Research article

Warmed-over flavour profiles, microbial changes, shelf-life and check-all-that-apply sensory analysis of cooked minced pork treated with varying levels of *Moringa oleifera* leaf and root powder

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A B S T R A C T

This study investigated warmed-over flavour profiles, microbial changes, shelf-life and sensory characteristics of minced cooked pork treated with *Moringa oleifera (M. oleifera)* root and leaf powder during refrigerated storage at 4 °C. A total of 8 treatments (control = no antioxidant; 0.5ML = 0.5% *M. oleifera* leaf; 1ML = 1% *M. oleifera* leaf; 0.5MR = 0.5 % *M. oleifera* root; 1MR = 1% *M. oleifera* root; 0.5MLR = 0.5% *M. oleifera* leaf and root mixed; 1MLR = 1% *M. oleifera* leaf and root mixed; BHT = 0.02% butylated hydroxytoluene) were evaluated. The minimum inhibitory concentration (MIC) of the plant extracts against the test bacteria was determined using the serial dilution in 96 well microtiter plates technique. Warmed-over flavour profiles were determined using the test for carbonyls assay where hexanal was used as a marker for warmed-over flavour. The check-all-that-apply sensory tool was used to characterise minced cooked pork treated with different antioxidants according to warmed-over flavour taste and odour intensities. The results showed that the antibacterial assay of the extracts exhibited a broad-spectrum of activity against the tested bacteria. The leaf extracts demonstrated better activity against both gram-negative and gram-positive bacteria, with most of the MICs at less than 1 mg/mL, while the root performed better against gram-negative bacteria compared to gram-positive bacteria. There was a significant rapid increase in the warmed-over flavour profiles of the control compared to the *M. oleifera* and BHT treated pork. The pork samples which had *M. oleifera* leaf, root, and their combination at inclusion levels of 1% and 0.5% displayed lower warmed-over flavour profiles that fell in the range (1.0–1.46 mg hexanal/100g fat) throughout the storage period. Consumer sensory evaluation revealed that pork samples treated with the highest inclusion level (1%) of the *M. oleifera* leaf powder received the lowest consumer rating scores for appearance. Based on these results, adding *M. oleifera* leaf and root powder can decrease warmed-over flavour development and improve the shelf-life of processed pork. Furthermore, the incorporation of *M. oleifera* root powder can potentially be more acceptable to consumers because of its colour compared to the leaf, which gives the product a green colour that may not be pleasant for some consumers. This suggests that the inclusion of the root powder at 1% may be well accepted for consumption by consumers.

1. Introduction

Warmed-over flavour is a product of lipid oxidation, which decreases the shelf-life and acceptance of processed or cooked pork and pork products. Meat that has developed warmed-over flavour characteristically possess undesirable odour, deteriorated nutritional value, flavour, texture, colour and potentially exudes toxic compounds (Skowyra et al., 2015). These unpleasant characteristics often lead to food rejection by consumers and results in economic losses (Iulietto et al., 2015). In recent years, increased awareness of health risks and benefits associated with food has birthed a situation where consumers are progressively dictating the products offered in the market (Bedale et al., 2016; Hung and Verbeke 2019).

Consumers are currently demanding more of ready-to-eat convenience foods which are produced using natural compounds but still possess exciting sensory and textural properties (Brennan et al., 2013;

Lungu et al., 2020). Due to these increasing consumer expectations concerning quality, convenience, safety, and extended shelf-life (Djenane and Roncalés 2018), natural antioxidants are currently being explored as potentially safe and cost-effective enhancers of muscle foods' quality and shelf-life.

Natural antioxidants mainly consist of plant phenolics that can be derived in all parts of plants including seeds, roots, leaves and barks (Embuscado, 2015). The antioxidant activities of these phenolic compounds are mainly attributed to their multifunctional ability to act as reducing agents and free radical terminators (Batista et al., 2016; Barku 2019). This means that plant phenolics can eliminate the accumulation of reactive oxygen species (ROS) like peroxyl radical and hydroxyl radical during lipid oxidation (Nimse and Pal 2015). In this way, plant phenolics can also protect the human body from the carcinogenic and toxic health effects that are supposedly associated with the presence of (ROS).

To successfully use plant-based antioxidants in meat and meat product development, there is a need to characterize and predict how the product formulated using these kinds of antioxidants will be perceived in the market. Consumer acceptance of meat and meat products is highly influenced by factors associated with the appearance, texture, flavour, and taste (Xazela et al., 2017). A practical approach in understanding how consumers would perceive pork products treated with varying levels of plant-based antioxidants is critical for the development, promotion of new products and the reformulation of existing ones. Moreover, how consumer expectations are met influences their loyalty and purchasing decisions of various foods (Font-i-Furnols and Guerrero 2014; Baba et al., 2016). There is, however, still a gap in research that focuses on how consumers perceive pork products treated with different plant-derived antioxidants. Also, to back up perceptions of consumers, chemical analysis of warmed-over flavour in cooked pork is of utmost importance to quantify the efficacy of these plant-derived antioxidants in impeding warmed-over flavour development. This also helps to gain an insight into the sensory and chemical analysis differences and inter-relationships (Byrne et al., 2001).

Expressing secondary products of lipid oxidation as warmed-over flavour profiles, by measuring the hexanal content, has been the primary tool for the chemical evaluation of warmed-over flavour development in meat under refrigerated storage (Jayathilakan et al., 2007).

This study seeks to determine the acceptable *M. oleifera* leaf and root powder inclusion levels which can potentially improve the shelf-life, reduce the development of warmed-over flavors, and maintain consumer acceptance of cooked pork during refrigerated storage. Studies have shown that *Moringa oleifera* possesses antimicrobial and antioxidant properties due to its inherent bioactive compounds. In addition, *M. oleifera* leaves contain proteins, minerals and all the essential amino acids. Vitamins A, B, beta-carotene, pyridoxine, nicotinic acid, C, D, and E are also present in *M. oleifera* (Thapa et al., 2019). All these antioxidant and nutritional properties of *M. oleifera* qualify it to potentially play a double role of improving the shelf-life at the same time presenting healthy functional ready-to-eat meat products to consumers. The inclusion of *M. oleifera* leaf meal has been reported to improve shelf-life stability in ground beef (Falowo et al., 2017; Mashau et al., 2021) and pork patties (Muthukumar et al., 2014). This has been attributed to the presence of polyphenolic compounds. While extensive research has been done on the use of *M. oleifera* leaf powder, there is still little or no information on the effect of *M. oleifera* root powder on warmed-over flavour profiles and sensory characteristics of minced pork. The present study uses *M. oleifera* in its powder form because it is a cheaper, more natural, and practical form compared to extracted forms which involve expensive and time-consuming extraction procedures.

