sustainability

MDPI

Article

Quality and Oxidative Changes of Minced Cooked Pork Incorporated with *Moringa oleifera* Leaf and Root Powder

Nobuhle S. Lungu [1,*], Anthony J. Afolayan [1], Ronald S. Thomas [2,†] and Emrobowansan M. Idamokoro [3]

[1] Department of Botany, University of Fort Hare, Private Bag X 1314, Alice 5700, South Africa; Aafolayan@ufh.ac.za
[2] Agricultural Research Council-Animal Production Institute (Nutrition Building), Private Bag X 2, Irene 0062, South Africa; ronaldt@arc.agric.za
[3] Department of Economic and Business Sciences, Faculty of Commerce and Administration Vision, Walter Sisulu University, Private Bag X 1, Mthatha 5117, South Africa; mondayidamokoro@gmail.com
* Correspondence: nslungu@gmail.com; Tel.: +27-786-530-766
† The author passed away after the paper was accepted.

Abstract: Consumers are currently demanding meat products produced using natural additives due to their potential health benefits. Consequently, there has been a progressive interest in the search for more natural antioxidant sources. The aim of this study was to evaluate the effect of varying levels of *M. oleifera* root and leaf powder on the oxidative stability and quality of cooked minced pork during eight days of refrigerated storage at 4 °C. The leaves contained significantly higher ($p < 0.05$) concentrations of total phenols (50.08 ± 0.01 mg GAE/g), while the roots contained the highest amounts of flavonoids (375.15 ± 0.19 mg QE/g) and proanthocyanidins (132.62 ± 0.4 mg CE/g). Both plant parts demonstrated good 2,2-Diphenyl-2-picrylhydrazyl (DPPH) and 2,2 íazino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radical scavenging activity. Minced cooked pork incorporated with *M. oleifera* leaf and root powder had significantly lower pH and thiobarbituric acid-reactive substances (TBARS) values compared to the control ($p < 0.05$). The ferric reducing antioxidant power (FRAP) was also significantly higher in the samples that incorporated antioxidants compared to the control ($p < 0.05$). Findings from this study suggest that *M. oleifera* leaves and roots are potent antioxidant sources which can be incorporated in meat to improve quality attributes during storage.

Keywords: natural antioxidants; meat; consumer health; shelf-life

Citation: Lungu, N.S.; Afolayan, A.J.; Thomas, R.S.; Idamokoro, E.M. Quality and Oxidative Changes of Minced Cooked Pork Incorporated with *Moringa oleifera* Leaf and Root Powder. *Sustainability* **2021**, *13*, 10126. https://doi.org/10.3390/su131810126

Academic Editors: Márcio Carocho and Patricia Morales

Received: 1 June 2021
Accepted: 23 June 2021
Published: 10 September 2021

1. Introduction

Lipid oxidation remains a major concern to the meat industry, whose primary objective is to produce safe, nutritious, appetizing and healthy meat products that satisfy consumers [1]. To enhance meat quality and introduce exciting sensory characteristics that capture consumer attention, the meat industry has substantially grown in the area of processing. However, during processing, meat is subjected to various technological procedures including heat treatment or cooking, mincing, exposure to light, freezing and salt addition, which speed up the rate of oxidative reactions [2,3]. The mincing and cooking of meat before refrigerated storage have been reported to disrupt muscle cell membranes, paving the way for the reactions of unsaturated lipids with pro-oxidant substances like non-heme iron [4]. Moreover, radical-induced lipid oxidation that occurs in high-temperature cooking conditions contributes to the formation of potentially harmful end products, such as 4-hydroxynonenal (4-HNE) and malonaldehyde (MDA) [5].

In this era, where the meat processing industry is exponentially growing due to the increased demand for ready-to-eat meat and meat products by consumers, it is imperative to develop sustainable strategies for improving the shelf-life of such products. Efforts have been made to minimize the extent of oxidative degradation and antimicrobial spoilage

in processed meat and meat products through the use of natural and synthetic antioxidants [6]. However, health concerns regarding the association of synthetic antioxidants with potential toxicological effects have prompted consumers to demand meat and meat products produced with minimal or no synthetic additives. These demands have reinforced the need to search for alternative antioxidants that will improve the shelf-life of processed meats without any negative health implications.

In this regard, plant-based antioxidants have been identified as potential synthetic antioxidant replacers [7]. Of particular interest in this study is the *Moringa oleifera* plant, which has been reported to possess an impressive range of antimicrobial and antioxidant properties [8]. The *M. oleifera* plant has been extensively researched as a medicinal plant and animal feed additive in goats [9,10], cattle [11,12], chickens [13,14] and pigs [15], where it exhibited some antioxidant activity on the meat produced from these species. The antioxidant and antimicrobial properties of *M. oleifera* are attributed to its polyphenolic compounds, which can terminate chain inhibition through scavenging initiating radicals, breaking chain reactions, disintegrating peroxides, reducing localized oxygen concentrations and binding chain-initiating catalysts [16]. The *M. oleifera* plant has earned names including "miracle tree", tree of life, and God's gift to man [17,18], due to its reported effectiveness in treating bacterial infections, fungal infections and some gastro-intestinal and hematological disorders; reducing inflammation; improving heart function; as well as boosting the immune system of people living with human immunodeficiency virus (HIV) [17]. The *M. oleifera* plant is rich in minerals and nutrients, including amino acids, vitamin C, calcium and potassium [19–21]. Hence, it has been used to improve nutrition in some developing countries [19]. These characteristics and other documented health benefits of *M. oleifera* makes it suitable to be potentially used in meat and meat products processing to develop functional food products for enhancing human health.

Moreover, *M. oleifera* is a sustainable, fast-growing, drought-resistant tree which is easy to cultivate and manage; therefore, there is a potential role for the plant to promote the sustainable development goal of good health and well-being. There has been, however, limited research on the potential of *M. oleifera* as an antioxidant and shelf-life enhancer in minced cooked pork. In addition, the research has focused more on the use of the leaves, leaving the roots behind. Therefore, the objective of this study was to investigate the effects of varying levels of *M. oleifera* leaves and roots, and their combination, on minced cooked pork's oxidative stability and quality. The *M. oleifera* used in this study was cultivated in South Africa; therefore, it was first subjected to phytochemical screening and in vitro antioxidant activity determination to validate it. Among the various meat types that are available in the market, pork meat and meat products contain relatively higher amounts of polyunsaturated fatty acids [22,23], which makes them highly susceptible to oxidative degradation. Hence, this study focused on pork meat.

2. Materials and Methods

2.1. Plant Harvesting and Meat Sample Collection

The leaves and roots of the *Moringa oleifera* plant used in this study were harvested from a certified moringa farm known as Lefakong farm, situated in Boosplas, North West Province, South Africa. The farm area lies within latitude 25°19′21.5″ S, 28°14′35.9″ E. The leaves were harvested by cutting the leaf stems from the branches using a knife. The roots were harvested by digging and uprooting the tree. The harvested fresh *M. oleifera* leaves and roots were first cleaned to remove dust and then air-dried at room temperature under well-aerated conditions without direct sunlight to prevent the denaturation of important phytoconstituents. The dried plant samples were ground into powder and stored in airtight containers at room temperature until needed for extraction. Pork samples were obtained at 24 h post-mortem from the East London commercial abattoir, Eastern Cape, South Africa. The abattoir adheres to all animal handling and meat safety regulations. Fresh pork muscle (muscularis longissimus thoracis et lumborum, LTL) was cut from each pig carcass.

2.2. Preparation of Extracts

The ground plant parts were separately extracted in duplicates using acetone, ethanol and distilled water as the solvents. Briefly, the ground leaf sample was weighed into 3 different conical flasks (200 g sample for each flask) containing (2 L) acetone, ethanol and distilled water, respectively. The same procedure was repeated for the root sample. Thereafter, the mixtures were shaken (Orbital Incubator Shaker, Gallenkamp, London, UK) for 24 h. This was followed by the filtration of extracts under pressure using a Buchner funnel and Whatman No. 1 filter paper. The ethanol and acetone extracts were concentrated to dryness under pressure using a rotary evaporator (Strike 202 Steroglass, Perugia, Italy), while the aqueous extracts were dried under reduced pressure in a freeze drier. The extracts were then stored at 4 °C until further analyses. All the experiments were carried out in triplicate.

Chemicals and Reagents

All reagents and chemicals, including Folin–Ciocalteu, trichloroacetic acid (TCA), gallic acid, rutin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis (3-ethylobenzothiazoline-6-sulphonic acid diammonium salt) (ABTS), BHT acetone and ethanol, were purchased from Merck and Sigma-Aldrich, Gauteng, South Africa. All the reagents and chemicals that were used in this study were of analytical grade.

2.3. Phytochemical Screening

2.3.1. Determination of Phenolic Content

Total phenolic content was evaluated using a modified Folin–Ciocalteu method as described by Unuofin et al. [24], where 0.5 mL of the root and leaf plant extracts (1 mg/mL) and standard gallic acid (0.02–0.1 mg/mL) and the control were separately measured into different test tubes. The measured contents were then mixed with 2.5 mL of 10% Folin–Ciocalteu reagent. The mixture was allowed to react for 5 min. Subsequently, 2 mL of 7.5% (*w*/*v*) anhydrous sodium carbonate (Na_2CO_3) was added to the solution, vortexed and incubated at 40 °C for 30 min. This was followed by the measuring of absorbance at 765 nm using a spectrophotometer (AJI-C03 UV-VIS). The experiment was carried out in triplicate. The control was made following the same procedure but replacing the plant extract with methanol. The total phenol content was deduced from the gallic acid standard/calibration graph equation reading: y = 22.953x + 0.0013, R^2 = 0.9981. The following formula was used in the calculation of the total phenol content:

$$C = c \times V/m$$

where C = total content of phenolic compounds in mg/g plant extract in gallic acid equivalent (GAE) or mg GAE/g extract, c = the concentration of gallic acid established from the calibration curve in mg/mL, V = the volume of extract in mL, and *m* = the weight of extract used in the assay in g.

2.3.2. Determination of Proanthocyanidin Content

The technique described by Kibiti and Afolayan [25] was used to evaluate the proanthocyanidin content of the sample. In summary, 0.5 mL of different solvent (ethanol, acetone and distilled water) extracts of 1 mg/mL were added to a mixture of 3 mL of 4% (*w*/*v*) vanillin and 1.5 mL of hydrochloric acid, and the solution was vortexed. The solution was then incubated for 15 min at 27 °C, and absorbance was measured at 500 nm using a spectrophotometer. The experiment was done in triplicate. Proanthocyanidin content was extrapolated by using the calibration graph equation (y = 1.1283x + 0.1174, R^2 = 0.9793), obtained with catechin as a standard, and expressed as mg catechin equivalent (CE)/g using the formula cV/*m*, as described for the phenols above.

