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Biopreservative efficacy of grape (*Vitis vinifera*) and clementine mandarin orange (*Citrus reticulata*) by-product extracts in raw ground beef patties

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ABSTRACT

Beef patties were treated with 450 μg/g of extracts from grape (*Vitis vinifera*) seeds (GSE), pomace (GPE) or orange (*Citrus reticulata*) pomace (OPE) and compared to negative (no extract; CTR) and positive (sodium metabisulphite; SMB) controls for their effect on colour, lipid and protein oxidation and bacterial growth under simulated retail display conditions (4 °C) for 9 d, and sensory quality. Antioxidant activity and redness of beef patties increased in the order of CTR < OPE = GPE < GSE < SMB. The order of thiobarbituric acid reactive substances and carbonyl values were CTR > GPE = OPE > GSE > SBM, while that of bacterial counts were CTR > GSE = GPE > OPE > SMB. Retail display period had significant effect on all the shelf-life parameters. Overall, intensity of aroma, beef-like aroma and flavour in beef patties were highest in OPE. Results suggested that GSE and OPE could be commercially valorised as natural antioxidants and antibacterials in beef patties, respectively.

1. Introduction

Growing urbanisation and consumer preference towards convenience foods have led to an increase in demand for ready-to-eat foods, particularly processed meat products (Ansorena, Pereda, & Marcovich, 2018; Nikmaram et al., 2018). Satisfying this demand is a challenge as large quantities of food losses occur along the supply chain before reaching the consumer (FAO, 2019; Žugčić et al., 2019). Regarding meat, over one-fifth of the 263 million tons produced globally is either lost or wasted (FAO, 2017). Oxidative degradation processes and bacterial spoilage are the largest contributors to meat losses through reduction in shelf-life, sensory quality, nutritional and health value (Papuc, Goran, Predescu, Nicorescu, & Stefan, 2017). Processed meat products including burger patties are affected the most due to their high contents of lipids, water and salt, and large surface areas (Hygreeva, Pandey, & Radhakrishna, 2014), which increases the formation of reactive oxygen species (De Smet & Vossen, 2016; Oswell, Thippareddi, & Pegg, 2018). In spite of their susceptibility to oxidation, ground beef burger patties are the most popular meat products owing to their accessibility, affordability and preparatory versatility (Gordon-Larsen, Guilkey, & Popkin, 2011; Mapiye et al., 2014). Increased market competition and consumer demand have influenced a need to enhance the healthfulness, safety, and shelf-life of processed meat products (López-López et al., 2011). Consequently, preservatives mostly synthetic antioxidants and antibacterials are often incorporated in processed meat products to retard oxidative degradation processes and bacterial spoilage (Cunha et al., 2018; Kumar, Yadav, Ahmad, & Narsaiah, 2015).

Regrettably, synthetic preservatives (e.g., sulphites and nitrites) were recently shown to exhibit toxic and carcinogenic effects (D'Amore et al., 2020; Jiang & Xiong, 2016; Kumar et al., 2015). Currently, there is increasing consumer demand and preference in plant-derived preservatives because they are more potent (Jiang & Xiong, 2016; Mhalla et al., 2017), safe, nutritious, and healthy than synthetic preservatives (Lorenzo et al., 2018; Pogorzelska-Nowicka, Atanasov, Horbańczuk, & Wierzbicka, 2018). To this end, research to determine the biopreservative effects of bioactive phytochemicals isolated from spices, herbs, vegetables and fruits by-products such as grape seed or pomace (Peixoto et al., 2018; Sagdic et al., 2011) and citrus pomace (Bora, Kamle, Mahato, Tiwari, & Kumar, 2020; Contini et al., 2012) is gaining momentum worldwide.

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Grapes and citrus are the major fruit crops in the world, with an annual production of approximately 77.8 and 102 million metric tons, respectively (OIV, 2019; USDA, 2020). Of this, 20–30% and 50–70% end up as grape and orange pomace, respectively. These wastes pose serious processing, storage and disposal challenges which present huge costs to the fruit industry (Tayengwa, Chikwanha, Dugan, Mutsvangwa, & Mapiye, 2020). Recent research found that extracts from grape seed and pomace (Peixoto et al., 2018; Pfukwa et al., 2019), and clementine mandarin orange pomace (Bora et al., 2020; Pfukwa et al., 2019) are rich sources of a multitude and diverse polyphenols that have strong in vitro antioxidant and antibacterial properties, respectively. However, there is limited data about *in producto* biopreservative efficacy of extracts from these winery (Sagdic, Ozturk, Ozkan, et al., 2011) and citrus (Contini et al., 2012) by-products on oxidative and microbial stability of beef patties. To our knowledge, there are no studies that have compared the *in producto* biopreservative efficiency of extracts from these fruit by-products on shelf-life, textural properties and sensory quality of beef patties. Overall, valorisation of winery and citrus by-products as meat preservatives could provide dual benefits of reducing fruit wastes and meat losses. The objective of the current study was, therefore, to assess the biopreservative effects of grape seed, pomace and clementine mandarin orange pomace extracts in raw ground beef patties under simulated retail display conditions (4 °C for 9 d).

2. Materials and methods

2.1. Preparation and polyphenolic extraction of fruit extracts

Grape pomace (*Vitis vinifera* cv. Pinotage) was sourced from Welgevallen Cellar (Stellenbosch, South Africa). Clementine mandarin orange (*Citrus reticulata* Blanco cv. clementine) pomace were collected from Cape Fruit Processors (Pty) Ltd. (Citrusdal, South Africa). Grape seed extract (GSE) was a donation from Brenn-O-Kem (Pty) Ltd. (Wolseley, South Africa). Grape pomace extract (GPE) and orange pomace extract (OPE) were prepared using the solid–liquid extraction method at Brenn-O-Kem (Pty) Ltd. For the determination of phytochemical analysis, the samples were washed with boiling water to remove the excess fat then pre-extracted with 62% ethanol (1:10, *w/v*)]. The extract was recovered using the filter press (Model KFP639/20, Schenk, South Africa) method and subsequently vacuum-dried using a single stage, liquid ring vacuum pump (Model MHF-150, Nash, Wisconsin, USA) to get rid of the solvent. The dried extracts were finely ground with a 0.1-μm mesh and, separately weighed and re-suspended in 62% ethanol before the determination of bioactive phytochemicals.

2.2. Phytochemical profiling of the fruit by-product

Phytochemical compositions of fruit by-product extracts were determined using high-resolution Ultra-Performance Liquid Chromatograph-Mass Spectrometry (UPLC-MS; Lai et al., 2018). Data were processed using MS-DIAL and MS-FINDER (RIKEN Centre for Sustainable Resource Science: Metabolome Informatics Research Team, Kanagawa, Japan) (Lai et al., 2018; Tsugawa et al., 2015). Results were expressed as concentration in mg catechin /kg (mg CE/kg). The contents of total phenolic and tannin extracts were determined using the Folin-Ciocalteu method (Makkar, 2003). The results were presented as grams of gallic acid equivalents per hundred grams of dry matter (g GAE/100 g DM). The total flavonoids were measured based on the method described by Yang, Martinson, and Liu (2009) and results reported as g CE/100 g of extract. Quantification of anthocyanins were determined by the pH-differential method (Wrolstad & Giusti, 2001) and content expressed as grams cyanidin-3-glucoside equivalent per 100 g extract. Proanthocyanidins were quantified using the procedure of Porter, Hrstich, and Chan (1986), and were expressed as grams of cyanidin equivalent per hundred grams of extract DM. Total carotenoids were quantified according to the procedures of Rodrigo, Cilla, Barberá, and Zacarías (2015). The total content of carotenoids was expressed as g β-carotene per 100 g of extract. Results were an average of three replicates.