2. Materials and methods

2.1. Harvesting of Moringa oleifera and collection of meat samples

Moringa oleifera and meat samples were collected and processed as described by Lungu et al. (2021). In summary, *Moringa oleifera* leaves and roots (voucher number MAP/004/2019) were harvested from the Lefakong *Moringa* farm in Boosplas, North West Province, South Africa. Prior to being air-dried in well-ventilated conditions at room temperature without direct sunlight exposure, dust was removed from the freshly harvested *M. oleifera* leaves and roots. The drying was done without direct sunlight exposure to preserve vital phytoconstituents. Thereafter, the plant samples were ground into powder (which can pass through a 2 mm sieve) and packed in tightly sealed storage containers at room temperature (20 °C) up to the point of need for analyses. Fresh *Muscularis longissimus thoracis et lumborum,* LTL samples were collected at 24 h *post-mortem* from the East London commercial abattoir in Eastern Cape, South Africa. East London abattoir complies with the Meat Safety Act (Act No. 40 of 2000) and adheres to standard animal handling procedures. An ethical clearance was sought from the University of Fort Hare Animal Research Ethics Committee (AREC) to collect meat samples from the abattoir. The study was granted ethical clearance certificate number: AFO031SLUN01/19/A.

2.1.1. Chemicals and reagents

This study used chemicals of analytical grade. The synthetic antioxidant BHT was obtained from Sigma Aldrich chemicals, Gauteng South Africa. Other chemicals including Benzene, Sodium Hydroxide pellets, Tricloracetic acid (TCA), Ethanol, 1,2-Dinitrophernyl hydrazine and Hexane were also obtained from Sigma Aldrich chemicals, Gauteng, South Africa.

2.1.2. Meat sample preparation

Meat samples were prepared using the method described by Lungu et al. (2021). An electric meat mincer (Trespade 22 EL Plus, Torino, Italy) was used to mince pork samples. Before the inclusion of *M. oleifera* and synthetic antioxidant, the minced pork samples were separated for each experiment. Thereafter, pork samples were randomly allocated to one of these treatment groups: control with no additives; 0.5% *M. oleifera* leaf powder/0.5ML; 1% *M. oleifera* leaf powder/1ML; 0.5% *M. oleifera* root powder/0.5MR; 1% *M. oleifera* root powder/1MR; 0.5% mixed *M. oleifera* leaf and root powder/0.5MLR; 1% mixed *M. oleifera* leaf and root powder/1MLR; 0.02% Butylated hydroxytoluene (BHT). For each treatment, the mixing process was replicated 4 times. The different treatment meat samples were packed into polypropylene bags and cooked for 45 min under atmospheric pressure in a water bath (Pura Julabo GmbH, Seelbach, Germany) at 78 °C. The pork samples were evaluated on days 0, 2, 4, 6 and 8. Samples for day zero were immediately cooled and evaluated while samples for evaluation on day 2–8 were kept under refrigerated storage at 4 °C. On their respective days of analysis, these samples were first re-heated to reach a core temperature of 70 °C and then allowed to cool down at ambient temperature for 5 min prior to evaluations. To reduce carry-over and taste adaptation on sensory judgements, panelists were instructed to refrain from consuming food 1 h before conducting oral evaluations. Samples from different treatments were marked with different codes. To cleanse the palate in between evaluating different samples the panelists were given distilled water.

2.1.3. Selection of panelists

Permission to conduct the study was granted by the University of Fort Hare Research Ethics committee. A 12-member semi-trained consumer panel made up of four males and eight females aged between 21 to 50 years from the University of Fort Hare meat science research group, Alice, South Africa was used in this study. The panel was selected from a group of individuals who eat pork and had knowledge on meat science. Some of the participants had previous experience as panelists in sensory evaluation. The selection was based on willingness and availability to participate in the sensory evaluation on every day required. All participants signed an informed consent form prior to participating in the study. The informed consent clearly outlined the purpose of the research and explicitly stated that participation was on free-will basis and that participants could withdraw at any given point without being questioned or given a penalty.

The attributes appearance, odour and taste were assessed using a 9-point hedonic scale ranging from dislike extremely to like extremely as described by (Maqsood et al., 2016). The panelists were also asked to indicate their overall liking for each sample on a check-all-that-apply evaluation sheet. The terms that were used to describe the warmed-over flavour in the pork were adopted from Byrne et al. (1999).

2.2. Chemical analysis

2.2.1. Total carbonyls assay

Warmed-over flavour profile presented as mg of n-hexanal per 100g fat was monitored by the method described by Reddy et al. (2014). Briefly, 5g of minced cooked meat samples were extracted in 50 ml of carbonyl free benzene. 5ml of the resultant sample filtrate was then mixed with 3 ml of 3–4% trichloracetic acid (in benzene) and 5 ml of 0.05% DNPH solution (in benzene). The solution was incubated at 60 °C for half an hour. After cooling for about 10 min, 10 ml of 4% alcoholic potassium hydroxide was added and volume was topped up to 50 ml with ethanol. The solution was left to stand for 10 min, thereafter, absorbance was read at 480 nm. A standard curve was drawn using hexanal (50–250 mg) in 5 ml benzene. Warmed-over profiles, in terms of hexanal, were calculated with the aid of the standard curve.

2.3. Antimicrobial assay

2.3.1. The rationale for micro-organism selection

The selection of bacteria for this study was informed by their history as opportunistic pathogens in humans and animals and their link with the spoilage of food products.

2.3.2. Microorganism strains

Bacterial isolates used in this study were reference strains obtained from the Microbiology Laboratory, University of Fort hare, Alice, South Africa. The study used eight bacterial strains (four gram-positive and four gram-negative). The gram-positive strains were *Bacillus pumilus* (ATCC 14884), *Bacillus Subtilis* (laboratory isolate), *Staphylococcus aureus* (ATCC 6538) and *Staphylococcus epidermis* (laboratory isolate), while the four gram-negative strains included *Escherechia coli* (ATCC 8739), *Proteus vulgaris* (ATCC 6830), *Shigella flexneri* (laboratory isolate) and *Klebsiella pneumoniae* (ATCC 25922).