2.3.3. Determination of Flavonoid Content

The aluminum chloride colorimetric assay described by Ohikena et al. [26] was used to determine the flavonoid content. Briefly, 0.5 mL aliquots of the solvent extracts at different concentrations (0.2–1 mg/mL) were pipetted into different test tubes. This was followed by the addition of 2 mL of distilled water to each test tube and 0.15 mL of 5% sodium nitrite thereafter. The solution was allowed to react for 6 min. Quercetin standard reaction mixtures were prepared using the same method. After which, 0.15 mL of $AlCl_3$ (10%) was added to the solution and left to stand for another 5 min, then lastly, 1 mL of 1 M sodium hydroxide was added. The solution was topped up to 5 mL using distilled water. The absorbance was measured at 420 nm. The experiment was done in triplicate. The flavonoid content was then calculated using the calibration curve equation obtained with quercetin as a standard ($y = 22.953x + 0.0013$, $R^2 = 0.9981$) and expressed as mg of quercetin equivalent (QE/g).

2.4. Antioxidant Assays

Antioxidant capacities of the plant extracts were evaluated using DPPH radical scavenging and ABTS radical scavenging assays.

2.4.1. DPPH Radical Scavenging Assay

The scavenging ability of the plant extract on DPPH was determined using the method described by Ohikena et al. [26], with modifications of the concentrations. Briefly, 1 mL of DPPH prepared in methanol (0.135 mM) was mixed with 1 mL of varying concentrations ranging from 0.2 mg/mL–1 mg/mL of plant extracts and standards (Rutin and BHT). The mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. A control containing DPPH and methanol was also prepared. The absorbance was measured at 517 nm using a spectrophotometer. The scavenging ability of the plant extracts was calculated as: DPPH scavenging activity (%) = [(Absorbance of the control − Absorbance of the sample)/(Absorbance of the control)] × 100.

2.4.2. ABTS Radical Scavenging Assay

The method described by Akinrinde et al. [27] was adopted to determine the ABTS radical scavenging activity of the different leaf and root extracts. The working solution was prepared by mixing 7 mM ABTS solution and 2.45 mM potassium persulfate solution in equal amounts (1:1). The mixture was allowed to react for 12 h in the dark at room temperature to produce a green-colored ABTS radical ($ABTS^+$). The resultant solution was further diluted by mixing 1 mL of the $ABTS^+$ solution with 50 mL of methanol until an absorbance of 0.700 ± 0.01 at 734 nm was reached. Thereafter, 1 mL of plant extracts and standards at different concentrations (0.02 mg/mL–0.32 mg/mL) were separately mixed with 1 mL of the $ABTS^+$ solution. After 7 min, the absorbance was measured at 734 nm. The percentage of $ABTS^+$ inhibition was calculated using the following equation: ABTS radical scavenging activity (%) = [(Absorbance of the control - Absorbance of the sample)/(Absorbance of the control)] × 100.

2.5. Meat Sample Preparation

Pork meat samples were minced with a sterile meat grinder (Trespade 22 EL Plus, Torino, Italy). The ground pork was divided into different portions for each experiment prior to the addition of the test compounds. The pork samples were then randomly assigned to each of the following treatments: (1) control (no additives), (2) 0.5ML (0.5% *M. oleifera* leaf powder), (3) 1ML (1% *M. oleifera* leaf powder), (4) 0.5% MR (0.5% *M. oleifera* root powder), (5) 1MR (1% *M. oleifera* root powder), (6) 0.5% MLR (0.5% combined *M. oleifera* leaf and root powder), (7) 1MLR (1% combined *M. oleifera* leaf and root powder), and (8) SYN (0.02% BHT). Each treatment had four replicates. The meat samples with no antioxidants and those mixed with natural and synthetic antioxidants were then packed into polypropylene pouches and cooked in a water bath at 78 °C under atmospheric

pressure for 45 min. After heat treatment, the cooked samples were cooled to room temperature. The samples for day zero (first day of analysis) were immediately taken for analysis, while the remaining treatment samples were stored in oxygen-permeable bags and stored at 4 °C for analysis at storage days 2, 4, 6 and 8.

2.6. pH Measurement

The pH of the cooked minced pork samples was determined as described by Falowo et al. [28] with slight modifications. Briefly, 25 mL of distilled water was used to homogenize a 5 g portion of the pork sample for 60 s using a homogenizer (Model Polytron® PT 2500 E Stand Dispersion Device, Kinematica AG, Switzerland). The pH values were then measured using a standardized electrode attached to a digital pH meter (CRISON Instruments S.A., Alella, Spain). The pH measurements were taken in four replicates per each treatment and storage day.

2.7. Instrumental Color Measurement

The color changes of cooked minced pork during refrigerated storage were measured on days 0, 2, 4, 6 and 8 using a Hunter Lab Minolta colorimeter (45/0 BYK-Gardener GmbH, USA) with a 20 mm diameter measurement area, illuminant D65- day light and 10° standard observer. The CIE co-ordinates for L* = lightness (0 = dark, 100 = light); a* = redness (red-green spectrum); and b* = yellowness (yellow-blue spectrum) were measured perpendicular to the minced pork surface from three randomly selected points. All the color parameters (L*, a*, and b*) were taken from the mean of readings recorded from four samples per treatment. Before any measurements were taken, the color guide was first calibrated using the green, black, and white standards.

Chroma was calculated using the formular $C^* = \sqrt{a^{*2} + b^{*2}}$.

2.8. Lipid Oxidation Determination

Lipid oxidation was determined using the acid precipitation method of measuring thiobarbituric acid reactive substances (TBARS) described by Descalzo et al. [29] with some slight modifications. In summary, two grams of each meat sample were homogenized for 20 s with 6.25 mL Tricloracetic acid and 6.25 mL distilled water using a homogenizer (Model Polytron® PT 2500 E Stand Dispersion Device, Kinematica AG, Switzerland). A standard was prepared in duplicate by adding 0, 5, 10 and 20 µL of Tetramethoxypropan (TMP, 0.001 M) TMP in 1 mL of distilled water. Three test tubes were prepared, and to each, 1 mL of filtered slurry was added. One mL of TBA was then added to each standard and to 2 tubes of the 3 sample test tubes, while 1 mL of distilled water was added to the third sample test tube to act as a turbidity blank. All tubes were capped, vortexed and incubated in a water bath at 70 °C for 1 h. After that, the samples were allowed to cool and absorbance was read at 530 nm. The TBARS were expressed as mg of malondialdehyde (MDA)/kg meat.

Ferric Reducing Antioxidant Power Determination

Ferric antioxidant reducing power (FRAP) was determined following the procedure described by Arshad et al. [30]. Briefly, a 200 µL homogenized meat filtrate sample was mixed with 500 µL sodium phosphate buffer (0.2 M, pH 6.6) and 500 µL potassium ferricyanide (1%). The resultant mixture was incubated at 50 °C for 20 min. After addition of 2.5 mL trichloroacetic acid (TCA, (10%)), the mixture was centrifuged at 2200× *g* for 10 min. The upper layer (500 µL) was mixed with 500 µL distilled water and 100 µL ferricchloride (0.1%), and the absorbance was measured at 700 nm.

2.9. Statistical Analysis

Data was first tested for normality using the Ryan–Joiner's test. The data was normally distributed. One-way analysis of variance (ANOVA) was performed to determine the differences in means (±SD) of the phytochemicals and antioxidant activities between the

leaf and root. The results were expressed as mean standard deviation (SD) of the three replicates. Data on pH, color, TBARS and FRAP was analyzed using a generalized linear model where plant parts and storage day were the sources of variations. Significance was tested at $p < 0.05$. Data analysis was performed using the statistical package program Minitab, release version 17.

3. Results

3.1. Total Phytochemical Content

The total phenols, proanthocyanidins and flavonoids content of the *M. oleifera* leaf and root extracts are shown in Table 1. The results from this study revealed that the phytochemical compounds in question differed significantly ($p < 0.05$) between the two plant parts and among the three solvents that were used. From this study, the acetone leaf extracts were found to contain significantly higher ($p < 0.05$) concentrations of total phenol (50.08 ± 0.01 mg GAE/g), while the root acetone extract contained the highest amounts of flavonoids (375.15 ± 0.19 mg QE/g) and proanthocynanidins (132.62 ± 0.4 mg CE/g).

Table 1. Polyphenolic content of various solvent extracts of *M. oleifera* leaf and root (mean ± SD).

Solvent	Total Phenols (mg GAE/g)		Flavonoids (mg QE/g)		Proanthocyanidins (mg CE/g)	
	Leaf	Root	Leaf	Root	Leaf	Root
Acetone	50.08 ± 0.0 ^{a}	19.72 ± 0.13 ^{a}	145.13 ± 0.39 ^{a}	375.51 ± 0.19 ^{a}	45.91 ± 0.67 ^{a}	132.62 ± 0.44 ^{a}
Aqueous	8.65 ± 0.01 ^{c}	4.42 ± 0.02 ^{c}	40.44 ± 0.19 ^{c}	77.82 ± 0.34 ^{c}	24.05 ± 0.44 ^{c}	26.70 ± 0.44 ^{c}
Ethanol	39.20 ± 0.01 ^{b}	18.36 ± 0.02 ^{b}	76.015 ± 0.19 ^{b}	284.49 ± 0.34 ^{b}	33.94.72 ± 0.26 ^{b}	118.12 ± 0.51 ^{b}

mg GAE/g = milligram gallic acid equivalent per gram of extract; mg QE/g = milligram quercetin equivalent per gram of extract; mg CE/g = milligram catechin equivalent per gram of extract. Means in the same column with different letters $^{a–c}$ are significantly different ($p < 0.05$).

3.2. DPPH Free Radical Scavenging Assay

The DPPH radical scavenging activity of the different solvent extracts of the two plant parts and standards (BHT and Rutin) are shown (Figure 1). At the maximum concentration tested (1 mg/mL), all the DPPH radical scavenging activities of the root acetone, water and ethanol extracts were not significantly different ($p < 0.05$) from the leaf acetone extract and BHT scavenging activities. The aforementioned samples recorded the highest activities ranging between (93.28 ± 0.2) to (96.74 ± 0.1). Overall, the root extracts showed better DPPH scavenging activity compared to the leaf extracts.