2.3. Preparation and storage of beef patties

Lean beef loins [left and right *longissimus thoracis et lumborum* muscles] and subcutaneous fat of eight steers (<1 year old) were purchased from a commercial abattoir (Hermon, South Africa) after chilling 24 h. The loins and fat were vacuum packaged and transported under cold storage conditions (4 °C) to the Department of Animal Sciences' Meat Science laboratory (Stellenbosch University, South Africa). On arrival at the laboratory, all visible fat and connective tissues were manually trimmed and removed from the loins under hygienic condition. A total of eight meat batches, each batch comprising of loins (i.e., left and right, and corresponding subcutaneous fat) from one steer were formed. Loins and fat for each batch were then cut into cubes (2 cm^3) and subdivided into 5 portions. Five treatments were then created by adding either water (control, CTR), 450 μg/g of sodium metabisulphite (SMB, a commercial preservative), GSE, GPE or OPE to each meat portion. Thereafter, each portion with all ingredients (Table 1) was thoroughly mixed by hands and ground using a Tre Spade Electric Mincer (Munaaz Catering Equipment (Pty) Ltd., Cape Town, South Africa) with a 6 mm plate. All the treatments had same contribution of 73.6% lean meat, 18.4% fat, 1.2% binder, 0.3% emulsifier, and 2% salt (Table 1). However, the control had 5% deionized water, whilst SMB and the extract treatments had 4.950% deionized water and 0.0450% sodium metabisulphite and the respective fruit by-product extracts (Table 1). The fruit by-product extracts concentration of 450 μg/g matched the maximum permitted level of sodium metabisulphite in processed meats (South African government regulation Section 15(1) of the Foodstuffs, Cosmetics and Disinfectant Act 54 of 1972). A handheld patty-maker (model PMB0100/0130; BUTCHERQUIP, Johannesburg, South Africa) was used to mould beef patties (10 cm diameter and 1 cm thickness) from 100 g portions of the mixed formulations. A total of 640 beef patties (5 treatments × 8 batches × 16 samples) were formed.

Of the 16 patties formed for each treatment batch on day one, 12 were randomly selected, vacuum packaged and stored at −20 °C for determination of textural profile, cooking yield and sensory quality. The remaining 4 patties for each treatment batch were then used for proximate analysis and shelf-life evaluation. The proximate compositions of the patties are presented in Table 1. Each of the four patties,

Table 1
Ingredients, formulations and proximate composition of beef patties treated with fruit by-product extracts.

Ingredients (g/kg)	Treatments					SD
	CTR	SMB	GSE	GPE	OPE	
Lean meat	1177.6	1177.6	1177.6	1177.6	1177.6	
Fat	294.4	294.4	294.4	294.4	294.4	
Potato starch (binder)	19.2	19.2	19.2	19.2	19.2	
Sodium triphosphate (emulsifier)	4.8	4.8	4.8	4.8	4.8	
Salt	24	24	24	24	24	
Distilled water	80	79.28	79.28	79.28	79.28	
Preservative/fruit extract	0	0.72	0.72	0.72	0.72	
Proximate composition (%)						
Moisture	62.7	62.4	63.3	62.1	62.4	0.78
Ash	2.7	2.6	2.3	2.8	2.5	0.15
Intramuscular fat	16.7	16.9	16.3	16.4	16.6	0.37
Crude protein	18.2	18.1	18.1	18.4	18.3	0.52

CTR: control; SMB: sodium metabisulphite; GSE: grape seed extract; GPE: grape pomace extract; OPE: Clementine mandarin orange pomace extract; SD: standard deviation. 0.72 g.

corresponding to d 1, 3, 5, 7 and 9 were placed onto a polystyrene rectangle tray like with a sterile stomacher bag (Cleansafe LABS, Cape Town, South Africa). The trays were wrapped with 10 μm-thick low-density polyethylene film (moisture vapor transfer rate: 585 g/m^2/24 h/1 atm., with oxygen permeability of 2500 cm^3/m^2/24 h/1 atm. and carbon dioxide permeability of 18,000 cm^3/ m^2/ 24 h/1 atm. (Vinyl films, Mpact, Cape Town, South Africa). White, fluorescent lights (Philips TL-D 58 W/33–640, cool white, 4600 Lumen) were used in the display cabinet (±4 °C), simulating retail conditions. After every 24 h, the trays were rotated daily to minimise variation of light intensity and temperature. Sampling of beef patties was conducted at the end of each retail display period. Before recording pH and colour coordinates, a 20 g sample was cut from each patty under hygienic conditions for quantification of microbial loads. The sample was vacuum-packed and stored at −20 °C and analysed for microbial counts within 72 h. For each patty, three readings of the colour coordinates were taken on three different locations from the surface making 24 observations per treatment. After averaging the replications per patty, eight observations were subjected to statistical analysis. The remaining 80 g of patty was divided into three parts for the determination of antioxidant activity, protein, and lipid oxidation and stored at −80 °C prior to analysis.

2.4. Evaluation of the stability of beef patties treated with fruit by-product extracts under retail display conditions

Prior to analysis of proximate, antioxidant activity and lipid and protein oxidation, all samples were thawed overnight at 4 °C and homogenised using a knife mill (Knifetec™ 1095, Höganäs., Sweden).

2.4.1. pH and colour measurements

Three values were taken for pH analysis from each beef patty using a pH meter fixed with an electrode (model: MP230, Mettler-Toledo, Greifensee, Switzerland). Thirty minutes after removal of the cling wrap, three readings for each colour coordinate [i.e., lightness (L*); redness (a*) and yellowness (b*)] were taken on non-overlapping points on the surface of each beef patty. Colour measurements were performed using the colour-guide 45/0 Spectrophotometer (BYK-209, Gardner GmbH, Gerestried, Germany) with an aperture diameter of 11-mm, D_{65} illuminant and a 10° observer angle. The hue angle (H*) and chroma (C*) values were calculated from a* and b* colour coordinates using the following equations: $H^* = \tan^{-1}$ (b*/a*) and $C^* = (a^{*2} + b^{*2})^{1/2}$. Hue angle represents discolouration while C* is correlated in the intensity of red colour.

2.4.2. Proximate composition determination

Methods 934.01, 942.05 and 968.06 were used to determine moisture, ash and crude protein (CP) contents of the homogenised beef patties (AOAC, 2002). Total fat was measured by extracting with chloroform/methanol (2/1 *v/v*) as described by Lee, Trevino, and Chaiyawat (1996). Crude protein was determined on the defatted samples. All measurements were conducted in triplicate.