2.3.3. Antimicrobial microdilution assay

The serial dilution in 96 well microtiter plates technique described by Eloff (1998) was used to determine the minimum inhibitory concerntration (MIC) of the plant extracts against the test bacteria. In summary, overnight cultures of the tested bacteria were produced by inoculating 100 μl of the bacteria in 10 mL MHB (Mueller-Hinton Broth) medium and incubating at 37 °C for 24 h. The next day, overnight subculture stock was diluted with (MHB) medium at a concentration of 1.100 (200 μL bacteria: 19.8 mL MHB). This was done to ensure that the bacteria were at the start of the log phase when the experiment commenced. The plant extracts were then dissolved in 50 mg/mL of their corresponding extracting solvents. 100 μL of sterile distilled water was then pipetted into the wells. Then, 100 μL of the plant extract was only added to row A of the wells. This was mixed using a pipette and followed by serial two-fold dilutions. 100 μL of the diluted bacteria was then added to the wells and covered. The microplates were then incubated overnight at 37 °C. Minimum inhibitory concentrations values were recorded thereafter. Neomycin, which is a standard antibiotic, was used as the control.

2.4. Statistical analysis

Warmed-over profiles data obtained were subjected to analysis of variance (ANOVA) using Tukey's test to evaluate the statistical significance of the treatment means and significance was established at ($p < 0.05$). This was done using the Minitab statistical analysis tool. The sensory data was processed using the XLSTAT version 2018.5 statistical software CATA and shelf-life tool. Regression analysis was performed to establish the relationship between the warmed-over profiles and shelf-life sensory data.
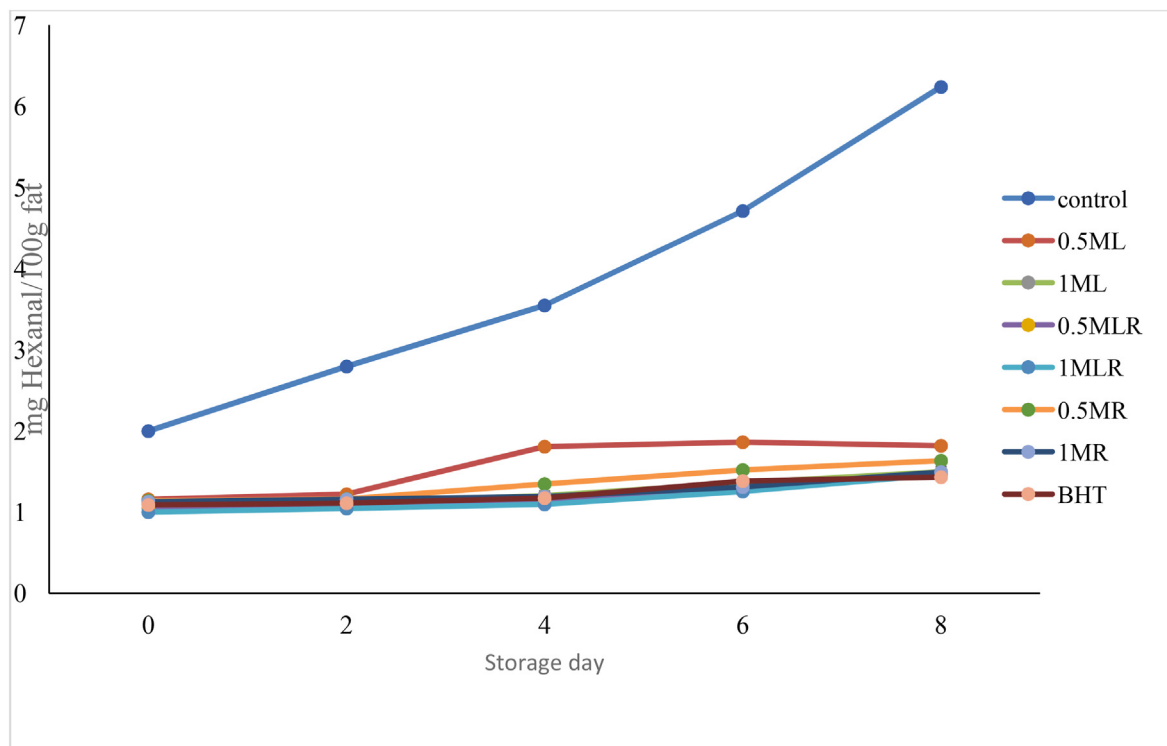

Figure 1. Warmed-over flavour profiles (mg of hexanal/100 g fat) of cooked minced pork in the treated with synthetic (BHT) and natural antioxidants (*M.oleifera* leaf and root) during storage at 4 °C, Control = no antioxidants, 0.5ML = 0.5% leaf, 1ML = 1% leaf, 0.5MLR = 0.5% leaf and root, 1MLR = 1% leaf and root, 0.5MR = 0.5% root, 1MR = 1% root, BHT = 0.02% Butylated hydroxytoluene, ($p < 0.05$).

Table 1. Antimicrobial activities (Minimum Inhibitory Concentration – MIC mg/mL) of *M. oleifera* leaf and root extracts on selected bacteria as determined by the microdilution method.

Plant part	Micro-organism (MIC mg/mL)								
	Gram-negative Gram-positive								
	Solvent	KP	SF	PV	EC	BS	SA	SE	BP
Leaf	Acetone	**0.19**	1.56	**0.19**	**0.78**	**0.098**	˙0.098	**0.12**	**078**
	Water	**0.78**	3.12	1.56	0.78	1.56	**0.39**	**0.78**	**0.78**
	Ethanol	**0.098**	**0.39**	**0.098**	**0.098**	**0.195**	˙0.098	˙0.098	**0.78**
Roots	Acetone	3.12	**0.78**	**0.39**	**0.78**	1.56	1.56	3.12	6.25
	Water	**0.78**	**0.78**	**0.39**	**0.78**	6.25	6.65	3.12	6.25
	Ethanol	3.12	6.25	6.25	3.12	6.25	6.25	3.12	3.12
Neomycin		**0.195**	**0.78**	˙0.098	˙0.098	1.56	**0.195**	˙0.098	˙0.098