3.3. ABTS Radical Scavenging Assay

The scavenging activities of the different solvent extracts and standards on ABTS radicals are shown (Figure 2). The ABTS scavenging activity of the plant extracts and standards ranged from 23% to 92%, with the root water extract exhibiting the least activity, while rutin demonstrated the highest activity. At the highest concentration (0.32 mg/mL), root ethanol and root water extracts exhibited high scavenging activity (91.54 ± 0.4% and 91.37 ± 0.5%, respectively), which was not significantly different ($p < 0.05$) to that of the test compounds BHT (91.54 ± 0.5%) and Rutin (92.04 ± 0.2%).

3.4. Effect of M. oleifera Leaf and Root Powder on pH of Pre-Cooked Minced Pork under Refrigerated Storage at 4 °C

The changes in cooked minced pork pH values over the five experimental storage days at 4 °C for the different treatments are shown in Table 2. The results showed that at day zero, the pH values ranged from 6.02 ± 0.02 to 6.09 ± 0.01 across the treatments. There were no significant differences ($p > 0.05$) among the control, BHT and all *M. oleifera*-powder-treated pork samples, except the 0.5%-leaf-powder-treated pork. On day 8, the results displayed pH values ranging from 6.13 ± 0.01 to 6.25 ± 0.01 across all treatments. The pH values varied significantly ($p < 0.05$) across all treatments. Overall, the results showed that the pH values were increasing with storage days for all the treatments. In addition, the pH

values of the pork samples treated with the *M. oleifera* leaf and root powder were generally lower compared to the control and the BHT-treated samples.

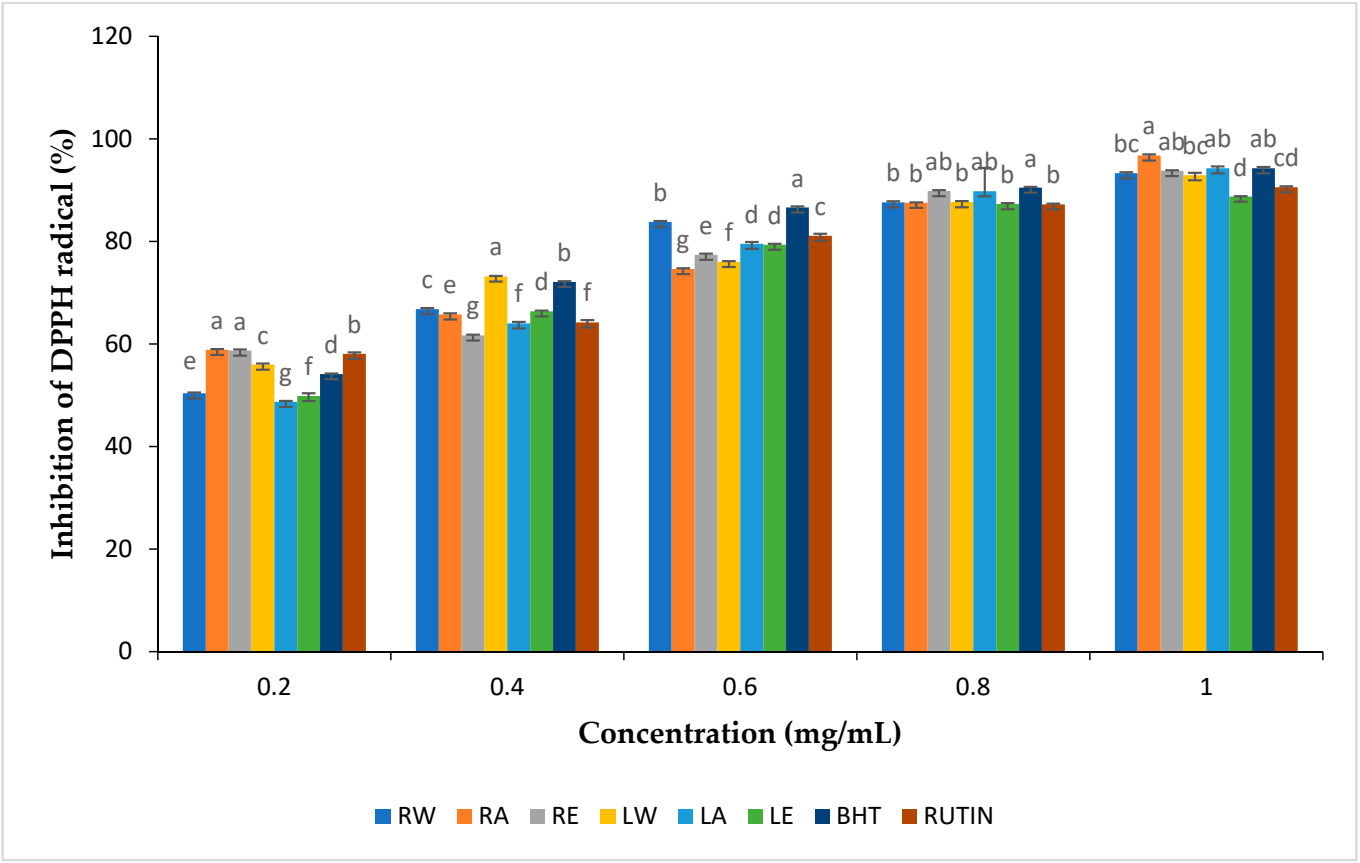

Figure 1. DPPH radical scavenging activity of different extracts of *M. oleifera* leaf and root. Values are expressed as mean ± standard deviation of the three replicates. Bars in the same concentration with different letters are significantly different ($p < 0.05$). RW = root water; RA = root acetone; RE = root ethanol; LW = leaf water; LA = leaf acetone; LE = leaf ethanol; BHT = butylated hydroxyl toluene.

3.5. Effect of M. oleifera Leaf and Root Powder on Colour of Cooked Minced Pork during Storage 4 °C

The effects of storage time and the addition of the tested natural and synthetic antioxidants on Hunter lab color (L, a and b) of minced cooked pork are shown in Figures 3–5, respectively. The results showed that there were significant differences ($p < 0.05$) in lightness (L*) across all the treatments and over the storage days. Overall, the control group displayed slightly higher values than the pork samples treated with *M. oleifera*. The BHT-treated samples exhibited lower L* values for all the storage days. The results also showed that L* values were slightly increasing with storage days across all treatments. Redness (a*) also varied significantly ($p < 0.05$) across the treatments. The values were randomly fluctuating with no particular traceable trend across treatments. However, there was a notable gradual decreasing trend with increase in storage days for all the treatments. Similarly, the yellowness (b*) values showed a decreasing trend with increasing storage days and also significant differences ($p < 0.05$) among the treatments. Chroma values are shown in (Figure 6). Chroma showed a significant ($p < 0.05$) decrease across all treatment groups during storage.

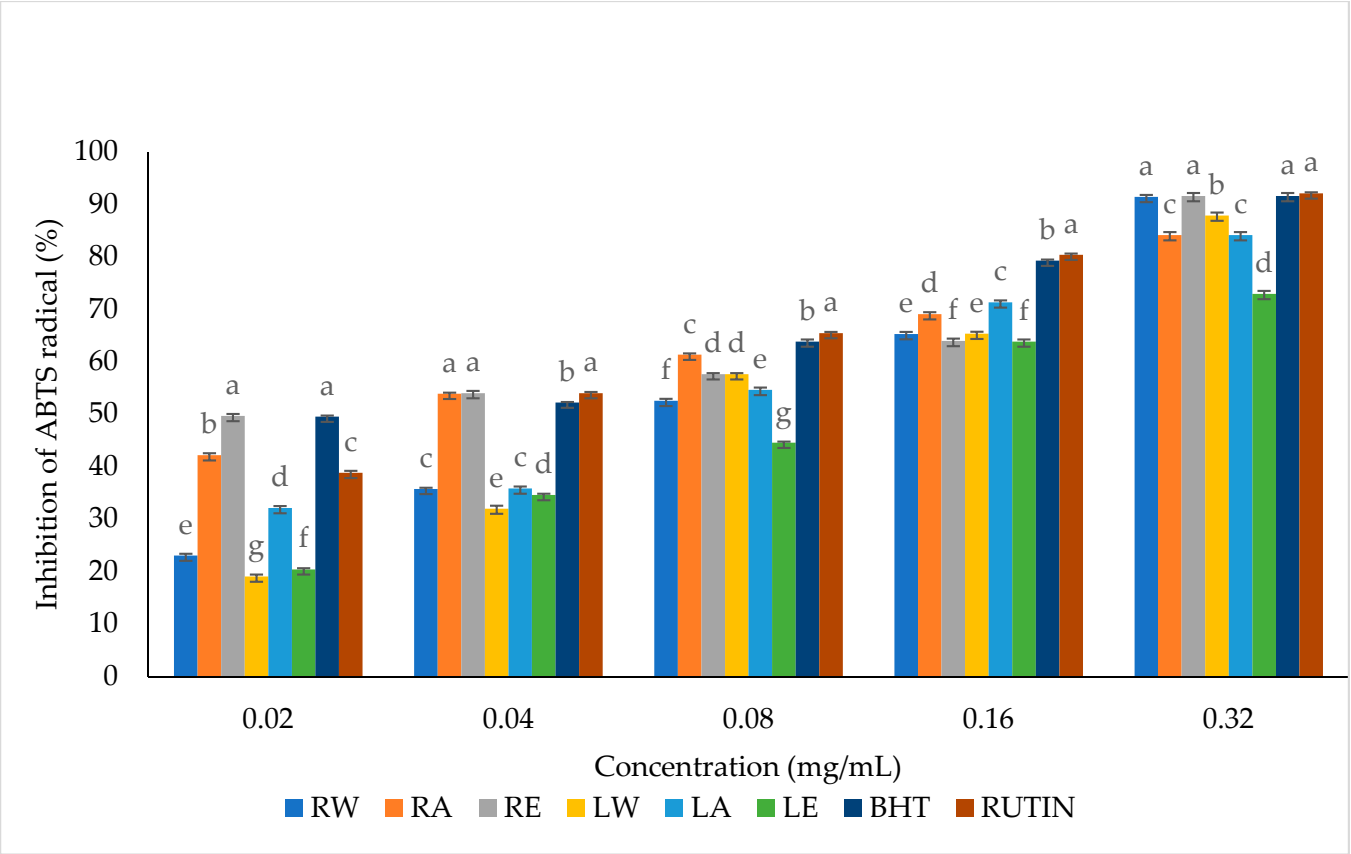

Figure 2. ABTS radical scavenging activity of different extracts of *M. oleifera* leaves and roots. Values are expressed as mean ± standard deviation of the three replicates. Bars in the same concentration with different letters are significantly different ($p < 0.05$). RW = root water; RA = root acetone; RE = root ethanol; LW = leaf water; LA = leaf acetone; LE = leaf ethanol; BHT = butylated hydroxyl toluene.