2.4.3. Antioxidant activity determination

Antioxidant activity analysis of the beef patties was conducted using the ferric reducing antioxidant power (FRAP) method (North, Dalle Zotte, & Hoffman, 2019). Duplicate beef patty samples for each experimental unit weighing 1 ± 0.050 g were homogenised (T18 digital ULTRA TURRAX®, IKA®, Staufen im Breisgau, Germany) at 9000 × rpm for 120 min in 5 mL of potassium phosphate buffer (pH 7.2). A 20 μL supernatant obtained by centrifuging the homogenate at 4024 ×*g* for 30 min was combined with 180 μL FRAP reagent [300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-Tris[2-pyridyl]-*s*-triazine and 20 mM ferric chloride, *v/v/v* (10:1:1)] in a 96-well microplate. The plates with samples were shaken for 3 s at an incubation temperature of 37 °C before absorbance was read at 593 nm (Spectrostar Nano®, BMG Labtech, Ortenberg, Germany). The activity of FRAP was evaluated by comparison to a ferrous sulphate standard curve [0.1–0.8 mM, $R^2 \geq 99$], and the results were expressed as mM of Fe^{2+} equivalent/ kg of beef patty.

2.4.4. Lipid oxidation analysis

The degree of lipid oxidation in beef patties was determined by estimation of malondialdehyde (MDA) content using the thiobarbituric acid reactive substances (TBARS) according to the modified method of Mukumbo et al. (2018). In brief, 2 g (in duplicate) of each sample were homogenised (T18 digital ULTRA TURRAX®, IKA®, Staufen im Breisgau, Germany) in 12.5 mL of 2.8% trichloroacetic acid and distilled water (1:1; *v/v*) for 20 s. The homogenate was filtered through a Whatman no. 1 filter paper. One millilitre (in duplicate) of the filtrate was combined with 1 mL of 0.02 M thiobarbituric acid in a 10 mL centrifuge tube. For each sample, 2 mL of distilled water were added to the third tube (i.e., turbidity blank). The tubes were then incubated in a water bath at 70 °C for 1 h. Absorbance was read at 530 nm (Spectrostar Nano®, BMG Labtech, Ortenberg, Germany) using 1,1,3,3-tetramethoxypropane standard curve (0–20 μM, $R^2 \geq 99$). Results were expressed as mg of MDA equivalent/ kg beef patty.

2.4.5. Protein carbonyl assay

The extent of protein oxidation was measured as carbonyl content in beef patties using Sigma-Aldrich Protein Carbonyl Colorimetric Assay Kit (St Louis, MO, USA; Sigma-Aldrich, 2015) following the manufacturer's procedure. Carbonyl content was determined by using derivatisation with 2,4-dinitrophenyl hydrazine (DNPH) and was quantified by a spectrophotometric assay at 375 nm. The carbonyl content was computed based on the molar absorptivity for protein hydrazones (22,000 $M^{-1}.cm^{-1}$). Protein content was calculated from the absorption of 562 nm using bovine serum albumin, as a standard. The results of total carbonyl content were expressed as nmol DNPH/ mg protein. All the beef patties were analysed in duplicates.

2.4.6. Bacterial load determination

Ten grams of thawed beef (24 h at 4 °C) taken from different points of the patty were homogenised in 90 mL of sterile peptone water (0.1% *w/w*) using a BagMixer 400 lab blender (INTERSCIENCE, 78,860 st Nom; France) for 120 s at 25 °C (Tayengwa et al., 2020). Thereafter, 5-fold serial dilutions (with 0.1% sterile peptone water) of the homogenates were prepared. Subsequently, 1 mL from each of the previously prepared serial dilutions was separately plated in duplicates on 3 M™ Petrifilm™ plates (3 M, South Africa Pty Ltd., Cape Town, South Africa). Microbial loads were quantified using specific 3 M™ Petrifilm™ Count Plates, Lactic acid bacteria (LAB; 3 M™ Petrifilm™ Lactic Acid Bacteria Count Plates), *Escherichia coli* and total coliform counts (*E. coli* and TCC; 3 M™ Petrifilm™ *E. coli*/Coliform Count Plates), *Listeria monocytogenes* counts (3 M™ Petrifilm™ Environmental Listeria Plates) and total aerobic counts (TVC; 3 M™ Petrifilm™ Aerobic Count Plates) according to the manufacturer's guidelines.

2.4.7. Cooking yield

All beef patties for cooking yield, textural profile and sensory evaluation were thawed overnight at 4 °C and blotted dry before being weighed. Beef patties were cooked using a non-stick cooking spray for approximately 4 min and 30 s (medium level of doneness) as described by (Berry, 1994) with modifications based on preliminary trial. Cooking was done using a Silex S-Tronic 165 GR grill (Model: S-Tronic 165, Silex, Hamburg, Germany) equipped with ribbed, cast iron plates (voltage: 400 V; power consumption: 6.0 kW; fusing: 3 × 16 Amp; grill surface: 360 × 360 mm; height closed: 450 mm; height open: 675 mm; depth open: 670 mm; area for place: 400 × 670 mm 56 kg) set at a depth of 14 mm with the bottom plate at 143 °C and the top plate at 154 °C for 10 min. Cooked weights were recorded for the determination of cooking yield using the following equation: cooking yield (%) = (weight of cooked beef patty/ weight of raw patty) × 100 (Berry, 1994).

2.4.8. Descriptive sensory analysis

A nine-member trained panel (women aged between 26 and 69 years) with previous experience in sensory evaluation of meat following the American Meat Science Association (2015) guidelines was used for the Descriptive Sensory Analysis (DSA). Six patties from each batch within a treatment were used for the training phase and the other six patties for testing. The training phase for the panellists was conducted over a 4-day period with two treatment batches and reference standards per day (Table 2). The training of panellists was conducted in two groups per day (two sessions per group, four sessions in total, each session lasted for 1 h). The panellists had to be divided randomly into two groups of four and five individuals each, as per COVID-19 regulations under alert level one of the Republic of South Africa [Disaster Management Act No. 27 of 2002, Amendment of Regulation issues in terms of Section 27(2)] where a maximum of eight individuals were allowed in the sensory evaluation area.

Beef patties were cooked as described previously, and each cooked patty was cut into eight equal wedges and covered with aluminium foil squares of 14.5 cm × 14.5 cm. Of these wedges, three were used for DSA and five for textural profile analysis. The DSA wedges were then warmed using a Hobart convection oven (Paris, France) preheated at 60 °C for 8 min based on American Meat Science Association (2015) guidelines. Wedges were then blind coded with 3-digit random numbers. Each panel member received a glass containing three wrapped wedges from each patty served at 60 °C.

