

**Summary** Essential oils possessing antioxidant characteristics have acquired broad interest as an alternative to synthetic food antioxidants like butylated hydroxytoluene (BHT). In this study, mānuka (with 5, 25 and 40% triketone content) (MO), rosemary (RO) and kānuka (KO) oils were characterised and screened through DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric radical absorbing power) assays for their antioxidant efficacies. Different triketone levels were selected to examine their effect on the antioxidant activity of MO. All MOs showed higher phenolic content and antioxidant activities than KO and RO. Based on the obtained results, the MO with 25% triketone content and RO were chosen to study their antioxidant effects in pastes prepared from New Zealand normal (3% fat) and wagyu (12% fat) beef during refrigerated storage (7 days). No significant effect of the oils was observed on lipid oxidation in normal pastes during storage. However, MO and BHT significantly reduced lipid oxidation in wagyu pastes, showing the potential of mānuka oil as a natural antioxidant in high-fat meat products.

**Keywords** Antioxidant properties, kānuka oil, mānuka oil, meat, natural preservatives, rosemary oil, manuka.

**Introduction**

The reduced particle size and increased surface area during meat processing operations such as grinding and comminution expose ground meat products like meat paste to iron (which behaves as a potent oxidation catalyst), enzymes and water and make them more susceptible to deteriorative oxidative changes (Lee *et al.*, 2005; McBride *et al.*, 2007). Chemical antioxidants, such as butylated hydroxytoluene (BHT), propyl gallate, *tert*-butylhydroquinone (TBHQ) and butylated hydroxyanisole (BHA) are usually added in meat products to alleviate any oxidation reactions (Lee *et al.*, 2005). However, consumer awareness and interest are growing toward the use of natural preservatives in foods (Ünal *et al.*, 2014; Kaur *et al.*, 2021). Different plant products, particularly essential oils and their bioactive compounds have been considered as green and safe alternatives to chemical preservatives (Li *et al.*, 2014; Mehdizadeh *et al.*, 2022). Extensive research studies have reported the usage of essential oils in food products; however, research on understanding the antioxidant and antimicrobial potential of mānuka (MO) and

kānuka oil (KO) from a food applications viewpoint is scarce. *Leptospermum scoparium* (mānuka) and *Kunzea ericoides* (kānuka) are the most widely distributed flowering plants native to New Zealand and some parts of Australia. These have been utilised in beverages and medicinal purposes in the Māori culture since ancient times (Porter & Wilkins, 1999; Kwon *et al.*, 2013). The antimicrobial characteristics of the oils obtained from the leaves of these plants, especially MO, have been reported widely in the literature (Porter & Wilkins, 1999; Kwon *et al.*, 2013). The antioxidant potential of MO has been attributed to the presence of sesquiterpene compounds (Kwon *et al.*, 2013). When individual components in the MO were tested for their antioxidant potential, only  $\gamma$ -terpinene and terpinen-4-ol exhibited antioxidant activity (Lis-Balchin *et al.*, 2000; Lis-Balchin, 2006). The higher antioxidant potential of MO than that of KO has been reported by Lis-Balchin & Hart (1998). In these studies, the MO exerted a more consistent antioxidant effect on mice's skin than tea tree (*Melaleuca alternifolia*) or kānuka oils (Lis-Balchin, 2006).

However, the antioxidant effects of essential oils can be unpredictable depending on the concentration used, the food matrix's compositional characteristics and the

\*Correspondent: E-mail: [L.Kaur@massey.ac.nz](mailto:L.Kaur@massey.ac.nz)

tested foods' fatty acid composition (Estévez & Cava, 2006; de Oliveira *et al.*, 2011). The antioxidant effects of sage and rosemary essential oils (RO) on the oxidative stability of liver pâtés have been shown by Estévez *et al.* (2007). In their work, both essential oils (at 1000 ppm concentration) protected lipid and protein oxidation of liver pâtés made from Iberian pigs, while the same oils increased the oxidative deterioration of such constituents in liver pâtés made from white pigs. The probable reason could be the difference in the fat composition of meat from both pigs (Estévez *et al.*, 2004). The meat products prepared from wagyu and other beef breeds are considerably different in terms of their fat content and composition (Zhang, 2015), which may affect the antioxidant efficacies of added essential oils but has never been studied.

This study evaluated the potential of MO and ROs as a substitute for chemical antioxidants like butylated hydroxytoluene (BHT) in low and high-fat meat products.

Preliminary experiments involved characterisation and screening of MO (with 5%, 25% and 40% triketone content) and KO using DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric radical absorbing power) assays for their antioxidant efficacies and comparing them with a commonly used natural antioxidant, RO (Kaur *et al.*, 2021) and a chemical antioxidant, BHT (Lee *et al.*, 2005). Based on the results, the antioxidant effects of selected MO and RO were determined on lipid oxidation, pH, and colour changes in meat pastes from New Zealand normal and wagyu beef during refrigerated storage. The antimicrobial effects of these oils were also determined, the results for which will be presented in another paper.

## Materials and methods

### Materials

The steam-distilled MO and KO were kindly supplied by Tairawhiti Pharmaceuticals Ltd. (Te Araroa, New Zealand). The steam-distilled RO was from "Now Foods" (Auckland, New Zealand). The vacuum-packed normal and wagyu beef tenderloins purchased from the Gourmet Butchery, Napier (New Zealand), were stored in the freezer at  $-20^{\circ}\text{C}$  and thawed overnight prior to the analysis. Mānuka oil with triketone content of 5%, 25% and 40% have been referred to as MO 1, 2, and 3, respectively.