Table 2. Effect of *Moringa oleifera* leaf and root powder on pH of cooked pork during storage at 4 °C.

Storage	Treatments								Overall
Day	Control	0.5ML	1ML	0.5MR	1MR	0.5MLR	1MLR	BHT	Mean
0	6.08 ^{Cabc} ± 0.01	6.02 ^{Cd} ± 0.02	6.05 ^{Cabcd} ± 0.01	6.08 ^{Cab} ± 0.01	6.03 ^{Ccd} ± 0.05	6.04 ^{Cbcd} ± 0.01	6.09 ^{Ca} ± 0.01	6.08 ^{Ca} ± 0.01	5.93 ± 0.33
2	6.09 ^{Ca} ± 0.01	6.01 ^{Cd} ± 0.01	6.04 ^{Cbc} ± 0.01	6.08 ^{Ca} ± 0.01	6.03 ^{Ccd} ± 0.01	6.05 ^{Cb} ± 0.01	6.09 ^{Ca} ± 0.01	6.09 ^{Ca} ± 0.01	6.06 ± 0.03
4	6.13 ^{Bbc} ± 0.01	6.09 ^{Bd} ± 0.01	6.09 ^{Bd} ± 0.01	6.12 ^{Bc} ± 0.01	6.08 ^{Bd} ± 0.01	6.14 ^{Bab} ± 0.01	6.15 ^{Ba} ± 0.01	6.10 ^{Cd} ± 0.01	6.11 ± 0.02
6	6.13 ^{Bbc} ± 0.01	6.09 ^{Bd} ± 0.01	6.09 ^{Bd} ± 0.01	6.14 ^{Bbc} ± 0.01	6.09 ^{ABd} ± 0.01	6.14 ^{Bb} ± 0.05	6.16 ^{Ba} ± 0.01	6.12 ^{Bc} ± 0.01	6.12 ± 0.03
8	6.20 ^{Ac} ± 0.01	6.15 ^{Ae} ± 0.01	6.13 ^{Af} ± 0.01	6.18 ^{Ad} ± 0.01	6.14 ^{Aef} ± 0.01	6.22 ^{Ab} ± 0.01	6.25 ^{Aa} ± 0.01	6.17 ^{Ad} ± 0.01	6.18 ± 0.04
Overall Mean	6.12 ± 0.04	6.07 ± 0.05	6.08 ± 0.03	6.12 ± 0.04	6.07 ± 0.04	6.12 ± 0.07	6.15 ± 0.06	6.11 ± 0.04	

Values are mean ± SD of triplicate samples. Means with different superscripts $^{a–f}$ within a row indicate significant differences ($p < 0.05$). Means with different superscripts $^{A–C}$ within a column indicates significant differences ($p < 0.05$). Control = no antioxidant; 0.5ML = 0.5% *M. oleifera* leaf powder; 1ML = 1% *M. oleifera* leaf powder; 0.5MR = 0.5% *M. oleifera* root powder; 1MR = 0.5% *M. oleifera* root powder; 0.5MLR = 0.5% *M. oleifera* leaf and root powder; 1%MLR = 1% *M. oleifera* leaf and root powder; BHT = 0.02% butylated hydroxyl toluene.

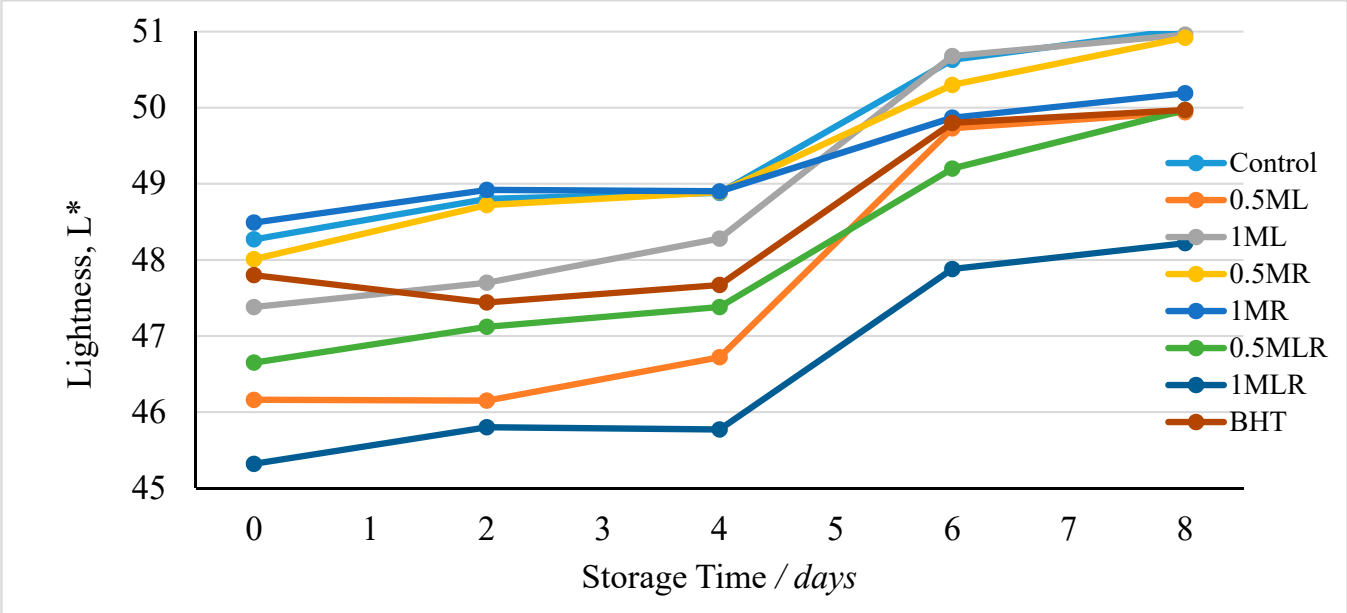

Figure 3. Effect of *M. oleifera* leaf and root powder on L* of pre-cooked pork during storage at 4 °C. Control = no antioxidant; 0.5ML = 0.5% *M. oleifera* leaf powder; 1ML = 1% *M. oleifera* leaf powder; 0.5MR = 0.5% *M. oleifera* root powder; 1MR = 0.5% *M. oleifera* root powder; 0.5MLR = 0.5% *M. oleifera* leaf and root powder; 1MLR = 1% *M. oleifera* leaf and root powder; BHT = 0.02% butylated hydroxytoluene.

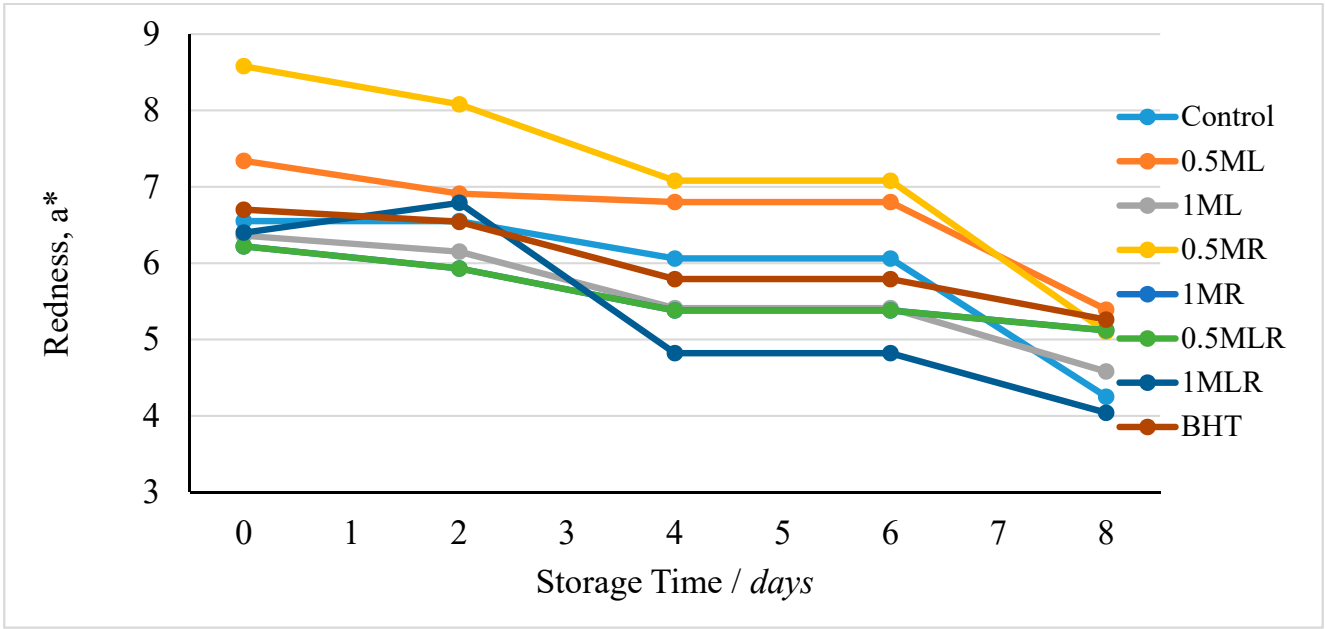

Figure 4. Effect of *M. oleifera* leaf and root powder on a* of cooked pork during storage at 4 °C. Control = no antioxidant; 0.5ML = 0.5% *M. oleifera* leaf powder; 1ML = 1% *M. oleifera* leaf powder; 0.5MR = 0.5% *M. oleifera* root powder; 1MR = 0.5% *M. oleifera* root powder; 0.5MLR = 0.5% *M. oleifera* leaf and root powder; 1MLR = 1% *M. oleifera* leaf and root powder; BHT = 0.02% butylated hydroxytoluene.