Kp- *Klebsiella pneumoniae*, SF- *Shigella flexneri*, PV- *Proteus vulgaris*, EC- *Escherchia Coli*, BS- *Bacillus subtilis*, SA- *Staphylococcus aureus*, SE- *Staphylococcus* epidermis, BP- *Bacillus pumilus*. Values in bold are considered to be active (MIC < 1 mg/mL).
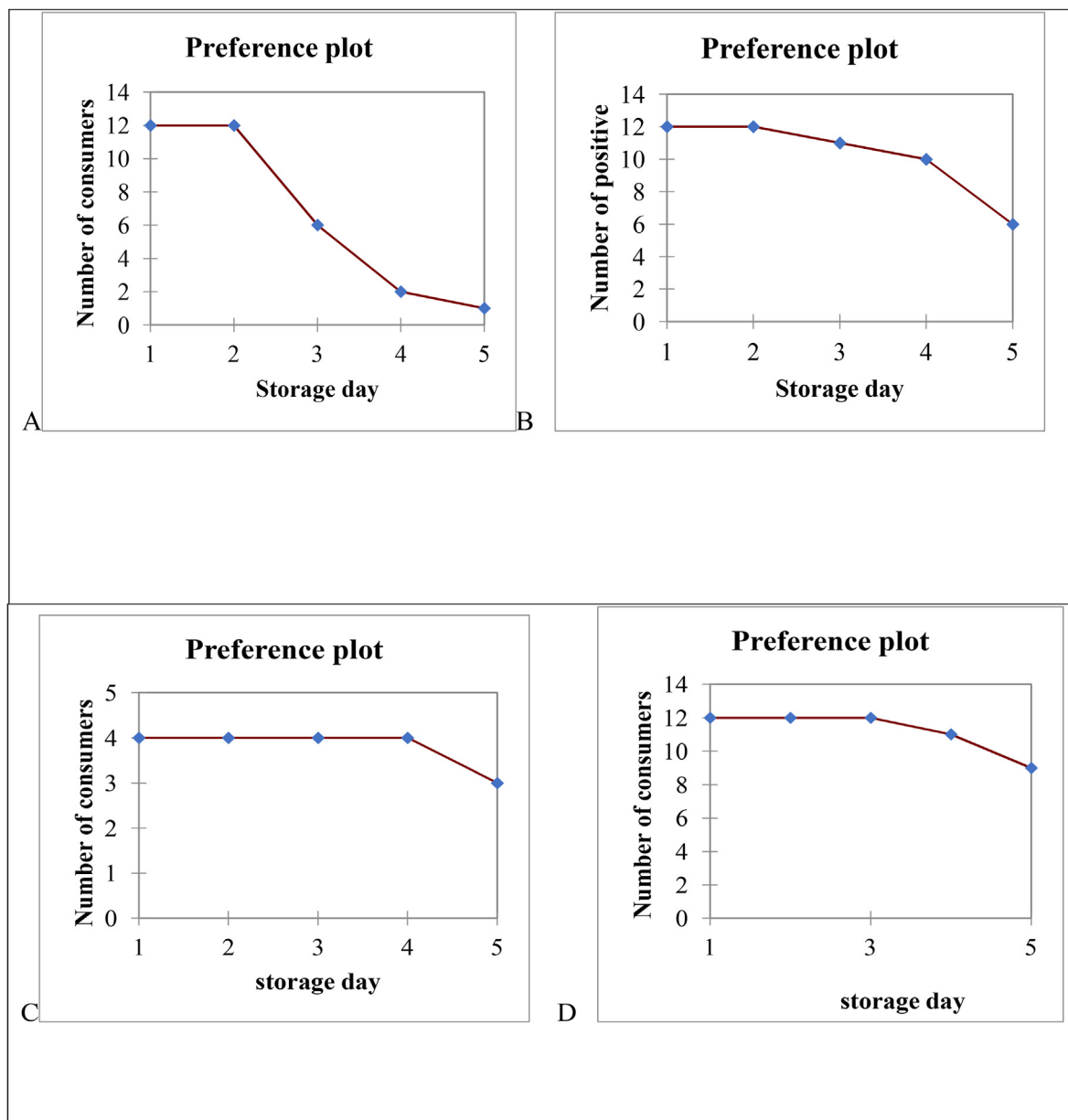

Figure 2. Consumer preference of cooked minced pork treated with synthetic (BHT) and natural antioxidants (*M. oleifera* leaves and roots) during storage at 4 °C (A) = pork with no antioxidants (B) = pork treated with 0.5% *M. oleifera* leaf powder (C) = pork treated with 1% *M. oleifera* leaf powder and (D) = pork treated 0.5% *M. oleifera* root powder.
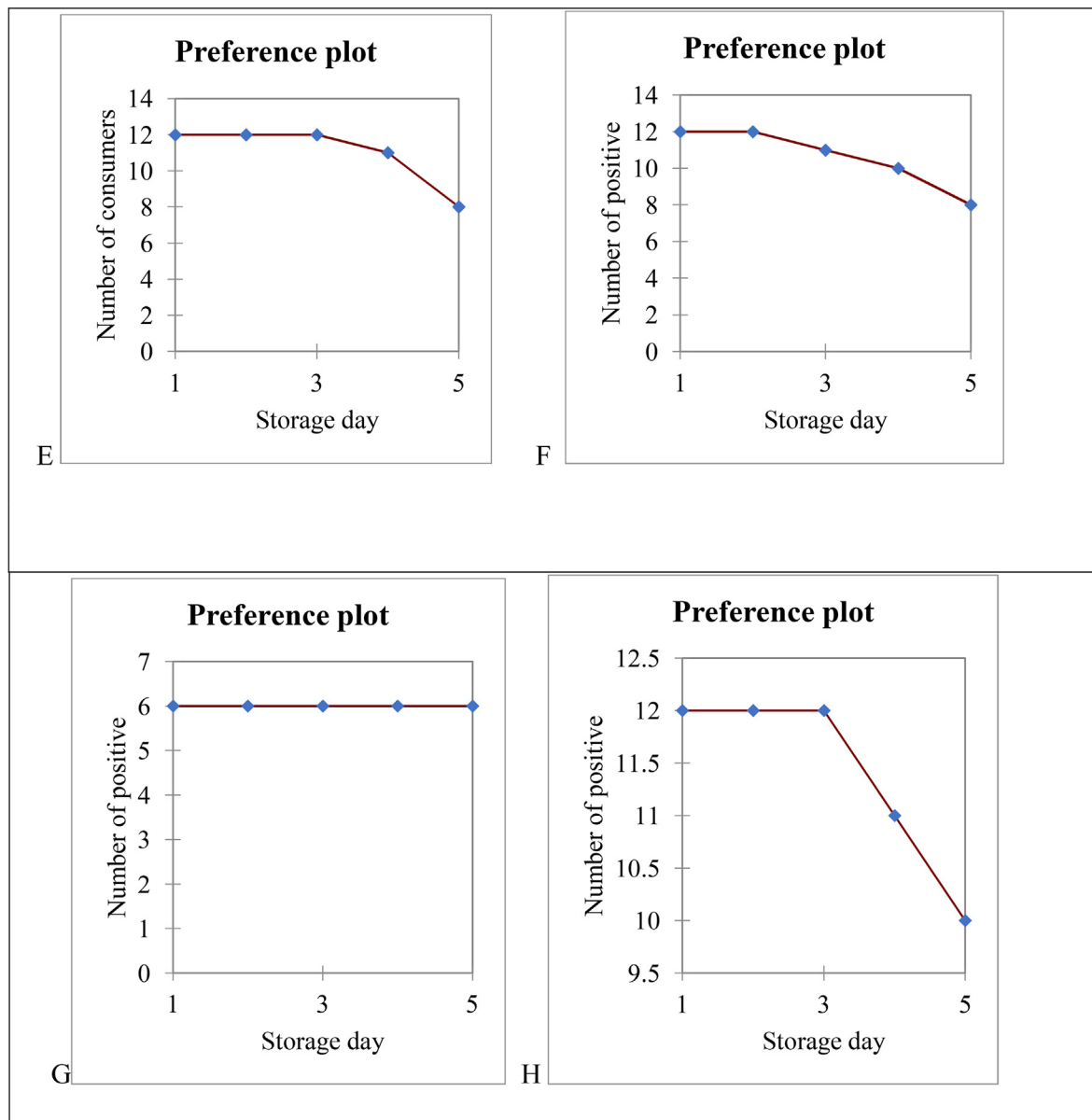
Figure 3. Consumer preference of cooked minced pork treated with synthetic (BHT) and natural antioxidants (*M. oleifera* leaves and roots) during storage at 4 °C (E) = pork with 1% *M. oleifera* root powder, (F) = pork treated with 0.5% *M. oleifera* leaf and root powder (G) = pork treated with 1% *M. oleifera* leaf and root powder and (H) = pork treated with BHT.