### Characterisation of oils

#### *Fourier transform infrared spectrometer (FTIR) analysis*

The infrared spectroscopy analysis of MO, KO and RO was performed with an iDr 7 ATR-FTIR spectrophotometer (Thermo Fisher Scientific, Waltham, MA,

USA), and spectra were obtained from 400 to  $4000\text{ cm}^{-1}$ .

#### *Total phenolic content (TPC) determination*

The total phenolic content of all the essential oils (MO, kākānuka and rosemary) was determined by following the method of Viuda-Martos *et al.* (2010) with slight modifications. Briefly, 0.5 mL of sample mixture (0.1 mL essential oil dissolved in methanol +0.4 mL RO water) and 1 mL of Folin–Ciocalteu reagent solution (1:10 in RO water). After thoroughly mixing and incubating at room temperature for 10 min, 2 mL of 15% sodium bicarbonate solution was added. The mixture was incubated at room temperature for 1 h in the dark, and absorbance was read at 740 nm using an Evolution 201 UV–Vis spectrophotometer (Thermo Scientific™, Madison, WI, USA). Gallic acid was used as a standard to plot the calibration curve. Each assay was carried out in triplicate.

#### **Determination of antioxidant potential of oils**

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was performed using the method of Torres-Martínez *et al.* (2017) with modifications. The essential oil (100  $\mu\text{L}$ , at different concentrations of 0.1 and 1% in methanol) was mixed with 2 mL of DPPH solution (0.5 mM). After thorough mixing, the prepared mixture was left in the dark for 20 min for incubation at room temperature, and then absorbance was measured against blank at 515 nm using an Evolution 201 UV–Vis spectrophotometer (Thermo Scientific™). FRAP assay was performed according to the method of Viuda-Martos *et al.* (2010). In the FRAP assay, gallic acid was used as standard control, and results were expressed in terms of  $\mu\text{g}$  gallic acid equivalents per mL of oil. Each assay was carried out in triplicate.

#### **Meat pastes preparation and storage conditions**

The normal and wagyu beef tenderloins were cut into small cubes using a sharp knife and then passed 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 hopper (Ohio, USA), attached with a knife and were ground for about 15 min. The paste was divided into six different lots for treatments with 2.5% MO, 2.5% RO, 300 mg/kg sodium nitrate, and 0.02% BHT, followed by mixing in a mixer (Kogan, 1600 W, New Zealand) for about 15 min at room temperature. A control sample without any preservative/treatment was also prepared from each meat system. All prepared samples were packed in zip-lock bags (100  $\times$  155 mm) and stored at  $4^{\circ}\text{C}$  in a dark room. Samples were removed at different time intervals of 0, 1, 4,

and 7 days and analysed for colour, pH and lipid oxidation. Three replications were performed for each sample.

### Meat pastes analysis

The 2-Thiobarbituric acid (TBA) values were determined as per the method of Botsoglou *et al.* (1994). The changes in the pH of meat paste samples during the 0- and 7-day storage period were estimated by following the AOAC method (AOAC, 2005). The colour of the meat paste samples was checked by using the Minolta colourimeter (Chroma meter, CR 400, Hong Kong, China). Briefly, equipment was calibrated first by using a white tile, and then each meat sample was scanned for colour values ( $L^*$ ,  $a^*$  and  $b^*$ ). Each analysis was performed in triplicates.

### Statistical analysis

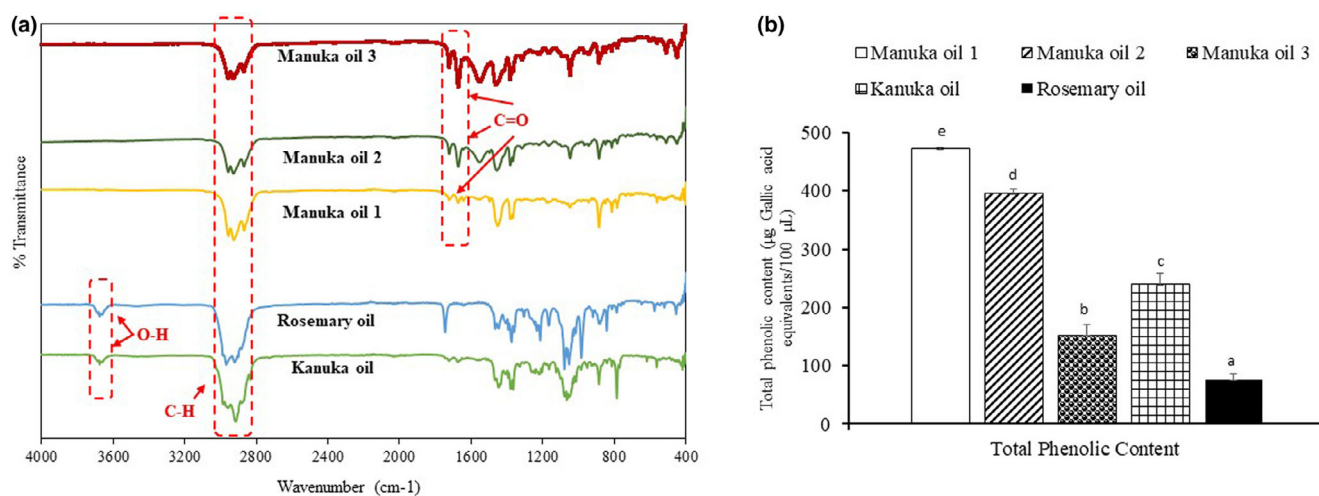
All tests in this study were performed in triplicate. Statistical evaluation was performed using a general linear model in Minitab Version 19.2020.2.0 (Minitab Inc., State College, PA, USA) to compare the effects of different treatments in normal and wagyu meat. The comparison was made between different meats, storage periods, and treatments. When at least one treatment was statistically different, a one-way analysis of variance (ANOVA), followed by the Tukey method analysis at a 95% confidence interval, was done to determine the significant effects into treatments.