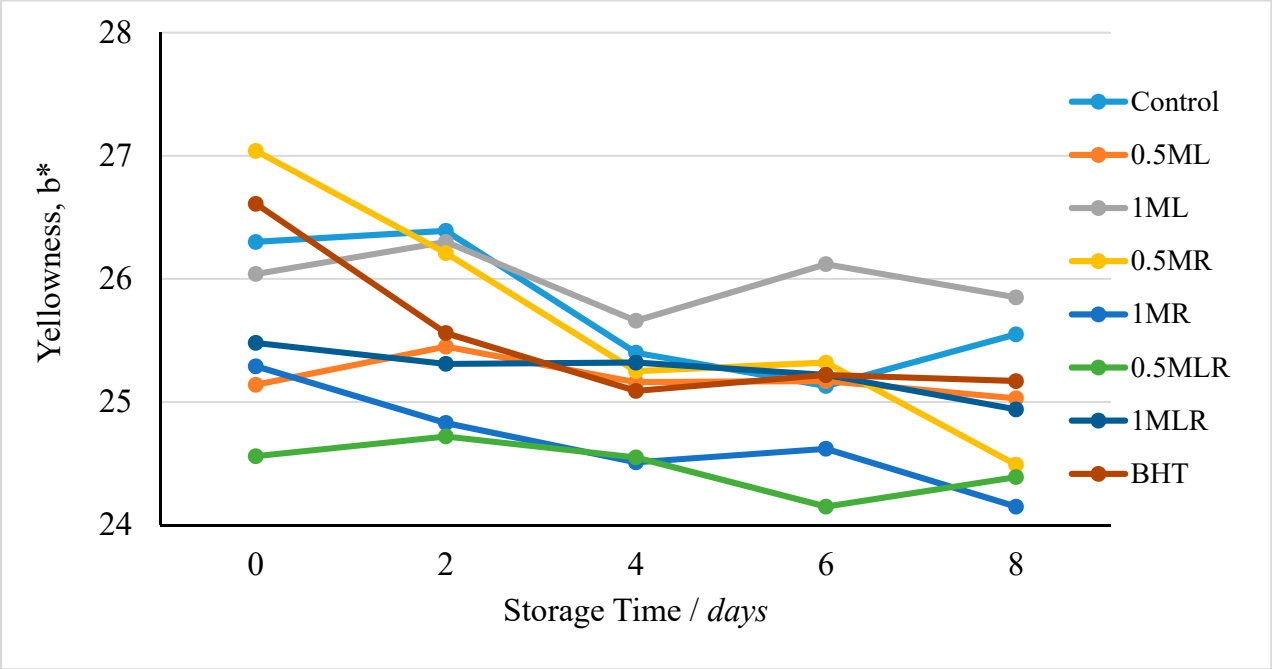

Figure 5. Effect of *M. oleifera* leaf and root powder on b* of cooked pork during storage at 4 °C. Control = no antioxidant; 0.5ML = 0.5% *M. oleifera* leaf powder; 1ML = 1% *M. oleifera* leaf powder; 0.5MR = 0.5% *M. oleifera* root powder; 1MR = 0.5% *M. oleifera* root powder; 0.5MLR = 0.5% *M. oleifera* leaf and root powder; 1MLR = 1% *M. oleifera* leaf and root powder; BHT = 0.02% butylated hydroxytoluene.

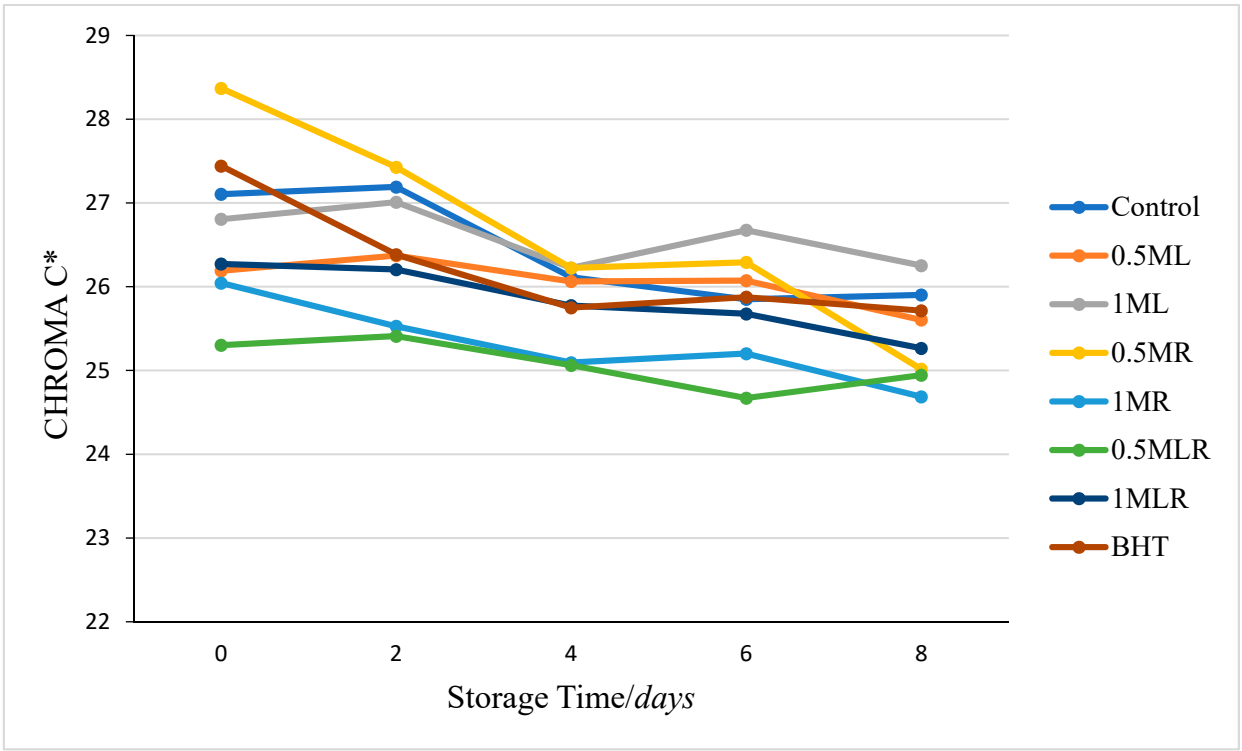

Figure 6. Effect of *M. oleifera* leaf and root powder on C* of cooked pork during storage at 4 °C. Control = no antioxidant; 0.5ML = 0.5% *M. oleifera* leaf powder; 1ML = 1% *M. oleifera* leaf powder; 0.5MR = 0.5% *M. oleifera* root powder; 1MR = 0.5% *M. oleifera* root powder; 0.5MLR = 0.5% *M. oleifera* leaf and root powder; 1MLR = 1% *M. oleifera* leaf and root powder; BHT = 0.02% butylated hydroxytoluene.

3.6. Effect of M. oleifera Leaf and Root Powder on Lipid Oxidation of Minced Pre-Cooked Pork during Storage at 4 °C

The effect of *M. oleifera* leaf, root and BHT inclusion on lipid oxidation of pre-cooked minced pork during storage at 4 °C is presented in Table 3. The results were expressed in terms of TBARS. The results showed that on day zero, there were no significant differences among the control, 0.5% ML-, 1% ML- and 0.5% MR-treated pork samples. Over the storage days, the samples treated with the plant powder and BHT exhibited significantly lower TBARS values when compared to the control ($p < 0.05$). On day 8, the control had 1.02 ± 0.01 MDA/kg meat while the 1% MLR had 0.50 ± 0.03 MDA/kg meat. Throughout the storage period, antioxidant treatments did not exceed 0.78 ± 0.01 MDA/kg. When comparing the two plant parts, the 1% MR had lower TBARS from day 0 compared to the leaf. The combination of the leaf and root also had TBARS values comparable to BHT. Overall, the FRAP values decreased as follows: Control > 0.5ML > 1ML > 0.5MR > 1MR > 0.5MLR > BHT > 1MLR.

Table 3. Effect of *M. oleifera* leaf and root powder on TBARS of cooked pork during storage at 4 °C.

Storage	Treatments								Overall
Day	**Control**	**0.5ML**	**1ML**	**0.5MR**	**1MR**	**0.5MLR**	**1MLR**	**BHT**	**Mean**
0	0.25^{Ba} ± 0.01	0.25^{Ba} ± 0.01	0.24^{Ba} ± 0.01	0.23^{Ba} ± 0.01	0.17^{Bb} ± 0.01	0.14^{Bc} ± 0.01	0.13^{Bc} ± 0.01	0.11^{Bd} ± 0.01	0.19 ± 0.06
2	0.34^{Ba} ± 0.01	0.32^{ABab} ± 0.01	0.30^{ABb} ± 0.01	0.29^{Bbc} ± 0.04	0.27^{ABc} ± 0.01	0.23^{ABd} ± 0.01	0.18^{Be} ± 0.03	0.14^{Bf} ± 0.01	0.23 ± 0.07
4	0.61^{Ba} ± 0.03	0.55^{ABb} ± 0.02	0.46^{ABc} ± 0.01	0.41^{ABd} ± 0.01	0.33^{ABe} ± 0.01	0.47^{Ac} ± 0.01	0.27^{ABf} ± 0.01	0.28^{ABf} ± 0.00	0.42 ± 0.12
6	0.73^{Ba} ± 0.00	0.70^{Ab} ± 0.01	$0.68^{AB\,c}$ ± 0.01	0.68^{Abc} ± 0.02	0.64^{Ad} ± 0.01	0.50^{Ae} ± 0.01	0.42^{Af} ± 0.01	0.52^{Ae} ± 0.01	0.61 ± 0.11
8	1.02^{Aa} ± 0.01	0.78^{Ab} ± 0.01	0.78^{Ab} ± 0.01	0.70^{Ac} ± 0.01	0.54^{Ad} ± 0.01	0.50^{Ae} ± 0.03	0.31^{Ag} ± 0.01	0.43^{Af} ± 0.00	0.63 ± 0.21
Overall Mean	0.59 ± 0.28	0.52 ± 0.21	0.49 ± 0.21	0.46 ± 20	0.39 ± 0.17	0.37 ± 0.15	0.26 ± 0.10	0.30 ± 0.16	

Values are mean ± SD of triplicate samples. Mean with different $^{a–g}$ superscripts within a row indicates significant differences ($p < 0.05$). Mean with $^{A, B}$ superscripts within a column indicates significant differences ($p < 0.05$). Control = no antioxidant; 0.5ML = 0.5% *M. oleifera* leaf powder; 1ML = 1% *M. oleifera* leaf powder; 0.5MR = 0.5% *M. oleifera* root powder; 1MR = 1% *M. oleifera* root powder; 0.5MLR = 0.5% *M. oleifera* leaf and root powder; 1MLR = 1% *M. oleifera* leaf and root powder; BHT = 0.02% butylated hydroxyl toluene

3.7. Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing antioxidant power (FRAP) of the tested natural and synthetic antioxidants is displayed in Figure 7. The results showed that FRAP was significantly lower ($p < 0.05$) in the control compared to the antioxidant treatments throughout the storage period. For all the antioxidants, the FRAP showed a decreasing trend with storage days ($p < 0.05$). The 1% root, 1% MLR and 1% ML treatment groups exhibited the highest FRAP values throughout the storage period. The 1% root FRAP values in particular, were the highest and almost comparable those of BHT, which were consistently the highest from the initial to the last storage day. Overall, FRAP values decreased as follows: Control > 0.5ML > 1ML > 1MR > 0.5MLR > 1MLR > 0.5MR > BHT.