2.5. Results

2.5.1. Warmed-over profiles of minced cooked pork treated with BHT and varying levels of M. oleifera leaf and root during storage at 4 °C

Warmed-over flavour profiles expressed as mg n-hexane/100g fat of minced cooked pork treated with natural and synthetic antioxidants during storage 4 °C are displayed (Figure 1). From the results, it was observed that the warmed-over profiles in the control group increased rapidly compared to other treatment groups. The root and leaf treatment groups (0.5 and 1% inclusion) exhibited lower warmed-over flavour profiles that fell in the range (1.0–1.46 mg hexanal/100g fat) throughout the storage period. On day zero, pork samples containing antioxidants had significantly different (p < 0.05) warmed-over flavour profiles from the control. On storage days two and four, the pork treated with antioxidants had varied significant differences (p < 0.05) among each other. On day 8, the control had the highest warmed-over profile of 6.23 ± 0.12 mg hexanal/100g fat while the samples treated with antioxidants decreased in this manner: 0.5ML > 1ML > 0.5MLR > 1MLR >0.5MR > 1R > BHT.

2.5.2. Antimicrobial activity

Table 1 shows the results of the antimicrobial activity of different solvent extracts of the *M. oleifera* leaves and roots. The results from this study reveal that the leaf extract exhibited a broad-spectrum of antibacterial activity against all the test bacteria, with most of the minimum inhibitory concentrations ranging at ‹ 1 mg/mL and this was seen mainly on the ethanol extracts. The root also showed good activity against the gram-negative bacteria when compared to its activity against the gram-positive bacteria. Remarkably, the aqueous extract of the root demonstrated better activity against the gram-positive bacteria while the acetone extract had better activity on the gram-positive bacteria. Overall, the leaf extracts exhibited better antibacterial activity on the test bacteria strains when compared to the root extracts.

2.5.3. Consumer overall liking or preference of the minced cooked pork samples treated with natural and synthetic antioxidants during storage at 4 °C

Shelf-life was determined by the number of consumers interested in the product. The shelf-life results of the non-antioxidant and antioxidant

treated minced cooked pork samples are shown (Figures 2 and 3). The results indicate that on day zero all consumers, preferred the minced cooked pork in all treatment groups except the 1ML and 1MLR treatment groups which were preferred by 33% and 50% of the consumers respectively. In addition, the results revealed that as from day four there was a sharp decrease in consumer preference for pork with no antioxidants. On day eight (8%) of the consumers liked the antioxidant-free pork while on an average (58%) of the consumers liked the antioxidant treated meat. Overall, the results showed that consumer preference of the meat decreased with increase in storage days.

2.5.4. *Relationship between warmed-over profiles and shelf-life of minced cooked pork*

The relationship between the warmed-over profiles and liking of pork samples is shown (Figure 4). The findings revealed an inverse association between warmed-over profiles and shelf-life, meaning that as warmed-over profiles increased shelf-life decreased.

2.6. *Check-all-that-apply of warmed-over flavor in samples treated with natural and synthetic antioxidants*

A check-all-that apply analysis was conducted to differentiate the intensities of warmed-over flavour and warmed-over taste among pork samples with and without antioxidants (Figure 5). The pork samples with no antioxidants and the one treated with 0.5 ML were described as high in warmed-over flavour taste and odour, while the BHT and 1MLR treated pork samples were categorized as low in warmed-over odour and taste. The rest of the treatment groups fell under medium warmed over-odour and taste.

2.7. *Sensory rating of appearance, color, and taste during storage*

The results of the consumer rating for attributes colour, taste and aroma on a scale (1–9) are shown in Table 2. Appearance had no significant differences ($p < 0.05$) on day zero across all treatment groups except for the 1ML treated pork which had a lower mean rating. The rating scores showed a decreasing trend as storage days increased.