## Results and discussion

### FTIR analysis of essential oils

As shown in the FTIR spectrum of the MO (1, 2 and 3), KO and RO (Fig. 1a), three intense peaks between 2800–3000  $\text{cm}^{-1}$  in the spectrum of all essential oil was observed, which could be due to the C-H stretching vibration of aliphatic  $\text{CH}_2$  bonds. However, due to H-bonded O-H group stretching, an intense peak between 3640 and 3720  $\text{cm}^{-1}$  was displayed in the spectra of KO and RO. It shows the presence of alcoholic compounds, like 1,8 cineole, linalool and terpinol, which are higher in kāmuka and RO than all MOs. In the KO spectra, an intense peak was observed at 885  $\text{cm}^{-1}$  due to the carbonyl (CH and  $\text{CH}_2$ ) groups bending, as presented in Fig. 1a, which could be attributed to the presence of  $\alpha$ -pinene, the major compound present in the oil. Mānuka and RO also exhibited this peak but were sustainably lower than the KO because alpha-pinene is present in lower quantities in the former oils. The presence of these chemical compounds was also confirmed by gas chromatography–mass spectrometry (GCMS) (data not shown).

Observing Fig. 1a, an absorption peak between 1690  $\text{cm}^{-1}$  and 1720  $\text{cm}^{-1}$  in the infrared spectrum of MOs is due to strong C = O stretching and attributed to the presence of  $\beta$ -triketones, including leptospermone, isoleptospermone, flavesone and grandiflorone (Liu, Tao, and Huang 2021). Characteristics peak of



**Figure 1** Fourier-transform infrared (FTIR) spectrum and total phenolic content of mānuka 1 (5% triketones), 2 (25% triketones), 3 (40% triketones), kāmuka and rosemary oils. In (a), the arrows correspond to the ketonic group (C=O) peaks in mānuka oil 1, 2 and 3, confirming the presence of  $\beta$ -triketones in these oils. An alcoholic group (–OH) peaks in kanuka and rosemary oil attributed to the alcoholic compounds like 1,8 cineole, linalool and terpinol in both oils. Mean values with different lowercase letters indicate a significant difference ( $P \leq 0.05$ ) between the samples in (b). Data are presented as Mean  $\pm$  SD.

**Table 1** Antioxidant activities of essential oils determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric radical absorbing power) assays

Concentration →	DPPH radical scavenging activity		Fe <sup>2+</sup> radical scavenging activity	
	0.1%	1%	0.1%	1%
MO 1	30.3 ± 0.7 <sup>c</sup>	54.8 ± 1.1 <sup>d</sup>	183.2 ± 2.9 <sup>e</sup>	209.5 ± 1.4 <sup>f</sup>
MO 2	35.7 ± 1.0 <sup>c</sup>	70.4 ± 0.1 <sup>f</sup>	164.5 ± 3.7 <sup>d</sup>	201.4 ± 0.4 <sup>e</sup>
MO 3	26.7 ± 1.4 <sup>c</sup>	67.9 ± 0.3 <sup>e</sup>	142.5 ± 0.4 <sup>c</sup>	183.5 ± 5.6 <sup>d</sup>
KO	6.9 ± 0.0 <sup>a</sup>	24.7 ± 0.7 <sup>b</sup>	23.5 ± 4.2 <sup>b</sup>	96.6 ± 5.2 <sup>c</sup>
RO	2.5 ± 1.1 <sup>a</sup>	13.0 ± 0.3 <sup>a</sup>	8.9 ± 0.7 <sup>a</sup>	24.2 ± 4.7 <sup>a</sup>
BHT	8.3 ± 0.4 <sup>b</sup>	42.7 ± 0.9 <sup>c</sup>	10.8 ± 0.1 <sup>a</sup>	45.3 ± 5.1 <sup>b</sup>

BHT- Butylated Hydroxytoluene; KO- Kānuka oil; MO 1- Mānuka oil with 5% triketone content; MO 2- Mānuka oil with 25% triketone content; MO 3- Mānuka oil with 40% triketone content; RO- Rosemary oil.

the keto group of camphor in RO displayed at 1746 cm<sup>-1</sup>, whereas peaks around 1375 cm<sup>-1</sup> and 1450 cm<sup>-1</sup> could be due to the ether group from the epoxy region of 1,8 cineole.