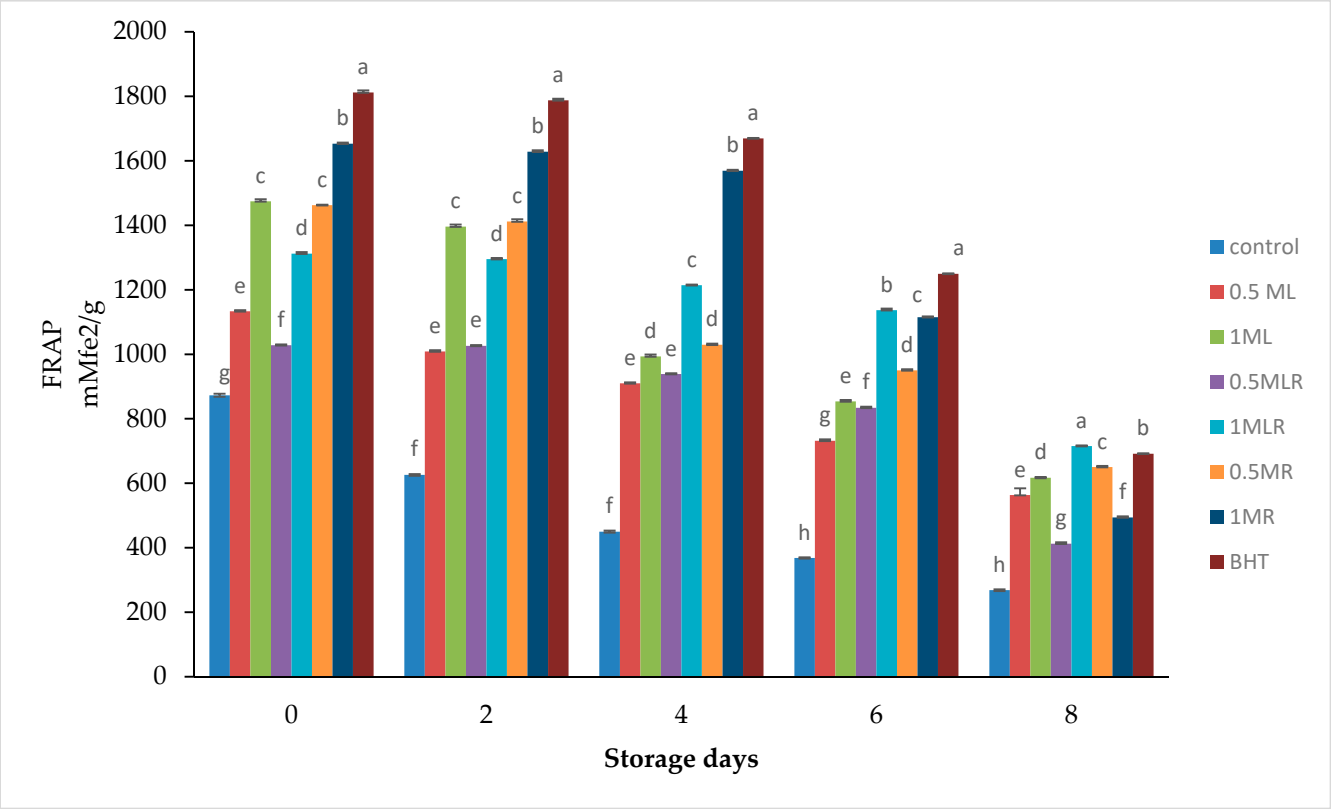

Figure 7. Ferric reducing antioxidant power (FRAP) of pre-cooked pork samples with varying levels of *M. oleifera* and 0.02% BHT incorporated. Means [a–h] that are different on the same storage day indicate significant differences ($p < 0.05$). Control = no antioxidant; LeafT0 = 0.5% *M. oleifera* leaf powder; LeafT01 = 1% *M. oleifera* leaf powder; rootT0 = 0.5% *M. oleifera* root powder; rootT01 = 1% *M. oleifera* root powder; LfxRtT0 = 0.5% *M. oleifera* leaf and root powder; LfxRtT01 = 1% *M. oleifera* leaf and root powder; SynT0 = 0.02% BHT.

4. Discussion

Phytochemicals

Plants characteristically produce polyphenolic compounds, which are secondary metabolites that play a role in the interaction of the cell with its environment to ensure that the existence of the organism in its ecosystems is sustained [31]. Secondary metabolites are involved in the protection of plants against biotic or abiotic stresses [32]. Such polyphenolic compounds include flavonoids and tannins. Research has shown that these polyphenols possess some antioxidant and antimicrobial activities. The antioxidant activity of polyphenolic compounds has been attributed to their redox properties, which allows them to act as reducing agents and as radical scavengers [24]. The results from this study showed that both the *M. oleifera* leaf and root extracts contained some appreciable amounts of polyphenols. Overall, the leaf had a higher total phenolic content compared to the root, which had higher flavonoids and proanthocynanidins. Tshabalala et al. [8] also reported higher phenolic and flavonoid contents in the leaves and roots of *M. oleifera*, respectively. On the contrary, Amaglo et al. [33] found that the leaves had the highest and most complex flavonoid concentrations. This was also similar to other studies [34,35] where the *M. oleifera* leaves were reported to possess more flavonoids when compared to the roots. These variations in the total contents of polyphenolic compounds reported from different studies could be attributed to factors like climate, harvesting time, plant genetics, stage of maturity, extraction methods used, different assay methods and standard molecules employed [35–37]. For this reason, a one-size-fits all approach in the use of *M. oleifera* as an antioxidant in the food industry (meat and meat products) or as a nutraceutical may not

be feasible. This suggests that it is crucial to quantify the compounds of the locally grown *M. oleifera* plants prior to practical application in any industry.

Antioxidant activity

For the antioxidant activity assays conducted, both the leaves and root extract exhibited strong antioxidant activity, which was almost equally comparable to that of the different standards (Rutin and BHT) used. These results are in line with findings of Falowo et al. [28], who reported high antioxidant activity of the *M. oleifera* leaves for the DPPH and ABTS assays. However, in this study the roots showed a slightly higher DPPH scavenging activity compared to the leaves. Although not so significantly different, these results corroborate findings by Tshabalala et al. [8], who also found better antioxidant activity in the roots. The high scavenging activities displayed by both the leaf and root could be attributed to the high polyphenolic contents reported earlier. According to Farasat et al. [38], there are strong positive significant correlations between free radical scavenging and contents of phenolics and flavonoids. Chai and Wong [39] also reported a similar positive correlation. This relationship could be due to phytochemical compounds like flavonoids being oxidized by radicals, yielding more stable, less-reactive radicals [40], in this way acting as antioxidants. The results from this study confirm that the *M. oleifera* leaves and roots have great free radical scavenging abilities and could potentially be used as natural sources of antioxidants. There is, however, a need to further characterize various compounds that are present in the plant extracts.

pH

On the initial storage day, there were no significant differences ($p < 0.05$) in the pH of the minced cooked pork treated with antioxidants and the control that had no antioxidants, except for the 0.5ML treatment group, which had slightly lower pH values. The pH values were found to be increasing with increasing storage days for all the treatments. These findings are similar to the findings of Muthukumar et al. [23], who reported increasing pH values with increasing storage days in cooked pork meat and patties treated with grape seed, bearberry extracts and *M. oleifera* leaf extract, respectively. Das et al. [41] also reported a similar trend in the pH of minced cooked goat meat treated with *Murraya Koenigii*. This observed increase of pH over storage days may be attributed to the buildup of metabolites due to bacterial activity on meat protein and amino acids [42]. When the glucose reservoirs in meat are depleted, bacteria start to utilize amino acids formed during protein breakdown, resulting in the production and accumulation of ammonia, which increases the pH [43,44]. The variations in pH across treatments could be attributed to the rate of utilization of amino acids by bacteria in the presence of the different natural and synthetic antioxidants at different concentrations. The higher pH values in the control throughout the storage period could possibly mean there was less bacterial activity inhibition compared to the natural- and synthetic-antioxidant-treated samples, which had the ability to slow down or inhibit microbial growth. This is also supported by Babuskin et al. [44], who reported that lower pH measured for chicken meat incorporated with spice extracts could have been due to the inhibitory effect of antimicrobial ingredients present in the natural spice extracts on the growth and propagation of spoilage microorganisms that break down basic nitrogen compounds.

Colour

The instrumental CIE values obtained for lightness (L*) varied across the treatment groups and over the storage days, with the control exhibiting slightly higher values compared to the antioxidant treatment groups. Similar results were also found by Akcan et al. [45], who reported lower L* values in cooked meatballs treated with *Laurus nobilis* and *Salvia officinalis*. Falowo et al. [46] found that the L* values of ground beef treated with *Ocimum basilicum L.* were lower than those of the control. In the present study, the lower L* values of *M. oleifera*-treated meats may have resulted from the inherent plant pigment materials, such as chlorophylls. The results from this study further revealed that the 1MLR treatment had the lowest L* values. Muthukumar et al. [23] also reported similar

results, where 600 ppm equivalent *M. oleifera* phenolics and 200 ppm BHT exhibited the lowest L* values in pre-cooked pork patties. The lightness values gradually increased with an increase in storage days, a trend that was also reported by Falowo et al. [28] for ground beef treated with *M. oleifera* leaves and *Bidens pilosa*.

The redness a* values showed an opposing trend of decrease with increasing storage days. The decrease in the redness values has been attributed to the oxidation of oxymyoglobin (OxyMb) to metmyoglobin (MetMb) [47]. This is supported by Yin and Cheng [48], who found higher MetMb concentrations in ground beef that had no antioxidants (control) compared to beef that was treated with garlic-derived compounds. In addition, the cooking of pork could have been a contributory factor to the reduction of redness, because internal cooking temperature has been reported to accelerate myoglobin denaturation. This is further supported by Fox [49], who stated that the red-brownish color of cooked meats is primarily determined by the occurrence of denatured-globin hemochromes produced as a result of high temperatures. The redness values in the antioxidant-treated samples were higher than that of the control. The 1MR treatment group exhibited the highest a* values. This could be attributed to the added antioxidants being able to stabilize the color of the pork. The yellowness b* values found from this present study were constant and decreased gradually over the storage days. Chroma values showed a significant decrease during storage across all treatment groups. A similar trend was reported by [50], and this could have been attributed to the increased a* values due to the addition of *M. oleifera*.