The chemical and physical reference standards were used during the training period for the panellists to be able to identify and differentiate sensory descriptors, and for the 100-point line scale calibration of each attribute by the panellists (Table 2). The initial list of reference standards was derived from American Meat Science Association (2015) and Braghieri et al. (2012), however, the intensities were decided on by consensus method during DSA training. A combination of ballot and consensus training methods were used during the training phase upon which flavour, aroma and texture attributes were decided (Table 2). The panellists did not detect the aroma or flavour associated with GSE, GPE, OPE and SMB solutions at 10 g/ 250 mL of distilled water during training and therefore, these attributes were excluded for the testing phase. Temperature of 21 ± 1 °C and humidity of 52 ± 2% were

Table 2
Reference standards, definitions and scales of final aroma, flavour and textured attributes used in descriptive sensory analysis of beef patties.

Aroma attributes	Attribute description	Reference standard used
0 = None, 100 = Prominent		
Overall aroma intensity	The intensity of the overall aromatics in the first few sniffs	BP 1 = 70
Beef-like aroma	Amount of beef aroma identity in the sample	BP 1 = 60–70; BP 2 = 60–70
Savoury aroma	Aromatics associated with salty, meaty and brothy characteristics	Liver = 10; BP 2 = 10; BP 1 = 20; Savoury 2 = 40; Savoury 1 = 80
Sweet-associated aroma	Aromatics associated with the impression of sweet	BP 1 = 20; Liver = 20–30; BP 2 = 30–40
Fatty aroma	Aromatics associated with cooked fat from a beef patty	BP 1 = 20
Liver-like aroma	Aromatics associated with pan fried beef ox liver	Liver = 70–80
Metallic aroma	Impression of slightly oxidised metal, such as iron, copper, and silver spoon or associated with raw meat (blood-like)	Metallic = 10; Liver = 30–50
Smoky-woody aroma	Dry, dusty aromatic reminiscent of burning wood	
Herbaceous aroma	Aromatics associated with a combination of dried herbs	BP 1 = 10
Rancid aroma	Aromatics commonly associated with oxidised fat and oils; may include cardboard, painty, varnish, and fishy	Rancid = 40

Flavour attributes	Attribute description	Reference standard used
0 = None, 100 = Prominent		
Beef-like flavour	Amount of beef flavour identity in the sample	BP 1 = 60; BP 2 = 50–65
Savoury flavour	Aromatics associated with salty, meaty and brothy characteristics	
Salty taste	Fundamental taste factor of which sodium chloride is typical	Salt = 5–10; BP 2 = 20; BP 1 = 20–30
Sweet-associated flavour	Combination of sweet taste and sweet aromatics; the aromatics associated with the impression of sweet	BP 1 = 10–20; BP 2 = 20–30; Liver = 30
Fatty flavour	Aromatics associated with cooked fat from a beef patty	BP 1 = 30; BP 2 = 30
Liver-like flavour	Aromatics associated with pan fried beef ox liver	Liver = 80
Metallic flavour	Impression of slightly oxidised metal, such as iron, copper, and silver spoon or associated with raw meat (blood-like)	BP 2 = 0; BP 1 = 0–10; Liver = 50–60
Rancid flavour	Flavour commonly associated with oxidised fat and oils; may include cardboard, painty, varnish, and fishy	
Peppery flavour	Aromatics commonly associated with black pepper	BP 2 = 10–15

Texture attributes	Attribute description	Reference standard used
Overall juiciness (0 = Dry, 100 = Extremely juicy	The impression of moisture during mastication	BP2 = 40–60
Mealiness (0 = None, 100 = Abundant)	The degree to which meat particles disintegrates into small gritty pieces in the mouth during the first 5 chews	Liver = 20–40
Residue (0 = None, 100 = Abundant)	The amount of meat particles left in the mouth after 10 chews	Liver = 10–20
Fatty coating (0 = None, 100 = Abundant)	The impression of fat coverage on the palate after mastication	BP 2 = 10–30; BP 1 = 20–30

Table 2 was adapted and modified from American Meat Science Association (2015) and Tayengwa, Chikwanha, Gouws, et al. (2020); Pan-fried beef patty (Woolworths Holdings Limited, Stellenbosch, SA) = BP 1; Pan-fried beef patty with fat (Woolworths Holdings Limited, Stellenbosch, SA) = BP 2; 5 mL of Marmite Yeast Extract (Woolworths Holdings Limited, Stellenbosch, SA) dissolved in 250 mL boiling water = Savoury 1; 5 mL of Beefy Bovril (meat and vegetable extract; Woolworths Holdings Limited, Stellenbosch, SA) dissolved in 250 mL boiling water = Savoury 2; Pan fried Ox liver (Woolworths Holdings Limited, Stellenbosch, SA) = Liver; 0.10% potassium chloride (Merck, Gauteng, SA) solution = Metallic; Microwaved sunflower oil (Woolworths Holdings Limited, Stellenbosch, SA) for 6 min at high = Rancid; 20% Iodated table salt (Woolworths Holdings Limited, Stellenbosch, SA) solution = Salt.

maintained in the preparation and testing rooms. Testing for the DSA was performed in individual tasting booths (76 cm wide × 51 cm depth). The booths had neutral-coloured walls and furniture, and standard lighting conditions (700–800 lx). Each booth was fitted with computers equipped with the Compusense five® (Compusense, Guelph, Canada) software program. Each batch (i.e., replicate) was assigned randomly to a testing session and 8 sessions were performed.

2.4.9. Texture profiling analysis test

Following the cooking procedures outlined in Section 2.4.4, beef patty texture profiling analysis (TPA; hardness, springiness, cohesiveness, gumminess and chewiness) was performed as described by Berry (1994). Five wedges from cooked patties as described above were analysed for each treatment batch. Each wedge was compressed twice to 75% of its original height using a 2 cm circular flat surface disk and the Instron Universal Testing Machine (model: 2519-107, Canton, Massachusetts 02021-1089, USA) with full scale load of 150 N (American Meat Science Association, 2015). The machine was equipped with Bluehill® 3 Universal Software following a double-bite method. The following parameters were analysed: hardness (N) as the peak force during the first compression cycle ("first bite"); cohesiveness as the ratio of the peak force area during the second compression to the peak force area during the first compression (Area2/Area1); springiness (elasticity, mm) as the height that the food recovers during the time elapsed between the end of the first compression and the start of the second compression; gumminess (N) was the product of hardness and cohesiveness, and chewiness (N × mm) was the product of gumminess and springiness. A crosshead speed of 100 mm/min was used.

2.5. Statistical analyses

All the data for the experiments were analysed by GLIMMIX procedures for repeated measures of SAS v. 9.4 (SAS Institute Inc., Cary, NC). For all the bacterial counts and scores of sensory, Shapiro-Wilk tests were conducted using PROC UNIVARIATE SAS v. 9.4 (SAS Institute Inc., Cary, NC) to test for normality of the residuals for each variable. The model used for statistical analyses of pH and shelf-life data of beef patties incorporated treatment, day and their interaction as the fixed effects, with batch and patty as the random effects. The model for sensory quality data incorporated treatment as the fixed effect, and panellist, session and batch were treated as random variables. Least square means test was used to determine the level of differences among treatments. Results were considered significantly different at $P \leq 0.05$.