#### Total phenolic content of the essential oils

The total phenolic contents (TPC) of MO (with different triketone contents- 5%, 25% and 40%), KO and RO are illustrated in Fig. 1b. Among all the tested oils, MO containing the lowest triketone content (5%) showed the highest TPC. The phenolic content decreased as the triketone content of the MOs increased. The MO having the highest triketone content (25%), exhibited the lowest total phenolic content but was not lower than the RO. The phenolic content and composition of RO have been widely studied (Viuda-Martos *et al.*, 2010; Kaur *et al.*, 2021). The values for total phenolic content for RO reported in the current study are similar to those reported previously (Viuda-Martos *et al.*, 2010).

#### Antioxidant potential of the essential oils

The antioxidant potential of the oil samples was evaluated through the DPPH and FRAP radical scavenging assays. The results of the DPPH test exhibited that essential oils had a potent concentration-dependent DPPH radical scavenging activity (Table 1). RO had the lowest DPPH radical scavenging activity at both tested concentrations than the KO and BHT. At all the other tested concentrations, MOs, particularly MO containing 40% triketones, showed better DPPH radical scavenging activity than all other tested samples. No significant effect of the triketone content was observed on the DPPH radical scavenging activity at the lowest tested concentration (0.1%) of MOs. The reason might be that the antioxidant activity of MO is due to sesquiterpene compounds, as Kwon *et al.* (2013) reported. In their

study, when individual components in the MO were tested for their antioxidant potential, only  $\gamma$ -terpinene and terpinen-4-ol showed antioxidant activity (Lis-Balchin *et al.*, 2000; Lis-Balchin, 2006).

Like the DPPH results, rosemary, KO and BHT showed lower antiradical activity than all MOs. Among MOs, the oil with the lowest triketone content had the highest FRAP values across all concentrations, as shown in Table 1. The possible reason could be the presence of higher amounts of phenols and flavonoids in that oil, which could have contributed to an enhanced FRAP activity. The higher antioxidant potential of MO than that of KO has been reported previously (Lis-Balchin & Hart, 1998). In these studies, the MO exerted a more consistent antioxidant effect on mice's skin than tea tree (*Melaleuca alternifolia*) or kānuka oils (Lis-Balchin *et al.*, 2000; Lis-Balchin, 2006).

#### Essential oil selection for adding in meat systems

Among the three MOs, only MO 2 with 25% triketone content was chosen to examine its effects against lipid oxidation, pH and colour changes in the meat systems. This selection was based on its enough antioxidant effect, antimicrobial activity (data not shown) and similarity of composition (triketones levels from 25% to 30%) to natural MO from East cape, New Zealand. The detailed data related to the antimicrobial and chemical composition will be published as part of another paper.

In the chemical composition of meat pastes, normal beef's initial fat, protein, and moisture contents were found to be 72.2%, 20.5% and 3.4%. However, the wagyu beef paste had lower moisture content (64.8%) and higher fat content (12.3%). The protein content of wagyu beef (19.4%) was almost similar to normal beef. Comparing the fat and moisture content of both beef pastes, it can be noted that fat content increased proportionally with a decrease in moisture content.

### Effects of the essential oils against lipid oxidation in meat systems

In this study, the lipid oxidation (TBARS) values of both meat pastes were significantly ( $P \leq 0.05$ ) influenced by the essential oil addition and storage time, as presented in Table 2.

All normal paste samples experienced an increase in TBARS values during the storage period, especially control samples, which showed the highest increase. However, in wagyu beef pastes, antioxidants treated pastes had significantly lower TBARS values than the control. The normal and wagyu beef meat pastes treated with BHT experienced no significant change in TBARS values throughout the storage, indicating the antioxidant effect of BHT against lipid oxidation. Interestingly, the MO and BHT treatments in wagyu pastes showed lower lipid oxidation than the control and therefore were the most effective in controlling lipid oxidation during storage.

During the storage, control wagyu pastes appeared more vulnerable to lipid oxidation than normal beef pastes, possibly due to their higher fat content. The higher unsaturated fatty acid and neutral lipid content in cooked wagyu beef have already been reported by Boylston *et al.* (1996). The lower TBARS values in essential oil-treated samples than controls could be attributed to the presence of bioactive constituents, which are responsible for the antioxidant activity, due

to their free-radical quenching ability through electron or proton donation mechanisms (Unal *et al.*, 2014; Pabast *et al.*, 2018). Kaur *et al.* (2021) reported that the presence of phenolic compounds in RO could be linked to its antioxidant activity. Regarding MO, Kwon *et al.* (2013) reported that  $\gamma$ -terpinene and terpinen-4-ol have antioxidant activity (Lis-Balchin, 2006).

Differences in the antioxidant activity of MO in two different types of meat pastes could be due to the difference in the fat content of meat pastes. Consistent with our results, Estévez *et al.* (2004) showed an unpredictable antioxidant effect of sage and rosemary oils in liver pâtés made from Iberian and white pigs, which may be due to the difference in the fatty acid composition of meat from both pigs (Estévez *et al.*, 2004). The difference in the fatty acid compositions could affect the physical state of lipids and, thereby texture of the prepared product. Thus, there may be changes in the dispersion of the essential oils in both meat systems, resulting in their different antioxidant activities (Estévez & Cava, 2006). Likewise, a recent study by Tomović *et al.* (2020) also showed a difference in the antioxidant potential of *Juniperus communis* essential oil, which was higher in 25%-fat containing dry-fermented sausages than the 15% fat sausages (Tomović *et al.*, 2020). Estévez *et al.* (2007) reported a higher antioxidant effect of sage and rosemary oils than the chemical antioxidant (BHT) in liver pâté samples.