3. Discussion

Oxidative degradation of cooked meat under refrigerated storage yields warmed-over flavour. This unpleasant odour is attributed to the compounds like ketones, alcohols and aldehydes that are produced from secondary lipid oxidation (Kim et al., 2016; Addeen et al., 2017). Aldehydes are significant decomposition by-products, and hexanal has been suggested as a suitable indicator for identifying WOF development since it can be easily detected because of its low odour threshold and is formed in quantities larger than other types of aldehydes (Resconi et al., 2013). Therefore, expressing secondary lipid oxidation compounds in terms of warmed-over flavour profiles through the determination of the hexanal content is an effective tool for measuring oxidation in lipids (Jayathi-lakan et al., 2007). The results from this study showed that warmed-over flavour profiles developed rapidly in the pork that had no antioxidants, while the pork that contained antioxidants (*M. oleifera* and BHT) had significantly slow ($p < 0.05$) WOF development throughout the storage period. Addition of the *M. oleifera* leaf powder and root powder significantly lowered the hexanal values. This means that *M. oleifera* had the ability to slow down or decrease lipid oxidation of the cooked pork during storage. Results from a related study by Lungu et al. (2021) where thiobarbituric acid-reactive substances (TBARS) and ferric reducing antioxidant power (FRAP) were measured showed that *M. oleifera* leaf and root powder treated pork samples had significantly lower (TBARS) values than the control ($p < 0.05$). Pork samples incorporated with antioxidants had significantly higher FRAP compared to the control ($p < 0.05$). The results concur with findings by Muthukumar et al. (2014) who found that *M. oleifera* leaf extracts reduced lipid oxidation in cooked pork patties during storage at 4 °C. Falowo et al. (2017) also reported that the *M. oleifera* leaf extract improved the oxidative stability of raw beef stored at the same storage temperature. Similarly, Mashau et al. (2021) reported
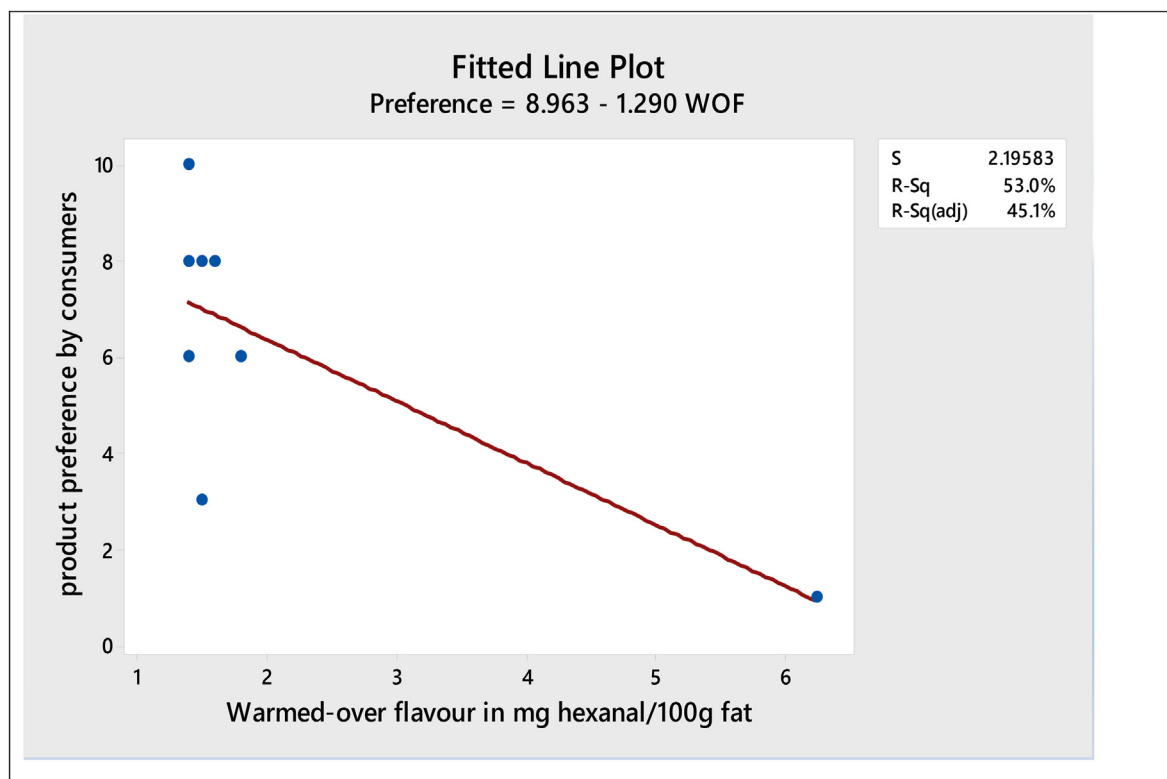

Figure 4. Association between shelf-life and warmed-over flavour profiles of cooked minced pork.
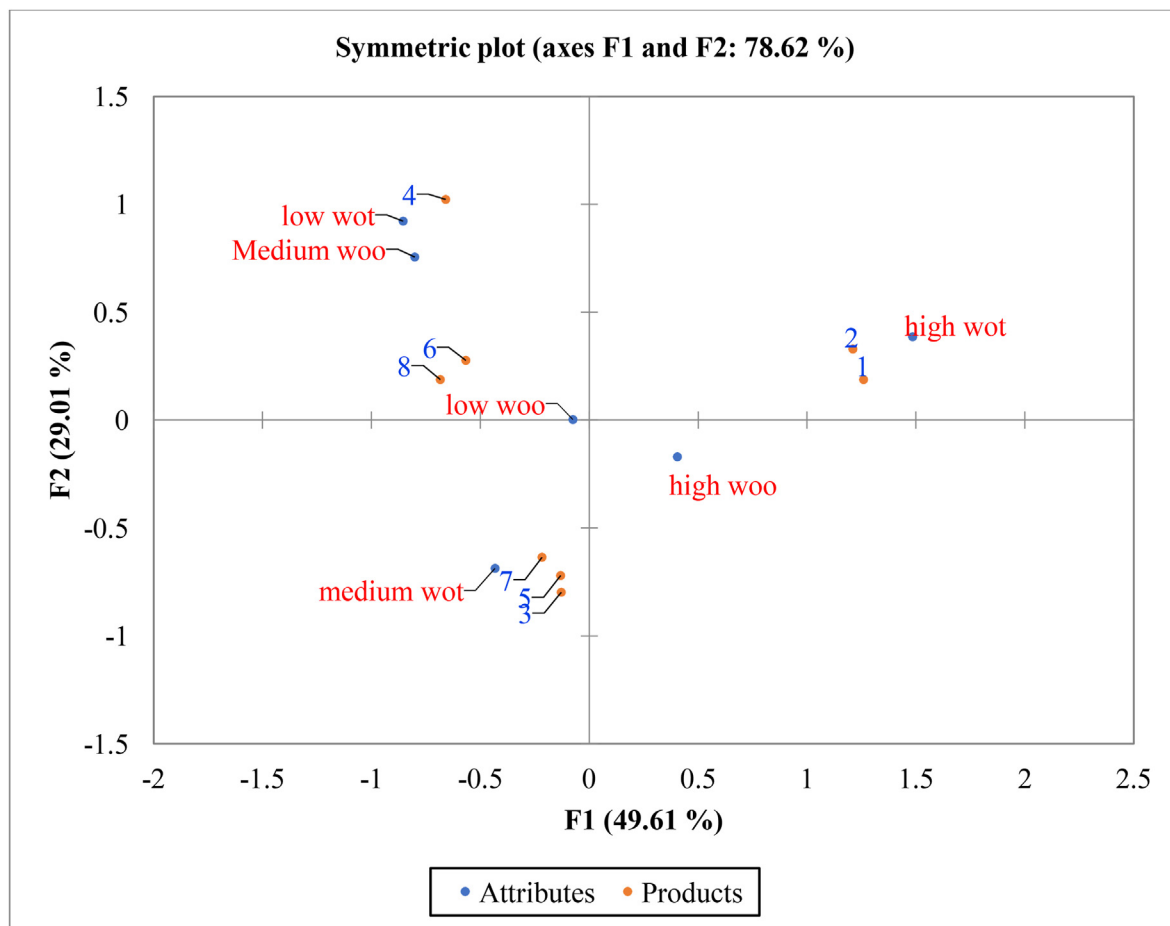
Figure 5. Classification of cooked minced treated with natural and synthetic antioxidants according to their intensities of warmed-over flavour and taste as denoted by the consumers. WOT-warmed-over taste, WOO- warmed-over odour. (1) control (no additives), (2) 0.5ML (0.5% *M. oleifera* leaf powder), (3) 1ML (1% *M. oleifera* leaf powder (4) 0.5MR (0.5% *M. oleifera* root powder) (5) 1MR (1% *M. oleifera* root powder) (6) 0.5MLR (0.5% mixed *M. oleifera* leaf and root powder) (7) 1MLR (1% mixed *M. oleifera* leaf and root powder) and 8) BHT.