Thiobarbituric Acid Substances (TBARS)

Common processing techniques such as mincing, cooking and salt addition enhance the formation of reactive oxygen species (ROS), making meat more susceptible to lipid oxidation. In recent years, owing to their health benefits and their potential use as natural food preservatives, plant-derived antioxidants have been proposed as a possible solution to the problem of lipid oxidation in processed meats. The results from the present study confirm the aforementioned hypothesis. Secondary oxidation products determined by the thiobarbituric acid reactive substances (TBARS) that are expressed as malonaldehyde/kg sample are a recognized measure of lipid oxidation in meat and meat products [51]. Lower TBARS are an indication of reduced lipid oxidation, and vice versa. The TBARS threshold values for detection of rancidity in cooked meats ranges from 1.0–2.2 (mg MDA/kg sample) [52,53]. The aforementioned values vary depending on the type of meat or meat products, as well as the procedure used in measuring TBARS. The results from this study revealed that the TBARS values of the control were significantly higher ($p < 0.05$) than those of samples treated with antioxidants for all the storage days. The results further showed that the pork samples in the control group had a rapid increase of the TBARS values compared to the antioxidant-treated pork samples, which means the antioxidants were able to retard lipid oxidation. These findings corroborate those of Jayawardana et al. [54], who reported significantly lower TBARS values in chicken sausages incorporated with 0.5, 0.75 and 1% *M. oleifera* leaf powder compared to the control treatment. In the present study, the TBARS values among the different *M. oleifera* leaves and roots treatments groups were significantly different ($p < 0.05$). These results are different from the findings of Mukumbo [55], who reported no significant differences in the TBARS values of pork droëwors treated with varying levels of *M. oleifera* leaf powder. This could be attributed to the *M. oleifera* being of Senegalese origin and the *M. oleifera* used in this study being grown in South Africa. Therefore, climatic and other factors could have played a role in plant composition variations, hence showing different antioxidant potentials. Notwithstanding, the *M. oleifera* plant's ability to reduce TBARS values could be attributed to its richness in phytochemicals that have the capacity to donate electrons and react with free radicals, converting them into more stable products, and in this way terminating free radical chain reactions [16].

With reference to the phytochemical content and in vitro antioxidant activity of the *M. oleifera* leaves and root extracts used in this study, one can confirm that *M. oleifera* inhibited lipid oxidation in meat due to its inherent phytochemical compounds which have the ability to scavenge free radicals. To the authors' knowledge, no studies have

been done to test the antioxidant capacity of the roots in pre-cooked meats. It is noteworthy that the present study revealed that the overall mean TBARS values of the MR treatments were lower than those of ML treatments. This is in agreement with findings by Tshabalala et al. [8], who found that the roots exhibited greater in vitro antioxidant potential compared to the leaves. The potential of the root as an antioxidant in meat and meat products remains largely unexamined, despite the substantial amount of literature highlighting its antioxidant properties. There is still a need for more studies that will focus on using the roots as an antioxidant in processed meats to further validate findings from this study. In summary, the TBARS values of the different treatments decreased in the following manner: Control > 0.5ML > 1ML > 0.5MR > 1MR > 0.5MLR > BHT > 1MLR.

Ferric Reducing Antioxidant Power (FRAP)

Transition metals can be utilized as catalysts that stimulate the formation of the first few radicals, and by so doing, initiating oxidative chain reactions. Ferrous ion is recognized to be a strong lipid oxidation pro-oxidant because of its high reactivity. The reducing power of a compound is one of the primary indicators of its potential antioxidant activity. The ability of the natural antioxidants to reduce ferricyanide (Fe^{3+}) complex to the ferrous form (Fe^{2+}) were examined in the present study. The results from this study showed that FRAP was significantly lower ($p < 0.05$) in the control compared to the antioxidant treatment groups throughout the storage period. These results are in line with findings of Kong et al. [56] who reported that spice extracts in cooked pork patties caused the reduction of the Fe^{3+} complex to the ferrous form. For all the samples treated with BHT and *M. oleifera*, FRAP showed a decreasing trend with storage days. The previously reported TBARS values are in agreement with the FRAP values, indicating an inverse relationship between the two which can be explained as follows: as the ferric reducing power of the antioxidants decreased, lipid oxidation was also slowly increasing. The 1MR, 1MLR and 1ML treatment groups exhibited the highest FRAP values throughout the storage period. The 1MR FRAP values, in particular, were comparable to those of BHT. This again is in agreement with earlier findings of the root being potentially a better source of antioxidants. In the present study, the high FRAP values in the natural-antioxidant-treated pork samples could be attributed to the chemical composition and structure of the plant phytochemicals such as flavones and flavonols, flavanones and dihydroflavonols, hidrolisated and condensed tannins, and total phenolics, which have been reported to have some antioxidant activity. Plant polyphenols have a structure which possesses a hydroxyl (OH) group, which has a considerable capability to chelate ferrous ion (Fe^{2+}) [56]. In support of the aforementioned statement, it has been previously proposed that natural phenolics, including rutin, quercetin, caffeic acid and catechin, could act as Fe^{2+} chelators [57].

5. Conclusions

The present study revealed that the application of *M. oleifera* leaves and roots can slow down the process of lipid oxidation in cooked minced meat. The *M. oleifera* leaf and root powder's antioxidant activities were comparable to that of synthetic antioxidant BHT. Generally, the root powder demonstrated superior antioxidant potential over the leaf powder. These slight differences in the antioxidant activities of the leaves and roots could be attributed to the varying contents of their inherent bioactive compounds. Notwithstanding, both the *M. oleifera* leaves and roots powders showed potent antioxidant capacities which can be beneficial to the meat processing industry for the improvement of quality attributes during storage. Findings from this study warrant further studies to perform other biochemical characterization of various *M. oleifera* compounds which may be beneficial in prolonging the shelf-life of meat.

Author Contributions: N.S.L. and A.J.A. conceptualized and designed the work. N.S.L. was accountable for conducting the research. N.S.L., A.J.A., R.S.T. and E.M.I. drafted and proofread the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by National Research Fund Collaborative Postgraduate Training Programme, grant number T693, and NRF Research Incentive grant, Project No. 979.

Institutional Review Board Statement: The study was ethically conducted. Ethical clearance (AFO031SLUN01/19/A) was granted by the University of Fort Hare Research Ethics Committee.

Informed Consent Statement: Not applicable.

Acknowledgments: The authors are grateful to the University of Fort Hare Govan Mbeki Research and Development Centre (GMRDC) meat science research group and the MPED Research Center for their support during this research.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Estévez, M. What's New in Meat Oxidation? In *New Aspects of Meat Quality*; Woodhead Publishing: Sawston, UK, 2017; pp. 91–109.
2. Soyer, A.; Özalp, B.; Dalmış, Ü.; Bilgin, V. Effects of freezing temperature and duration of frozen storage on lipid and protein oxidation in chicken meat. *Food Chem.* **2010**, *120*, 1025–1030. [CrossRef]
3. Turgut, S.S.; Işıkçı, F.; Soyer, A. Antioxidant activity of pomegranate peel extract on lipid and protein oxidation in beef meatballs during frozen storage. *Meat Sci.* **2017**, *129*, 111–119. [CrossRef]
4. Kim, S.J.; Min, S.C.; Shin, H.J.; Lee, Y.J.; Cho, A.R.; Kim, S.Y.; Han, J. Evaluation of the antioxidant activities and nutritional properties of ten edible plant extracts and their application to fresh ground beef. *Meat Sci.* **2013**, *93*, 715–722. [CrossRef]
5. Negre-Salvayre, A.; Coatrieux, C.; Ingueneau, C.; Salvayre, R. Advanced lipid peroxidation end products in oxidative damage to proteins. Potential role in diseases and therapeutic prospects for the inhibitors. *Brit. J. Pharmacol.* **2008**, *153*, 6–20. [CrossRef]
6. Kumar, Y.; Mehta, N.; Anurag, R.K.; Sethi, S.; Bashir, A.A.; Kumar, V.; Narsaiah, K. Improving Meat Safety through Reformulation Strategies: Natural Antioxidants and Antimicrobials. In *Reformulation as a Strategy for Developing Healthier Food Products*; Springer: Cham, Switzerland, 2019; pp. 251–289.
7. Kumar, Y.; Yadav, D.N.; Ahmad, T.; Narsaiah, K. Recent trends in the use of natural antioxidants for meat and meat products. *Compr. Rev. Food Sci. Food Saf.* **2015**, *14*, 796–812. [CrossRef]
8. Tshabalala, T.; Ndhlala, A.R.; Ncube, B.; Abdelgadir, H.A.; Van Staden, J. Potential substitution of the root with the leaf in the use of *Moringa oleifera* for antimicrobial, antidiabetic and antioxidant properties. *S. Afr. J. Bot.* **2019**. [CrossRef]
9. Moyo, B.; Oyedemi, S.; Masika, P.J.; Muchenje, V. Polyphenolic content and antioxidant properties of *Moringa oleifera* leaf extracts and enzymatic activity of liver from goats supplemented with *Moringa oleifera* leaves or sunflower seed cake. *Meat Sci.* **2012**, *91*, 441–447. [CrossRef] [PubMed]
10. Qwele, K.; Hugo, A.; Oyedemi, S.O.; Moyo, B.; Masika, P.J.; Muchenje, V. Chemical composition, fatty acid content and antioxidant potential of meat from goats supplemented with Moringa (*Moringa oleifera*) leaves, sunflower cake and grass hay. *Meat Sci.* **2013**, *93*, 455–462. [CrossRef] [PubMed]
11. Reyes-Sànchez, N.; Spörndly, E.; Ledin, I. Effect of feeding different levels of foliage of *Moringa oleifera* to creole dairy cows on intake, digestibility, milk production and composition. *Livest. Sci.* **2006**, *101*, 24–31. [CrossRef]
12. Mendieta-Araica, B.; Spörndly, R.; Reyes-Sànchez, N.; Spörndly, E. Moringa (Moringa oleifera) leaf meal as a source of protein in locally produced concentrates for dairy cows fed low protein diets in tropical areas. *Livest. Sci.* **2011**, *137*, 10–17. [CrossRef]
13. Wapi, C.; Nkukwana, T.T.; Hoffman, L.C.; Dzama, K.; Pieterse, E.; Mabusela, T.; Muchenje, V. Physico-chemical shelf-life indicators of meat from broilers given *Moringa oleifera* leaf meal. *S. Afr. J. Anim. Sci.* **2013**, *43* (Suppl. 1), 43–47. [CrossRef]
14. Nkukwana, T.T.; Muchenje, V.; Masika, P.J.; Hoffman, L.C.; Dzama, K.; Descalzo, A.M. Fatty acid composition and oxidative stability of breast meat from broiler chickens supplemented with *Moringa oleifera* leaf meal over a period of refrigeration. *Food Chem.* **2014**, *142*, 255–261. [CrossRef] [PubMed]
15. Mukumbo, F.E.; Maphosa, V.; Hugo, A.; Nkukwana, T.T.; Mabusela, T.P.; Muchenje, V. Effect of *Moringa oleifera* leaf meal on finisher pig growth performance, meat quality, shelf life and fatty acid composition of pork. *S. Afr. J. Anim. Sci.* **2014**, *44*, 388–400. [CrossRef]
16. Siddhuraju, P.; Becker, K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *J. Agric. Food Chem.* **2003**, *51*, 2144–2155. [CrossRef] [PubMed]
17. Anwar, F.; Sajid, L.; Muhammad, A.; Anwarul, H.G. Moringa oleifera: A Food plant with Multiple Medicinal Uses. *Phytother. Res.* **2007**, *21*, 17–25. [CrossRef]
18. Udechukwu, M.C.; Abbey, L.; Nwodo, U.; Udenigwe, C.C. Potential of *Moringa oleifera* seeds and leaves as functional food ingredients for human health promotion. *J. Food. Nutr. Res.* **2018**, *57*, 1–14.
19. Fahey, J.W. Moringa oleifera: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. *Trees Life J.* **2005**, *1*, 21205–22185.
20. Mahmood, K.T.; Mugal, T.; Haq, I.U. Moringa oleifera: A natural gift-A review. *J. Pharm. Sci. Res.* **2010**, *2*, 775.
21. Falowo, A.B.; Mukumbo, F.E.; Idamokoro, E.M.; Lorenzo, J.M.; Afolayan, A.J.; Muchenje, V. Multi-functional application of *Moringa oleifera* Lam. in nutrition and animal food products: A review. *Food Res. Int.* **2018**, *106*, 317–334. [CrossRef]