**Table 2** The changes in TBARS and pH values for wagyu and normal beef pastes with or without any added antioxidant agent during storage at 4 °C for 7 days

Parameters	Meat system	Treatments					SEM	P-value			
		MO	RO	SN	BHT	C		MO × RO	MO × SN	MO × BHT	MO × C
0th day											
TBARS	Wagyu	0.10 <sup>Bx</sup>	0.14 <sup>Ax</sup>	0.14 <sup>Ay</sup>	0.13 <sup>ABy</sup>	0.13 <sup>ABy</sup>	0.04	*	*	ns	ns
	Normal	0.11 <sup>Cx</sup>	0.07 <sup>Dy</sup>	0.22 <sup>Bx</sup>	0.29 <sup>Ax</sup>	0.24 <sup>Bx</sup>	0.01	**	**	**	**
pH	Wagyu	5.75 <sup>Bx</sup>	5.95 <sup>Ax</sup>	5.87 <sup>ABy</sup>	5.96 <sup>A</sup>	5.95 <sup>Ax</sup>	0.02	*	*	*	*
	Normal	5.42 <sup>Cy</sup>	5.63 <sup>By</sup>	5.67 <sup>Ax</sup>	5.78 <sup>B</sup>	5.72 <sup>ABy</sup>	0.01	**	**	**	**
7th day											
TBARS	Wagyu	0.20 <sup>By</sup>	0.30 <sup>BCx</sup>	0.39 <sup>Bx</sup>	0.20 <sup>Bx</sup>	0.59 <sup>Ax</sup>	0.02	**	**	ns	**
	Normal	0.49 <sup>Ax</sup>	0.30 <sup>Bx</sup>	0.47 <sup>Ax</sup>	0.33 <sup>Bx</sup>	0.51 <sup>Ay</sup>	0.19	**	ns	*	ns
pH	Wagyu	5.95 <sup>Dx</sup>	6.16 <sup>Bx</sup>	5.90 <sup>Dx</sup>	6.03 <sup>Cx</sup>	6.34 <sup>Ax</sup>	0.01	**	ns	ns	**
	Normal	5.41 <sup>By</sup>	5.63 <sup>Ay</sup>	5.26 <sup>Cy</sup>	5.39 <sup>By</sup>	5.43 <sup>By</sup>	0.01	**	**	ns	ns
Storage effect (0th × 7th day)											
TBARS	Wagyu	**	**	**	ns	**					
	Normal	**	**	**	ns	**					
pH	Wagyu	*	**	ns	**	**					
	Normal	ns	ns	**	**	**					

Treatments- MO-Mānuka oil, RO- Rosemary oil, SN- Sodium Nitrate, BHT- Butylated Hydroxytoluene, C- Control. MO×RO = comparison between mānuka oil and rosemary oil, MO×SN = comparison between mānuka oil and sodium nitrate, MO×BHT = comparison between mānuka oil and butylated hydroxytoluene, MO×C = comparison between mānuka oil and control, Storage effect (0th × 7th day) = comparison between 0th and 7th day. ns =  $P > 0.05$ , \* $P < 0.05$ , \*\* $P < 0.01$ . SEM- Standard error mean.

<sup>A-E</sup>Means within a row with the same superscript letters are not significantly different ( $P < 0.05$ ) between the treatments on the same storage day.

<sup>x,y</sup>Means within a column with the same superscript letters are not significantly different ( $P < 0.05$ ) between the meat systems.

### Effects of the essential oils on changes in pH values of meat systems

In this study, the pH values of both meat pastes were significantly ( $P \leq 0.05$ ) influenced by the essential oil addition and storage time (Table 2).

The normal and wagyu paste samples added with essential oils had lower changes in pH values than the control samples during storage. Although all normal paste samples showed a decrease in pH value as a function of the storage period, the essential oil-treated pastes had no significant changes in pH values. In the wagyu samples, an increase in pH values of control samples was observed during the storage period, while this increase was less significant in essential oils treated samples. The probable reason could be the microbial growth and meat enzyme inhibition activity of essential oils, thus preventing meat protein breakdown and consequent pH increase (Pabast *et al.*, 2018). The antimicrobial activity of MO and RO could be attributed to their naturally occurring bioactive compounds,  $\beta$ -triketones and 1, 8-cineole, respectively (Porter & Wilkins, 1999; Kaur *et al.*, 2021). Similar to our results, no significant difference in pH values of meat coated with chitosan coating containing free and

encapsulated essential oils was reported by Pabast *et al.* (2018). Ünal *et al.* (2014) also reported a lower increase in pH values of minced beef treated with oregano, sage and rosemary essential oils than controls.

Interestingly, differences in pH values of control normal and wagyu beef pastes could be due to the difference in chemical composition between the two meats, especially fat and moisture content, which may affect the water activity and subsequent microbial growth in both meats (Barmpalia-Davis *et al.*, 2009).

### Effect of the essential oils on the colour of meat systems

Depending on the addition of the antioxidants, both meat pastes exhibited different colour characteristics, as represented in Table 3.