Table 3
Polyphenolic composition of grape seed extract (GSE), grape pomace extract (GPE) and clementine orange pomace extract (OPE).*

Polyphenolic compounds[1]	Rt[2]	Mz[3]	Chemical formula	GSE	GPE	OPE	SEM
Flavones							
Keioside	14.6	623.2	$C_{28}H_{32}O_{16}$	ND*	ND*	6.8	0.02
Isoorientin 2"-O-rhamnoside	13.9	593.2	$C_{27}H_{30}O_{15}$	0.1	0.1	22.9	0.03
Luteolin 7-neohesperidoside	23.6	593.2	$C_{27}H_{30}O_{15}$	0.1	0.5	44.8	0.01
∑ Flavones				0.2	0.6	74.5	0.01
Flavanones							
Apigenosylide C;(−)-Apigenosylide C	21.8	711.3	$C_{37}H_{44}O_{14}$	ND*	0.7	41.3	0.03
Acacetin 7-(2G-rhamnosyl)-rutinoside; Apigenin 4	6.9	737.2	$C_{34}H_{42}O_{18}$	0.2	1.4	12.3	0.04
Quercetin 3-galactoside	17.4	463.1	$C_{21}H_{20}O_{12}$	1.8	8.9	0.1	0.02
Quercetin 3-glucosyl-galactosyl-glucoside	14.7	787.2	$C_{33}H_{40}O_{22}$	0.1	0.1	11.2	0.01
Naringin	18.3	579.2	$C_{27}H_{32}O_{14}$	0.5	0.7	79.9	0.03
Rutin	16.9	609.1	$C_{27}H_{30}O_{16}$	0.2	0.2	8.6	0.04
Hesperidin	19.7	609.2	$C_{28}H_{34}O_{15}$	0.1	6.4	76.6	0.04
Vitexin 2"-O-rhamnoside	12.4	577.1	$C_{27}H_{30}O_{14}$	7.1	0.4	0.2	0.04
∑ Flavanones				9.9	18.8	230.2	014
Flavanols							
Catechin	11.1	289.1	$C_{15}H_{14}O_6$	6.9	1.7	0.1	0.01
Epicatechin	13.2	289.1	$C_{15}H_{14}O_6$	28.9	4.3	0.1	0.01
Isorhamnetin 3-(6″-p-coumarylglucoside)	19.0	623.2	$C_{31}H_{28}O_{14}$	0.1	0.1	6.2	0.03
∑ Flavanols				35.9	6.1	6.4	0.01
Total phenols	–	–	–	9.7	2.4	2.2	0.10
Total flavonoids	–	–	–	1.6	1.7	3.9	0.01
Anthocyanins	–	–	–	0.5	0.3	0.1	0.01
Total carotenoids	–	–	–	0.3	0.3	0.1	0.01
Ascorbic acid	–	–	–	ND*	ND*	2.7	0.10
Hydroxybenzoic acids							
Gentisic acid	8.2	153.0	$C_7H_6O_4$	8.4	2.4	1.9	0.04
Hydroxycinnamic acids							
Plantamajoside	23.6	639.2	$C_{29}H_{36}O_{16}$	0.1	0.1	8.1	0.03
Hydroxyphenylpropanoic acids							
3-(3,4 Dihydroxyphenyl) lactic acid	14.9	197.0	$C_9H_{10}O_5$	20.1	12.2	0.2	0.01
Tranexamic Acid	8.1	156.1	$C_8H_{15}NO_2$	0.3	6.3	0.2	0.02
∑ Hydroxyphenylpropanoic acids				20.4	18.5	0.4	0.01
Limonoids							
Limonin 17-beta-D-glucoside	17.2	649.2	$C_{32}H_{42}O_{14}$	0.1	0.2	70.4	0.03
Benzenediols							
Hispolone	9.4	219.1	$C_{11}H_{12}N_2O_3$	1.8	2.8	10.6	0.01
Diterpenoids							
Crocin 3	19.9	651.3	$C_{32}H_{44}O_{14}$	0.1	0.1	22.7	0.01
Beta-Glucogallin	8.3	331.1	$C_{13}H_{16}O_{10}$	14.8	7.5	0.1	0.02
Total proanthocyanidins	–	–	–	59.4	33.2	5.2	0.6
Terpenoids							
Salvinorin A	12.0	431.2	$C_{23}H_{28}O_8$	0.5	1.8	38.7	0.03
pH of the extracts				4.3	3.8	3.4	0.02

Rt: retention time, Mz: molecular weight, SEM: standard error of mean; [1]Expressed as concentration in mg/kg vs Catechin. 2 Expressed as minutes. 3 Expressed as g/mol.

* ND: not detected.

3. Results

3.1. Polyphenolic profile of extracts from winery and citrus by-products

The polyphenolic composition of GSE, GPE and OPE are shown in Table 3. Clementine mandarin orange pomace extract had greater ($P \leq 0.05$) concentrations of individual and total flavonoids, flavones, flavanones, hydroxycinnamic acids, ascorbic acid, limonoids and diterpenoids compared to GSE and GPE. Grape seed extract had the greatest concentrations of anthocyanins, individual phenolic acids (i.e., hydroxybenzoic acids and hydroxyphenylpropanoic acids) and proanthocyanidins followed by GPE and OPE, respectively. Grape seed extract had greater concentrations of individual and total flavanols (i.e., catechin and epicatechin) and total phenols compared to GPE and GSE, whose concentrations did not differ from each other. The concentration of the identified benzenediols and terpenoids were in the following order: OPE > GPE > GSE. Grape seed extracts had the greatest pH followed by GPE and OPE.

3.2. pH and colour of extract-treated raw ground beef patties

The pH and colour of beef patties formulated using different types of extracts over the retail display period are presented in Table 4. There was no significant interaction ($P > 0.05$) between treatment and time for pH and colour parameters. The dynamics in pH and colour (except L*) were influenced by treatment and retail display period ($P \leq 0.05$). The CTR treatment had greater ($P \leq 0.05$) pH values than the other treatments. The redness (a*) values decreased in the order of SMB > GSE > GPE = OPE > CTR ($P \leq 0.05$). Extracts had similar ($P > 0.05$) b* values but were greater ($P \leq 0.05$) and lower ($P \leq 0.05$) than those for the CTR and SMB treatments, respectively. The beef patty discolouration, represented by the H* was greatest for the CTR treatment and least for the SMB treated patties, whereas the extract treated patties showed intermediate discolouration ($P \leq 0.05$). The SMB treatment had the greatest saturation (C*) values followed by the extracts and then the CTR treatment ($P \leq 0.05$). There was gradual decrease in a*, b* and C* colour parameter values, while the H* values increased during the retail display period ($P \leq 0.05$).