low TBARS values in beef treated with *M. oleifera* leaf powder. The ability of the *M. oleifera leaf* and root powder to reduce the warmed-over flavour profiles could be attributed to the presence of polyphenolic compounds (Siddhuraju and Becker 2003; Pakade et al., 2013) which possess a hydroxyl group that can donate hydrogen atoms that can bind and neutralize the free radicals which are involved in oxidation reactions (Falowo et al., 2014).

Jayathilakan et al. (2007), also reported low warmed-over profiles in cooked mutton, pork and beef treated with cloves and cinnamon and this was attributed to the ability of the plant-based spices to block the release of non-haem iron during cooking and storage. Similarly, the *M. oleifera* leaves and roots used in this study could have suppressed the release of non-haem iron which is a catalyst in the lipid oxidation reaction. To further support findings from this study (Ergezer and Serdaroğlu 2018), found plant phenolics to be effective against metmyoglobin formation in beef patties. At 1% inclusion, the *M. oleifera* root showed lower warmed-over flavour profiles compared to the leaf throughout the storage period. Generally, both plant parts are potentially good antioxidants in reducing warmed-over flavour development in cooked pork products during refrigerated storage.

Antibacterial activities of the leaf and root were measured using the microdilution, method of Eloff (1998). The results revealed that both plant parts had some antibacterial activity against the gram-negative and gram-positive bacteria strains used. The antimicrobial activity could be attributed to the abundance of phytochemicals including phenols, flavonoids. alkaloids and tannins which can disrupt the normal functioning of the bacterial cell wall (Kumar and Pandey, 2013; Farasat et al., 2014).

According to Malhotra and Mandal (2018), flavonoids exhibit antibacterial activity through their capacity to combine with extracellular and soluble proteins, as well as with bacterial cell walls, whereas tannins may inactivate microbial adhesions, enzymes, and cell membrane proteins. This is also supported by Aminzare et al. (2016) who stated that phenolic compounds alter the normal functions of cell membranes which include electron transfer, protein synthesis, nutrient exchange, and enzymatic activity. Given its complex phytochemical profile, *M. oleifera* is a promising preservative for emerging foods.

Both plant parts exhibited good activity against the gram-negative bacteria compared to gram-positive bacteria. A similar trend in the activity of plant extracts where results showed that the gram-negative bacteria were more susceptible was reported on *M. oleifera* (Moyo et al., 2012), *Vachellia Karoo* (Maphosa et al., 2019), *Lauridia tetragona* (Wintola and Afolayan, 2019), *Phragmanthera capitata (Sprengel) Balle (Loranthaceae)* (Ohikhena et al., 2017). The differences in the susceptibility of the gram-negative and the gram-positive bacteria is due to differences in their cell wall compositions (Nohynek et al., 2006). Generally, the extracts of *M. oleifera* leaf, particularly the acetone extract demonstrated superior antibacterial activity (MIC) against the tested bacteria compared to the *M. oleifera root* extracts. The antibacterial activity differences noted between the leaf and root extracts may be attributed to the differences in the contents of the soluble phenolic and polyphenolic compounds present in both plant parts (Igbinosa et al., 2009), since they all work in synergy to produce antibacterial activity.

While advances in using plant-based antioxidants in processed meat products are promising, sensory characteristics remain the most important

Table 2. Consumer rating of the attributes (appearance, taste and odour) of cooked minced pork during storage 4 °C.