Comparing redness values ( $a^*$ ) of control normal beef and wagyu beef paste on the 0th day, the former was less red than the latter. At the end of the storage period, no significant difference in redness value ( $a^*$ ) of wagyu beef treated with MO was observed (between the 0th and 7th day). As expected, the addition of sodium nitrate increased the  $a^*$  values of both meat samples, which could be linked to their antioxidant

**Table 3** The changes in colour ( $L^*$ ,  $a^*$  and  $b^*$ ) values for wagyu beef and normal beef paste with or without any added antioxidant agent during storage at 4 °C for 7 days

Parameters	Meat system	Treatments					SEM	<i>p</i> -value			
		MO	RO	SN	BHT	C		MO×RO	MO×SN	MO×BHT	MO×C
0th day											
$L^*$	Wagyu	43.61 <sup>By</sup>	42.88 <sup>By</sup>	44.82 <sup>Ax</sup>	44.56 <sup>Ax</sup>	44.10 <sup>Ax</sup>	0.24	ns	ns	ns	ns
	Normal	48.36 <sup>Ax</sup>	43.96 <sup>Bx</sup>	44.27 <sup>Bx</sup>	42.70 <sup>Cy</sup>	42.08 <sup>Cy</sup>	0.18	**	**	**	**
$a^*$	Wagyu	12.30 <sup>Ax</sup>	12.41 <sup>Ax</sup>	10.49 <sup>Cx</sup>	11.69 <sup>ABx</sup>	11.23 <sup>Bx</sup>	0.19	ns	**	ns	*
	Normal	6.17 <sup>Cy</sup>	7.34 <sup>By</sup>	9.74 <sup>Ax</sup>	7.27 <sup>By</sup>	7.59 <sup>By</sup>	0.15	**	*	*	*
$b^*$	Wagyu	10.72 <sup>Ay</sup>	9.76 <sup>Ay</sup>	10.21 <sup>Axy</sup>	10.32 <sup>Ax</sup>	10.56 <sup>Ax</sup>	0.21	ns	ns	*	ns
	Normal	12.28 <sup>Ax</sup>	10.19 <sup>Bx</sup>	8.83 <sup>BC</sup>	8.68 <sup>Cy</sup>	8.87 <sup>BCy</sup>	0.24	**	**	**	**
7th day											
$L^*$	Wagyu	43.82 <sup>Ay</sup>	44.32 <sup>Aay</sup>	43.77 <sup>Ay</sup>	43.76 <sup>Ay</sup>	43.77 <sup>Ax</sup>	0.29	ns	ns	ns	ns
	Normal	45.20 <sup>Ax</sup>	46.47 <sup>Ax</sup>	45.57 <sup>Ax</sup>	45.43 <sup>Ax</sup>	45.10 <sup>Ax</sup>	0.27	*	ns	ns	ns
$a^*$	Wagyu	12.24 <sup>ABx</sup>	10.48 <sup>Bx</sup>	17.45 <sup>Ax</sup>	13.01 <sup>ABx</sup>	11.43 <sup>ABx</sup>	1.16	*	*	ns	ns
	Normal	6.85 <sup>Ay</sup>	6.83 <sup>Ay</sup>	5.90 <sup>By</sup>	3.68 <sup>By</sup>	6.15 <sup>Ay</sup>	0.57	ns	**	ns	**
$b^*$	Wagyu	8.72 <sup>ABy</sup>	9.54 <sup>Ay</sup>	9.71 <sup>Ax</sup>	7.16 <sup>Cy</sup>	7.97 <sup>BCy</sup>	0.25	ns	ns	**	ns
	Normal	12.47 <sup>Ax</sup>	11.67 <sup>ABx</sup>	9.84 <sup>Cx</sup>	8.32 <sup>Dx</sup>	10.16 <sup>BCx</sup>	0.26	ns	**	**	**
Storage effect (0th × 7th day)											
$L^*$	Wagyu	ns	*	ns	*	ns					
	Normal	**	**	*	**	**					
$a^*$	Wagyu	ns	*	**	*	ns					
	Normal	*	ns	**	ns	**					
$b^*$	Wagyu	**	ns	ns	**	**					
	Normal	ns	**	ns	ns	**					

Treatments- MO-Mānuka oil, RO- Rosemary oil, SN- Sodium Nitrate, BHT- Butylated Hydroxytoluene, C- Control. MO×RO = comparison between mānuka oil and rosemary oil, MO×SN = comparison between mānuka oil and sodium nitrate, MO×BHT = comparison between mānuka oil and butylated hydroxytoluene, MO×C = comparison between mānuka oil and control, Storage effect (0th × 7th day) = comparison between 0th and 7th day. ns =  $P > 0.05$ ; \* $P < 0.05$ ; \*\* $P < 0.01$ . SEM- Standard error mean.

<sup>A-E</sup>Means within a row with the same superscript letters are not significantly different ( $P < 0.05$ ) between the treatments on the same storage day.

<sup>x-y</sup>Means within a column with the same superscript letters are not significantly different ( $P < 0.05$ ) between the meat systems.

effect and formation of stable red/pink colour pigment in sodium nitrate-added samples.