3.3. Effect of fruit by-product extracts on antioxidant activity, lipid and protein oxidation, and bacterial load of raw ground beef patties

Type of extract and time in retail display had effects ($P \leq 0.05$) on antioxidant activity, TBARS, carbonyls (Table 4) and bacterial loads (Table 5) but their interaction was not significant ($P > 0.05$). Sodium metabisulphite had a 34% greater antioxidant activity compared to the CTR, followed by GSE at 25%, and the lowest being GPE and OPE at 10% ($P \leq 0.05$; Table 4). The TBARS for CTR patties were more than threefold that of the SMB group, whereas TBARS for GSE and OPE were, respectively, 2.5 and 1.3 times less than CTR ($P \leq 0.05$; Table 4). With regards to carbonyl contents the following order was observed: CTR > GPE = OPE > GSE > SBM ($P \leq 0.05$; Table 4). In contrast, CTR had the greatest bacterial loads for LAB, TVC and TCC, followed by GSE and GPE that had similar counts, then OPE, and SMB in that order ($P \leq 0.05$; Table 5). Generally, antioxidant activity of beef patties declined ($P \leq 0.05$; Table 4) while TBARS, carbonyls (Table 4) and bacterial loads (Table 5) increased ($P \leq 0.05$) with time in retail display. None of the pathogenic microbes (*E. coli,* and *Listeria monocytogenes*) were detected in any of the beef patties during the time in retail display.

3.4. Cooking yield, texture profile and sensory quality of extract-treated raw ground beef patties

Results for cooking yield, texture profile (TPA) and sensory quality analyses of beef patties treated with extract from different fruit by-products are presented in Table 6. The attributes with mean values that were very low or close to zero were not presented in Table 6. Type of extract had no effect ($P > 0.05$) on cooking yield and TPA except for cohesiveness ($P \leq 0.05$). The CTR patties had the greatest cohesiveness values, with extracts having intermediate values and SMB the lowest ($P \leq 0.05$). Only, overall intensity aroma, beef-like aroma and flavour showed significant differences across treatments, with OPE beef patties having slightly greater ($P \leq 0.05$) values compared to other treatments.

4. Discussion

The weak acidic nature observed for the CTR patties compared to the other patties may be attributed to the bacterial degradation of meat proteins following depletion of the stored glucose and free amino acids (Bouarab-Chibane et al., 2017). Proteolysis produces ammonia, amines and other basic products that increase meat pH (Bouarab-Chibane et al., 2017; Muela, Sañudo, Campo, Medel, & Beltrán, 2010). The strong acidic nature for beef patties with added extracts and commercial preservative could be attributed to their antibacterial properties, which inhibits the growth and proliferation of spoilage microorganisms, particularly lactic acid bacteria (Wang, Ren, Liu, Zhu, & Wang, 2013) that subsequently reduce the breakdown of nitrogenous compounds (Bouarab-Chibane et al., 2017). Apart from the potential microbial

Table 4
Effect of extract and retail display time on pH, antioxidant activity, colour, lipid and protein oxidation of beef patties kept under retail display conditions.

Attribute	Treatment					Retail display period				SEM	*P*-Value		
	CTR	SMB	GSE	GPE	OPE	1	3	6	9		T	P	T × P
pH	5.7^{w}	$5.5^{\times}$	$5.5^{\times}$	$5.5^{\times}$	$5.5^{\times}$	5.7^{a}	5.7^{a}	5.6^{b}	5.5^{c}	0.02	0.003	0.001	0.301
Antioxidant activity[1]	139.9^{z}	210.4^{w}	$186.3^{\times}$	154.8^{y}	155.3^{y}	196.4^{a}	178.7^{b}	155.8^{c}	139.1^{d}	0.01	0.001	0.001	0.429
Colour coordinates													
Lightness (L*)	43.2	42.7	43.1	43.4	42.7	42.6	42.5	43.9	42.8	0.47	0.728	0.158	0.142
Redness (a*)	11.9^{z}	17.7^{w}	$14.7^{\times}$	13.2^{y}	13.4^{y}	21.1^{a}	14.5^{b}	12.6^{c}	10.5^{d}	0.32	0.001	0.001	0.431
Yellowness (b*)	13.9^{y}	17.3^{w}	$15.3^{\times}$	$15.2^{\times}$	$15.1^{\times}$	19.4^{a}	16.1^{b}	14.9^{c}	12.9^{d}	0.32	0.001	0.001	0,882
Hue angle (H*)	51.3^{w}	45.1^{y}	$48.1^{\times}$	$48.4^{\times}$	$48.3^{\times}$	42.5^{d}	46.3^{c}	50.1^{b}	54.6^{a}	0.59	0.001	0.001	0.776
Chroma (C*)	$19.8^{\times}$	24.6^{w}	$20.1^{\times}$	$19.8^{\times}$	$20.0^{\times}$	28.8^{a}	23.1^{b}	19.7^{c}	17.4^{d}	0.41	0.001	0.001	0.211
TBARS[2]	2.3^{w}	0.7^{z}	0.9^{y}	$1.3^{\times}$	$1.3^{\times}$	0.2^{d}	0.7^{c}	1.1^{b}	1.8^{a}	0.02	0.001	0.001	0.113
Carbonyls[3]	7.4^{w}	4.6^{z}	5.1^{y}	$5.6^{\times}$	$5.7^{\times}$	3.3^{d}	4.9^{c}	6.3^{b}	8.3^{a}	0.21	0.001	0.001	0.878

CTR: control; SMB: sodium metabisulphite; GSE: grape seed extract, GPE: grape pomace extract, OPE: clementine mandarin orange pomace extract, SEM: standard error of mean T: treatments; P: period; T × P: treatment and period interaction.
^{abcd}Within row, different superscripts indicate differences between time in retail display ($P \leq 0.05$).
^{wxyz}Within row, different superscripts indicate differences between treatments ($P \leq 0.05$).
[1] Expressed as mmol ferrous equivalent/kg beef patties.
[2] Expressed as mg malondialdehyde/kg beef patties.
[3] Expressed as nmol DNPH/mg protein.

Table 5
Effect of extract and retail display time on bacterial load of beef patties kept under retail display conditions.

Attribute	Treatment					Retail display period				SEM	P-Value		
	CTR	SMB	GSE	GPE	OPE	1	3	6	9		T	P	T × P
Total viable count[1]	4.3^{w}	1.9^{z}	3.4$^{\times}$	3.3$^{\times}$	2.7^{y}	2.6^{d}	2.8^{c}	3.0^{b}	4.5^{a}	0.09	0.001	0.001	0.287
Coliform count[1]	3.1^{w}	0.9^{z}	2.3$^{\times}$	2.1$^{\times}$	1.4^{y}	1.3^{d}	2.3^{c}	3.2^{b}	4.1^{a}	0.13	0.001	0.001	0.732
Lactic acid bacteria[1]	3.8^{w}	1.8^{z}	2.8$^{\times}$	2.7$^{\times}$	2.1^{y}	1.5^{d}	2.1^{c}	3.2^{b}	4.8^{a}	0.08	0.001	0.001	0.942

CTR: control; SMB: sodium metabisulphite; GSE: grape seed extract, GPE: grape pomace extract, OPE: clementine mandarin orange pomace extract, SEM: standard error of mean T: treatments; P: period; T x P: treatment and period interaction.
^{abcd}Within row, different superscripts indicate differences between time in retail display ($P \leq 0.05$).
^{wxyz}Within row, different superscripts indicate differences between treatments ($P \leq 0.05$).
[1] Expressed as logarithms of colony forming units per gram of beef patties.

Table 6
Effect of extracts on cooking yield, textural and sensory quality of cooked beef patties.