Storage day	Treatments								
	Attribute	Control	0.5ML	1ML	0.5MR	1MR	0.5MLR	1MLR	BHT
0	Appearance	6.2[a] ± 0.8	6.5[a] ± 0.8	4.0[c] ± 0.8	6.0[ab] ± 0.9	5.8[ab] ± 0.7	6.3[a] ± 0.5	5.2[b] ± 0.8	6.5[a] ± 0.7
	Taste	6.9[a] ± 0.3	6.7[ab] ± 0.5	6.5[ab] ± 0.7	6.2[b] ± 0.7	6.4[ab] ± 0.7	6.7[ab] ± 0.5	6.7a[b] ± 0.5	6.3[ab] ± 0.7
	Odour	6.9 ± 0.3	6.4[a] ± 0.5	6.5[abc] ± 0.5	5.8[bc] ± 0.6	6.6[ab] ± 0.7	5.7[c] ± 1.2	6.5[abc] ± 0.7	6.5[abc] ± 0.7
2	Appearance	6.2[abc] ± 0.7	6.8[ab] ± 0.8	6.8 ± 0.5	4.1 ± 0.9	5.8 ± 0.7	5.1 ± 0.7	5.7 ± 0.9	6.7 ± 0.5
	Taste	6.9 ± 0.3	6.7 ± 0.5	6.4[a] ± 0.8	6.8[a] ± 0.5	6.5[a] ± 0.5	6.3[a] ± 0.7	6.6[a] ± 0.5	6.4[a] ± 0.5
	Odour	6.9[a] ± 0.3	6.5[a] ± 0.5	6.2[ab] ± 0.7	5.1[c] ± 0.8	5.7[bc] ± 0.7	6.3[ab] ± 0.9	6.6[a] ± 0.5	6.6[a] ± 0.7
4	Appearance	5.6[ab] ± 0.7	5.9[a] ± 0.8	5.5[ab] ± 0.9	3.4[c] ± 0.7	5.7[a] ± 0.6	5.3[ab] ± 1.2	4.4[bc] ± 1.5	5.8[a] ± 0.9
	Taste	2.9[b] ± 0.8	4.9[a] ± 1.2	5.6[a] ± 0.7	5.2[a] ± 1.0	5.7[a] ± 0.7	5.5[a] ± 0.7	5.4[a] ± 0.5	5.5[a] ± 0.7
	Odour	4.0[b] ± 2.1	5.6[ab] ± 2.0	4.9[ab] ± 0.7	4.8[ab] ± 0.6	5.3[ab] ± 0.9	5.5[ab] ± 0.7	5.5[ab] ± 1.0	6.1[a] ± 0.8
6	Appearance	5.9[a] ± 0.7	5.7[a] ± 0.9	5.3[a] ± 0.9	3.8[b] ± 1.0	6.1[a] ± 0.7	5.8[a] ± 0.9	6.2[a] ± 0.7	6.3[a] ± 0.7
	Taste	2.1[d] ± 0.8	4.3[c] ± 0.9	4.3[c] ± 0.5	4.8[abc] ± 0.7	4.7[bc] ± 0.7	5.4[ab] ± 0.7	5.4[ab] ± 0.5	5.6[a] ± 0.5
	Odour	1.3[b] ± 0.5	4.8[a] ± 2.4	3.9[a] ± 0.9	4.7[a] ± 0.8	4.6[a] ± 1.1	3.9[a] ± 0.9	4.5[a] ± 1.0	3.8[a] ± 1.2
8	Appearance	5.8[a] ± 0.8	5.8[a] ± 0.9	5.9[a] ± 0.9	4.0[b] ± 0.7	4.3[b] ± 1.0	5.7[a] ± 0.9	6.1[a] ± 0.9	6.0[a] ± 0.9
	Taste	1.3[c] ± 0.5	4.2[b] ± 0.9	4.9[ab] ± 0.8	4.9[ab] ± 0.7	5.0[ab] ± 0.7	5.0[ab] ± 1.2	4.6[ab] ± 0.8	5.3[a] ± 0.7
	Odour	2.0[c] ± 1.5	4.5[ab] ± 1.5	2.8[bc] ± 1.4	5.2[a] ± 1.7	4.7[ab] ± 2.4	5.1[a] ± 1.6	5.0[a] ± 0.7	5.4[a] ± 0.9

Values are mean ± SD of triplicate samples; Means with different [a,b,c] along the same row indicate significant differences (p < 0.05).

factors influencing consumers' preference and purchasing decisions (Hung and Verbeke, 2019). Sensory attributes such as appearance, taste, flavour, and aroma have been recognized as the most important when it comes to the acceptability of food by consumers (Andersen et al., 2019). The aforementioned attributes are, however, deteriorated by enzymatic reactions, microbial spoilage and lipid oxidation during processing, handling and storage. The plant-based antioxidants were able to significantly improve (p < 0.05) the shelf-life of the meat for the storage days studied. This concurs with findings by Muthukumar et al. (2014) who reported that *M. oleifera* leaf improved the shelf-life of pork patties under refrigerated storage at 4 °C. In addition, Falowo et al. (2017) also reported that *M. oleifera* improved the shelf-life of raw beef. Shavisi et al. (2017) further showed that *Ziziphora clinopodioides* essential oil extended the shelf-life of minced beef during storage in refrigerated storage for a minimum of 11 days without any undesirable sensory characteristics. The ability of these plants to improve shelf-life could be attributed to their free radical scavenging and hydrogen donating nature (Das and Roychoudhury, 2014) as reported earlier. There was a significantly slow loss of shelf-life on the pork treated with antioxidants. Generally, this study revealed an inverse relationship between the warmed-over profiles and shelf-life, meaning that as warmed-over flavour profiles increased the shelf-life decreased. Similarly, López-Romero et al. (2018) reported that fresh flavour and fresh odour of cooked pork patties treated with *Agave angustifolia* extract decreased with storage days. The results of the improved shelf-life in this study are an indication that the plant additives managed to suppress the formation of secondary lipid oxidation products such as lactones, aldehydes, ketones, hexanal and alcohols which are responsible for off-odours.

The results for classifying the pork samples under different treatments revealed that consumers grouped the control and 0.5ML treated pork as high warmed-over flavour meats. This suggests that the 0.5ML inclusion level had antioxidant activity which was not good enough to highly inhibit warmed-over flavour development. While the 1% leaf inclusion seemed superior on the chemical analysis, consumers had a different perception on the appearance of the pork. The appearance attribute of pork treated with 1% *M. oleifera* leaf received lower liking scores. This could be attributed to the green colour that the leaf produced. In support of this, Teye et al. (2013) found that frankfurter sausages incorporated with higher *M. oleifera* leaf powder inclusions appeared green in colour and their acceptability was low. Similarly, Mashau et al. (2021) reported low ratings for colour in *M. oleifera leaf* treated ground beef and attributed this to the colour of *M. oleifera* leaf powder, which results from the presence chlorophyll. Zungu et al. (2019) also reported that the acceptability of snacks

decreased as *M. oleifera* leaf powder concentration was increased. The appearance of any food product influences its acceptance by consumers. If a new product's appearance differs from the standard products, consumers might see it as a sign of spoilage and therefore reject it (Teye et al., 2013). The root, on the other hand, received fairly good ratings for appearance. This suggests that the root could be a more suitable antioxidant that will not interfere with consumer attitude towards the appearance of the meat. Generally, consumer acceptance of the pork treated with BHT and *M. oleifera* was appreciable throughout this study. Since synthetic antioxidants are feared for their association with toxic health effects, this study suggests that the *M. oleifera* leaf and root powder may be potentially used as antioxidants in meat processing, with the root being more advantageous due to its colour.

4. Conclusion

The use of natural antioxidants in the production of meat and meat products is one of the promising technological advancements to meet consumer demands of healthy functional foods. The study showed that *M. oleifera* leaf and root powder extended the shelf-life of minced cooked pork. Phytochemicals such as phenols, alkaloids, flavonoids, and tannins present in *M. oleifera* possess antimicrobial properties, making it a potent preservative for emerging meat products. The addition of the *M. oleifera* leaf at inclusion level 1% was not well accepted by consumers who indicated the green colour on the product was not appealing. Therefore, it was suggested that the root powder may be an alternative, acceptable natural antioxidant for the control of lipid oxidation and improvement of meat shelf-life.

Declarations

Author contribution statement

Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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