Interestingly, different values of yellowness and blueness ( $b^*$ ) for control normal and wagyu beef pastes were observed in the initial phase of the storage period. It could be related to the concentration of  $\beta$ -carotene deposited in the fat of normal beef carcasses relative to other beef or breeds of cattle, including wagyu (Jaborek *et al.*, 2019). With MO treatment, differences in  $b^*$  values of wagyu beef pastes were observed during the storage, but this effect was not statistically significantly different from RO, and sodium nitrate-treated samples and their control counterparts. The sodium nitrate did not show any effect against the  $b^*$  values of both types of meat, while BHT decreased these values during the storage of wagyu beef paste (between the 0th and 7th day). Similar to  $b^*$  values, the effect of MO against the lightness ( $L^*$ ) values of wagyu beef paste were not significantly different from all other treatments. However, in normal beef, MO treatment resulted in significantly different lightness values than the other treated samples, on the 0th day and during the storage period (between the 0th and 7th day). The results of this study on colour changes agree with the previously reported study by Ünal *et al.* (2014). They reported higher  $L^*$  values in beef treated with RO than in control. Additionally, the initial difference in redness values of both meat could be linked to their pH, as it has been reported that meat colour gets darker with an increase in pH (Dixit *et al.*, 2021). The difference in  $b^*$  values of control normal and wagyu beef pastes in the initial phase of the storage period could be related to the concentration of  $\beta$ -carotene deposited in the fat of normal beef carcasses relative to other beef or breeds of cattle, including wagyu (Jaborek *et al.*, 2019). Similarly, a recent study by Tomović *et al.* (2020) also observed a significant effect of fat, *Juniperus communis* essential oil treatment and storage period on instrumental colour values of 25%-fat-containing dry-fermented sausages and 15% fat sausages.

## Conclusions

In conclusion, the results of the present study exhibit that MOs possessed higher antioxidant activity than RO or KO in all the antioxidant assays performed. In these free radical scavenging assays, a concentration-dependent increase in the antioxidant efficacy of essential oils and BHT was observed.

Further, when selected MO and RO were compared against BHT and sodium nitrate in wagyu and normal beef pastes, all treated meat systems showed a significant increase in lipid oxidation values during the 7-day refrigerated storage, except for BHT. The

MO addition, however, led to significant reduction of lipid oxidation in wagyu beef, possibly due to the lipo-solubility of bioactive compounds of essential oils. The results point toward the potential of MO as a green and natural preservative for the complete replacement of synthetic antioxidant agents such as BHT, mainly in high-fat meat systems. Although MO showed promising antioxidant characteristics in high-fat meat systems, future studies on its toxicity and consumer acceptability are required for its use in foods. Future research on the encapsulation and emulsification of MO is underway to overcome the challenges around its stability, solubility, taste and odour to facilitate a wide range of food applications.

## Acknowledgments

The authors would like to thank Massey University for granting the doctoral scholarship to the author R Kaur and the School of Food and Advanced Technology (Massey University) for funding this research. Open access publishing facilitated by Massey University, as part of the Wiley - Massey University agreement via the Council of Australian University Librarians.

## Author contributions

**Ramandeep Kaur:** Conceptualization (supporting); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); project administration (supporting); software (lead); validation (lead); visualization (supporting); writing – original draft (lead); writing – review and editing (lead). **Lovedeep Kaur:** Conceptualization (lead); funding acquisition (lead); methodology (equal); project administration (lead); resources (lead); supervision (lead); visualization (lead); writing – review and editing (equal). **Tanushree B. Gupta:** Conceptualization (supporting); investigation (supporting); supervision (supporting); visualization (supporting); writing – review and editing (supporting). **John Bronlund:** Conceptualization (supporting); investigation (supporting); methodology (supporting); supervision (supporting); visualization (supporting); writing – review and editing (supporting).

## Declaration of interest

The authors have no conflict of interest to declare.

## Ethical guidelines

Ethics approval was not required for this research.

## Peer review

The peer review history for this article is available at <https://publons.com/publon/10.1111/ijfs.16390>.

## Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## References

- AOAC, C. (2005). *Official Methods of Analysis of the Association of Analytical Chemists International*. Gaithersburg, MD: Official Methods.
- Barmpalia-Davis, I.M., Geornaras, I., Kendall, P.A. & Sofos, J.N. (2009). Effect of fat content on survival of listeria monocytogenes during simulated digestion of inoculated beef frankfurters stored at 7 °C. *Food Microbiology*, **26**, 483–490.
- Botsoglou, N.A., Fletouris, D.J., Papageorgiou, G.E., Vassilopoulos, V.N., Mantis, A.J. & Trakatellis, A.G. (1994). Rapid, sensitive, and specific Thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. *Journal of Agricultural and Food Chemistry*, **42**, 1931–1937.
- Boylston, T.D., Morgan, S.A., Johnson, K.A., Wright, R.W., Busboom, J.R. & Reeves, J.J. (1996). Volatile lipid oxidation products of Wagyu and domestic breeds of beef. *Journal of Agricultural and Food Chemistry*, **44**, 1091–1095.
- De Oliveira, T.L.C., De Araújo Soares, R., Ramos, E.M., Das Graças Cardoso, M., Alves, E. & Piccoli, R.H. (2011). Antimicrobial activity of *Satureja montana* L. essential oil against *Clostridium perfringens* type A inoculated in mortadella-type sausages formulated with different levels of sodium nitrite. *International Journal of Food Microbiology*, **144**, 546–555.
- Dixit, Y., Hitchman, S., Hicks, T.M. *et al.* (2021). Non-invasive spectroscopic and imaging systems for prediction of beef quality in a meat processing pilot plant. *Meat Science*, **181**, 108410.
- Estévez, M. & Cava, R. (2006). Effectiveness of rosemary essential oil as an inhibitor of lipid and protein oxidation: contradictory effects in different types of frankfurters. *Meat Science*, **72**, 348–355.
- Estévez, M., Morcuende, D., Ramírez, R., Ventanas, J. & Cava, R. (2004). Extensively reared Iberian pigs versus intensively reared white pigs for the manufacture of liver pâté. *Meat Science*, **67**, 453–461.
- Estévez, M., Ramírez, R., Ventanas, S. & Cava, R. (2007). Sage and rosemary essential oils versus BHT for the inhibition of lipid oxidative reactions in liver pâté. *LWT – Food Science and Technology*, **40**, 58–65.
- Jaborek, J.R., Zerby, H.N., Moeller, S.J., Fluharty, F.L. & Relling, A.E. (2019). Evaluation of feedlot performance, carcass characteristics, carcass retail cut distribution, Warner-Bratzler shear force, and fatty acid composition of purebred Jersey and crossbred Jersey steers. *Translational Animal Science*, **3**, 1475–1491.
- Kaur, R., Gupta, T.B., Bronlund, J. & Kaur, L. (2021). The potential of rosemary as a functional ingredient for meat products- A review. *Food Reviews International*, 1–21.
- Kwon, O.S., Jung, S.H. & Yang, B.S. (2013). Topical administration of manuka oil prevents UV-B irradiation-induced cutaneous photoaging in mice. *Evidence-Based Complementary and Alternative Medicine*, **2013**, 930857.
- Lee, S., Decker, E.A., Faustman, C. & Mancini, R.A. (2005). The effects of antioxidant combinations on color and lipid oxidation in n–3 oil fortified ground beef patties. *Meat Science*, **70**, 683–689.
- Li, Y., Fabiano-Tixier, A.-S. & Chemat, F. (2014). Essential oils as antioxidants. In: *Essential Oils as Reagents in Green Chemistry*. (edited by S. Sanjay Kumar). Pp. 21–27. New York, NY: Springer.
- Lis-Balchin, M. (2006). *Aromatherapy Science: A Guide for Healthcare Professionals*. London: Pharmaceutical Press.
- Lis-Balchin, M. & Hart, S. (1998). An investigation of the actions of the essential oils of Manuka (*Leptospermum scoparium*) and Kanuka (*Kunzea ericoides*), Myrtaceae on Guinea-pig smooth muscle. *Journal of Pharmacy and Pharmacology*, **50**, 809–811.
- Lis-Balchin, M., Hart, S.L. & Deans, S.G. (2000). Pharmacological and antimicrobial studies on different tea-tree oils (*Melaleuca alternifolia*, *Leptospermum scoparium* or Manuka and *Kunzea ericoides* or Kanuka), originating in Australia and New Zealand. *Phytotherapy Research*, **14**, 623–629.
- Mcbride, N.T.M., Hogan, S.A. & Kerry, J.P. (2007). Comparative addition of rosemary extract and additives on sensory and antioxidant properties of retail packaged beef. *International Journal of Food Science & Technology*, **42**, 1201–1207.
- Mehdizadeh, A., Shahidi, S.-A., Shariatifar, N., Shiran, M. & Ghorbani-Hasansaraei, A. (2022). Physicochemical characteristics and antioxidant activity of the chitosan/zein films incorporated with *Pulicaria gnaphalodes* L. extract-loaded nanoliposomes. *Journal of Food Measurement and Characterization*, **16**, 1252–1262.
- Pabast, M., Shariatifar, N., Beikzadeh, S. & Jahed, G. (2018). Effects of chitosan coatings incorporating with free or nano-encapsulated *Satureja* plant essential oil on quality characteristics of lamb meat. *Food Control*, **91**, 185–192.
- Porter, N.G. & Wilkins, A.L. (1999). Chemical, physical and antimicrobial properties of essential oils of *Leptospermum scoparium* and *Kunzea ericoides*. *Phytochemistry*, **50**, 407–415.
- Tomović, V., Šojić, B., Savanović, J. *et al.* (2020). New formulation towards healthier meat products: *Juniperus communis* L. essential oil as alternative for sodium nitrite in dry fermented sausages. *Foods*, **9**, 1066.
- Torres-Martínez, R., García-Rodríguez, Y.M., Ríos-Chávez, P. *et al.* (2017). Antioxidant activity of the essential oil and its major terpenes of *Satureja macrostema* (Moc. And Sessé ex Benth.) Briq. *Pharmacognosy Magazine*, **13**, S875.
- Ünal, K., Babaoglu, A.S. & Karakaya, M. (2014). Effect of oregano, sage and rosemary essential oils on lipid oxidation and color properties of minced beef during refrigerated storage. *Journal of Essential Oil Bearing Plants*, **17**, 797–805.
- Viuda-Martos, M., Ruiz Navajas, Y., Sánchez Zapata, E., Fernández-López, J. & Pérez-Álvarez, J.A. (2010). Antioxidant activity of essential oils of five spice plants widely used in a Mediterranean diet. *Flavour and Fragrance Journal*, **25**, 13–19.
- Zhang, R. (2015). *Characterisation of Bresaola Products Made from Beef, Veal, Wagyu, Mutton and Lamb*. Auckland: Auckland University of Technology.