Attribute	Treatment					SEM[1]	*P* value
	CTR	SMB	GSE	GPE	OPE		
Cooking yield (%)	81.2	80.4	81.7	80.9	81.6	0.58	0.514
Texture profile							
Hardness (N)	65.8	70.1	69.2	66.4	69.4	2.21	0.522
Cohesiveness	1.35^{a}	130^{ab}	1.32^{ab}	1.33^{ab}	1.32^{ab}	0.01	0.033
Springiness (mm)	5.97	5.4	5.51	5.35	5.11	0.28	0.283
Gumminess (N)	86.6	91.7	91.2	88.1	91.4	3.03	0.675
Chewiness (N × mm)	534	497	504	470	470	28.9	0.495
Aroma attributes							
Overall intensity	54.2^{b}	53.7^{b}	55.3^{ab}	55.4^{ab}	58.1^{a}	0.58	0.003
Beef-like	53.1^{b}	52.3^{b}	53.8^{ab}	54.6^{ab}	56.9^{a}	0.58	0.002
Savoury	24.2	25.2	25.2	25.5	26.3	0.58	0.226
Sweet associated	22.7	22.6	22.5	22.4	22.8	0.58	0.978
Fatty	23.2	23.7	23.2	23.5	23.8	0.58	0.926
Flavour attributes							
Beef-like	54.3^{b}	53.9^{b}	55.4^{ab}	56.4^{ab}	57.6^{a}	0.58	0.008
Savoury	27.3	27.1	28.1	28.4	28.3	0.58	0.375
Salty taste	25.7	25.8	25.1	25.9	25.6	0.58	0.832
Sweet associated	20.9	21.4	21.8	20.9	21.8	0.65	0.747
Fatty	24.1	22.3	23.7	23.1	24.3	0.58	0.161
Peppery	3.3	2.9	3.1	3.8	3.5	0.58	0.862
Texture attributes							
Overall juiciness	48.9	48.8	49.9	48.4	50.1	0.57	0.224
Residue	22.6	21.9	21.9	23.6	21.7	0.58	0.201
Fatty coating	24.9	24.7	24.7	24.9	24.8	0.58	0.993

GSE: grape seed extract, GPE: grape pomace extract; OPE: clementine mandarin orange pomace extract; SMB: sodium metabisulphite; SEM: standard error of mean.
^{ab}Least square means with different superscripts in the same row are significantly different ($P \leq 0.05$).
[1] SEM: standard error mean.

effects, the observed low pH of the extracts could have influenced the final pH of beef patties during retail display period. Overall, all pH values ranged between 5.5 and 5.8, which agree with other researchers (Bekhit, Geesink, Ilian, Morton, & Bickerstaffe, 2003; Hayes, Stepanyan, Allen, O'Grady, & Kerry, 2010) who incorporated phytochemical-rich preservatives in beef patties.

The finding that SMB-treated patties had enhanced antioxidant activity compared to other treatments could be due to the presence of sulphites in SMB, which act as oxygen scavengers and redox-active metal chelators (Feiner, 2006). Moreover, sulphites have been found to bind precursors involved in oxidative reactions preventing them from reacting with oxygen or by binding with compounds already oxidised to reverse oxygen effect (D'Amore et al., 2020; Feiner, 2006). Despite their efficacy as preservatives in ground meat, sulphites (e.g., sodium metabisulphite) have been reported to cause asthma and other symptoms of allergic responses such as skin rashes and irritations (D'Amore et al., 2020; Feiner, 2006; Vally & Misso, 2012). Furthermore, ingestion of sulphites has been positively correlated to adverse and toxic reactions, including vitamin deficiency and allergic diseases, and could result in dysbiotic events of the gut and oral microbiota (D'Amore et al., 2020; Irwin, Fisher, Graham, Malek, & Robidoux, 2017).

The greater antioxidant activity observed for beef patties from GSE as compared to GPE and OPE agrees with the in vitro antioxidant assays reported by Pfukwa et al. (2019). This could be mainly attributed to the greater contents of flavanols and proanthocyanidins in GSE than GPE and OPE, which exhibit great antioxidant potency (Bañón, Díaz, Rodríguez, Garrido, & Price, 2007; Pfukwa et al., 2019). Uchida et al. (1987) reported that proanthocyanidins have an antioxidant potency which is twenty times greater than α-tocopherol and fifty times greater than ascorbic acid. This antioxidant potency of flavanols and proanthocyanidins is ascribed to the existence of a catechol group on the B-ring that entraps free radicals and chelate transition metals (Shi, Yu, Pohorly, & Kakuda, 2003; Unusan, 2020). Moreover, flavanols and proanthocyanidins act as pro-oxidant enzyme inhibitors (Rauf et al., 2019; Unusan, 2020; Zuiter, 2014).

The observation that GPE and OPE patties had greater antioxidant activity relative to CTR patties could be due to the presence of flavonoids (i.e., flavones, flavanones and flavonols), phenolic acids (i.e., hydroxybenzoic acid and hydroxycinnamic acids) and ascorbic acid, which are known to increase antioxidant activity by scavenging the reactive oxygen species, chelating metal ions, reducing peroxide formation and sulphur radicals (Dasgupta, Solorzano, & Tong, 2010; Manessis et al., 2020; Nakao et al., 2011). The finding that the antioxidant capacity of fruit by-product extracts in beef patties decreased during the retail display time is supported by the fact that antioxidant potency of sulphur atoms and bioactive phytochemicals (i.e., proanthocyanidins,

flavonoids, ascorbic acid, limonoids) degrade/oxidise over time, leading to a reduction in their efficacy when applied to beef patties (Manessis et al., 2020; Nakao et al., 2011).

The lack of differences among treatments for beef patty L* is consistent with findings of Bañón et al. (2007), Bouarab-Chibane et al. (2017) and Reihani, Tan, Huda, and Easa (2014), who applied polyphenolic fruit extracts to beef patties. The observed trend that SMB and extracts performed better than CTR in terms of a*, b*, and C* values could be due to reduced conversion of oxymyoglobin to metmyoglobin by the antioxidant effects of sulphites and bioactive phytochemicals (Bouarab-Chibane et al., 2017; D'Amore et al., 2020; Faustman & Suman, 2017). The L* and a* values observed in the present study were within the threshold values of $\leq$46.3 and $\geq$14.5, respectively, which are deemed acceptable thresholds by consumers of fresh beef patties (Cooper, Suman, Wiegand, Schumacher, & Lorenzen, 2018; Holman, van de Ven, Mao, Coombs, & Hopkins, 2017). Lower H* values observed for beef patties treated with either commercial preservative (SMB) or citrus and winery by-product extracts compared to CTR could also be attributed to the protective effects of sulphites, flavonoids, ascorbic acid and limonoids (Bouarab-Chibane et al., 2017; Luciano et al., 2011).

The observed gradual decline in colour parameters (i.e., a*, b* and chroma) over retail display period could be attributed to oxidation of deoxymyoglobin and/or oxymyoglobin to metmyoglobin (Falowo, Fayemi, & Muchenje, 2014) owing to the degradation of the antioxidants. The gradual increase in H* values over time in retail display could be probably due to oxidative processes which subsequently results in production of Schiff pigments like lipofuschin from lipid and protein complexes (Bañón et al., 2007; Chelh, Gatellier, & Santé-Lhoutellier, 2007).

The observed increase in TBARS and carbonyls content in CTR patties could be due to the production of more MDA and carbonyls which are deemed as secondary products of lipid and protein oxidation (Bouarab-Chibane et al., 2017; Horbańczuk, Kurek, Atanasov, Brnčić, & Brnčić, 2019). The finding that the SMB and GSE had lower TBARS, and carbonyls contents compared to other extracts was anticipated and could be attributed to protective antioxidant effects of the commercial preservative and fruit extracts as discussed earlier. The increase in TBARS and carbonyls contents throughout the time in retail display could be because of the exposure to pro-oxidants in beef patties over time (Horbańczuk et al., 2019). It is worth pointing out that after 9 days of retail display, TBARS values of CTR beef patties surpassed 2 mg MDA/kg of beef patty recommended as a threshold for sensory detection of rancid flavours (Bouarab-Chibane et al., 2017; Campo et al., 2006; Horbańczuk et al., 2019).

The greatest antibacterial activity of the SMB compared to other treatments confirmed that they are more effective in limiting the bacterial growth as reported by Pfukwa et al. (2019). Sodium metabisulphite contains sulphites (i.e., sulphur dioxide), which act as bacteriostatic or bactericidal through disruption of bacterial cell wall (D'Amore et al., 2020; Irwin et al., 2017). The observation that patties from OPE had lower TVC, TCC and LAB counts compared to GSE and GPE could be due to antibacterial potential and chelating ability related to the presence of ascorbic acid, flavones, flavanones, terpenoids and limonoids in the former extract OPE (Zou, Xi, Hu, Nie, & Zhou, 2016). However, the mechanism of the antibacterial action of OPE bioactive phytochemicals has not yet been fully elucidated. Flavanones, flavones and limonoids have, however, been postulated to disrupt bacterial membranes, thereby reducing their viability, chelate metal ions required for growth of bacteria, and/or act as an inhibitor against enzyme deoxyribonucleic acid (DNA) gyrase. The latter is dependent on adenosine triphosphate required for its transcription, replication of DNA and chromosome segregation processes in bacteria (Apak, Özyürek, Güçlü, & Çapanoŋlu, 2016;Khan et al., 2018 ; Papuc et al., 2017). Ascorbic acid has been found to have inhibitory effects on growth of bacteria (Helgadóttir et al., 2017; Majtan, Sojka, Palenikova, Bucekova, & Majtan, 2020; Przekwas, Wiktorczyk, Budzyńska, Wałecka-Zacharska, & Gospodarek-Komkowska, 2020). Its suggested mechanisms of action include not only anti-quorum sensing activity (Majtan et al., 2020; Przekwas et al., 2020) and inhibition of extracellular polymeric substances production (Helgadóttir et al., 2017; Majtan et al., 2020), but also its ability to reduce the pH in the environment, providing unconducive conditions for the survival of the bacteria (Przekwas et al., 2020). The observation that GSE and GPE had lower bacterial counts than CTR patties could be due to the presence of phenols, flavanols and proanthocyanidins in GSE and GPE which act as bactericidal through disruption of bacterial cell wall (Khan et al., 2018; Papuc et al., 2017). Increase in LAB, TCC and TVC during retail display concurs with previous studies that reported increases in counts for the bacteria on beef patties (Kılıç, Şimşek, Claus, Karaca, & Bilecen, 2018; Sagdic, Ozturk, Yilmaz, & Yetim, 2011). This may be due to the reduced antioxidant and antibacterial activity of extracts as explained earlier.

The observation that citrus and winery by-products extracts did not affect texture profile parameters of beef patties except for cohesiveness, is in accordance with previous studies which formulated patties with polyphenolic extracts from fruit by-products (Bouarab-Chibane et al., 2017; Youssef & Barbut, 2011). The finding that beef patties containing positive control, citrus and winery by-product extracts had lower cohesiveness values compared to CTR patties suggests that the sulphites and bioactive phytochemicals may be related to low weight loss during cooking, leading to low protein concentration and the development of a less cohesive protein matrix (Pogorzelska-Nowicka et al., 2018; Youssef & Barbut, 2011). Nevertheless, their mechanism of action is still unclear and warrants further investigation.

The findings that OPE patties had improved overall aroma intensity, beef-like aroma and flavour compared to other treatments were consistent with previous studies (Horbańczuk et al., 2019; Nissen, Byrne, Bertelsen, & Skibsted, 2004), which preserved patties with plant extracts rich in flavonoids and ascorbic acid. These results could be linked to synergistic action of ascorbic acid and flavonoids found in OPE, which have been reported to maintain flavour and aroma of meat (Lahučky, Bahelka, Novotná, & Vašičková, 2005; Mitsumoto, O'Grady, Kerry, & Buckley, 2005). It is, however, postulated that flavanones, flavones and ascorbic acid in the OPE could have resulted in the formation of aliphatic hydrocarbons and heterocyclic compounds, especially those containing sulphur which are important flavour and aroma compounds produced in the Maillard reaction during cooking of patties (Van, Hwang, Jeong, & Touseef, 2012). However, the mechanisms of action of ascorbic acid and flavonoids on flavour and aroma attributes are not clearly known and merit investigation.

Based on the current findings, OPE and GSE could be considered for application in meat matrix as natural and sustainable antioxidants and antibacterials in that order. However, purification of OPE and GSE is recommended for future studies as it enhances bioefficacy in the meat matrix by increasing the adsorption and desorption of bioactive phytochemicals during extraction (Soto, Moure, Domínguez, & Parajó, 2011; Yangui & Abderrabba, 2018). Furthermore, determining the optimum concentration of the purified GSE and OPE required to enhance oxidative and microbial stability of beef patties without negatively affecting safety and eating quality is recommended. The purified GSE and OPE could be added in combination to provide either additive or synergistic effects in enhancing shelf-life of beef products and consequently reducing losses along the supply chain. To this end, determination of the costs of producing pure GSE and OPE is important for their uptake by the meat industry.

5. Conclusions

Grape seed extract was more effective than GPE and OPE in retarding lipid and protein oxidation. The OPE was the most potent antibacterial extract for inhibiting bacterial growth, and enhancing overall aroma intensity, beef-like aroma and flavour of beef patties. Therefore, GSE and OPE can be used as natural and sustainable preservatives to increase

resistance of biomolecules to oxidative processes and bacterial spoilage of beef patties. Follow-up studies are warranted to determine the optimum concentrations of the purified GSE and OPE required to extend shelf-life of beef patties without compromising their safety, sensory quality and profitability.

Author contribution

Conceptualisation, Funding acquisition, Project management, Supervision, Editing - C.M.; Writing original draft, Investigation - T.B.; Supervision, Editing – O.C.C.; Sensory evaluation, Editing - J.M; Bacterial evaluation, Editing - P.A.G.; Editing – T.T., M.M. and O.A.F. All authors have read and agreed to the published version of the manuscript.

Conflict of interests

The authors declare no conflict of interest.

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