22. Rhee, K.S.; Anderson, L.M.; Sams, A.R. Lipid oxidation potential of beef, chicken, and pork. *J. Food Sci.* **1996**, *61*, 8–12. [CrossRef]
23. Muthukumar, M.; Naveena, B.M.; Vaithiyanathan, S.; Sen, A.R.; Sureshkumar, K. Effect of incorporation of *Moringa oleifera* leaves extract on quality of ground pork patties. *J. Food Sci. Technol.* **2014**, *51*, 3172–3180. [CrossRef] [PubMed]
24. Unuofin, J.O.; Otunola, G.A.; Afolayan, A.J. Phytochemical screening and in vitro evaluation of antioxidant and antimicrobial activities of *Kedrostis africana* (L.) *Cogn. Asian Pac. J. Trop. Biomed.* **2017**, *7*, 901–908. [CrossRef]
25. Kibiti, C.M.; Afolayan, A.J. Preliminary phytochemical screening and biological activities of Bulbine abyssinica used in the folk medicine in the Eastern Cape Province, South Africa. *Evid.-Based Complement. Altern. Med.* **2015**. [CrossRef] [PubMed]
26. Ohikhena, F.U.; Wintola, O.A.; Afolayan, A.J. Quantitative phytochemical constituents and antioxidant activities of the Mistletoe, Phragmanthera capitata (Sprengel) Balle extracted with different solvents. *Pharmacogn. Res.* **2018**, *10*, 16.
27. Akinrinde, A.S.; Afolayan, A.J.; Bradley, G. Phytochemical composition and antioxidant activities of Dianthus Thunbergii hooper and Hypoxis Argentea harv ex baker: Plants used for the management of diabetes mellitus in Eastern Cape, South Africa. *Pharmacogn. Maga.* **2018**, *14*, 195.
28. Falowo, A.B.; Muchenje, V.; Hugo, A.; Aiyegoro, O.A.; Fayemi, P.O. Antioxidant activities of *Moringa oleifera* L. and *Bidens pilosa* L. leaf extracts and their effects on oxidative stability of ground raw beef during refrigeration storage. *CyTA-J. Food* **2017**, *15*, 249–256. [CrossRef]
29. Descalzo, A.M.; Insani, E.M.; Biolatti, A.; Sancho, A.M.; García, P.T.; Pensel, N.A.; Josifovich, J.A. Influence of pasture or grain-based diets supplemented with vitamin E on antioxidant/oxidative balance of Argentine beef. *Meat Sci.* **2005**, *70*, 35–44. [CrossRef]
30. Arshad, M.S.; Anjum, F.M.; Khan, M.I.; Shahid, M.; Akhtar, S.; Sohaib, M. Wheat germ oil enrichment in broiler feed with α-lipoic acid to enhance the antioxidant potential and lipid stability of meat. *Lipids Health Dis.* **2013**, *12*, 164. [CrossRef] [PubMed]
31. Pagare, S.; Bhatia, M.; Tripathi, N.; Pagare, S.; Bansal, Y.K. Secondary metabolites of plants and their role: Overview. *Curr. Trends Biotechnol. Pharm.* **2015**, *11*, 705–719.
32. Bhattacharya, A.; Sood, P.; Citovsky, V. The roles of plant phenolics in defence and communication during Agrobacterium and Rhizobium infection. Molecular Plant Pathology Concentration of plant extracts for bacteria. *Mol. Plant Pathol.* **2010**, *64*, 711–713.
33. Amaglo, N.K.; Bennett, R.N.; Curto, R.B.L.; Rosa, E.A.; Turco, V.L.; Giuffrida, A.; Curto, A.L.; Crea, F.; Timpo, G.M. Profiling selected phytochemicals and nutrients in different tissues of the multipurpose tree *Moringa oleifera* L., grown in Ghana. *Food Chem.* **2010**, *122*, 1047–1054. [CrossRef]
34. Taylor, J.L.S.; Van Staden, J. The effect of age, season and growth conditions on anti-inflammatory activity in *Eucomis autumnalis* (Mill.) Chitt. Plant extracts. *Plant Growth Regul.* **2001**, *34*, 39–47. [CrossRef]
35. Xu, Y.B.; Chen, G.L.; Guo, M.Q. Antioxidant and anti-inflammatory activities of the crude extracts of *Moringa oleifera* from Kenya and their correlations with flavonoids. *Antioxidants* **2019**, *8*, 296. [CrossRef]
36. Ncube, B.; Finnie, J.F.; Van Staden, J. Quality from the field: The impact of environmental factors as quality determinants in medicinal plants. *S. Afr. J. Bot.* **2012**, *82*, 11–20. [CrossRef]
37. Leone, A.; Fiorillo, G.; Criscuoli, F.; Ravasenghi, S.; Santagostini, L.; Fico, G.; Spadafranca, A.; Battezzati, A.; Schiraldi, A.; Pozzi, F.; et al. Nutritional characterization and phenolic profiling of *Moringa oleifera* leaves grown in Chad, Sahrawi Refugee Camps, and Haiti. *Int. J. Mol. Sci.* **2015**, *16*, 18923–18937. [CrossRef] [PubMed]
38. Farasat, M.; Khavari-Nejad, R.A.; Nabavi, S.M.B.; Namjooyan, F. Antioxidant activity, total phenolics and flavonoid contents of some edible green seaweeds from northern coasts of the Persian Gulf. *Iran. J. Pharm. Res.* **2014**, *13*, 163.
39. Chai, T.T.; Wong, F.C. Whole-plant profiling of total phenolic and flavonoid contents, antioxidant capacity and nitric oxide scavenging capacity of Turnera subulata. *J. Med. Plants Res.* **2012**, *6*, 1730–1735.
40. Nijveldt, R.J.; Van Nood, E.L.S.; Van Hoorn, D.E.; Boelens, P.G.; Van Norren, K.; Van Leeuwen, P.A. 2001. Flavonoids: A review of probable mechanisms of action and potential applications. *Am. J. Clin. Nutr.* **2001**, *74*, 418–425. [CrossRef]
41. Das, A.K.; Rajkumar, V.; Dwivedi, D.K. Antioxidant effect of curry leaf (*Murraya koenigii*) powder on quality of ground and cooked goat meat. *Int. Food Res. J.* **2011**, *18*, 563–569.
42. Shah, M.A.; Bosco, S.J.D.; Mir, S.A. Effect of *Moringa oleifera* leaf extract on the physicochemical properties of modified atmosphere packaged raw beef. *Food Packag. Shelf Life* **2015**, *3*, 31–38. [CrossRef]
43. Gill, C.O. Meat spoilage and evaluation of the potential storage life of fresh meat. *J. Food Protect.* **1983**, *46*, 444–452. [CrossRef]
44. Babuskin, S.; Babu, P.A.S.; Sasikala, M.; Sabina, K.; Archana, G.; Sivarajan, M.; Sukumar, M. Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. *Int. J. Food Microbiol.* **2014**, *171*, 32–40.
45. Akcan, T.; Estévez, M.; Serdaroğlu, M. Antioxidant protection of cooked meatballs during frozen storage by whey protein edible films with phytochemicals from Laurus nobilis L. and Salvia officinalis. *LWT* **2017**, *77*, 323–331. [CrossRef]
46. Falowo, A.B.; Mukumbo, F.E.; Idamokoro, E.M.; Afolayan, A.J.; Muchenje, V. Phytochemical Constituents and Antioxidant Activity of Sweet Basil (*Ocimum basilicum* L.) Essential Oil on Ground Beef from Boran and Nguni Cattle. *Int. J. Food Sci.* **2019**. [CrossRef] [PubMed]
47. Faustman, C.; Sun, Q.; Mancini, R.; Suman, S.P. Myoglobin and lipid oxidation interactions: Mechanistic bases and control. *Meat. Sci.* **2010**, *86*, 86–94. [CrossRef] [PubMed]
48. Yin, M.C.; Cheng, W.S. Antioxidant and antimicrobial effects of four garlic-derived organosulfur compounds in ground beef. *Meat Sci.* **2003**, *63*, 23–28. [CrossRef]

49. Fox, J.B., Jr. The Pigments of Meat. In *The Science of Meat and Meat Products*, 3rd ed.; Price, J., Schweigert, B.S., Eds.; Food and Nutrition Press: Westport, CT, USA, 1987; pp. 193–216.
50. Mashau, M.E.; Ramatsetse, K.E.; Ramasha, S.E. Effects of adding *Moringa oleifera* leaves Powder on the Nutritional Properties, Lipid Oxidation and Microbial Growth in ground beef during storage. *Appl. Sci.* **2021**, *11*, 2944. [CrossRef]
51. Pal, J.; Raju, C.V.; Lakshmisha, I.P.; Pandey, G.; Raj, R.; Singh, R.R. Antioxidant activity of pomegranate peel extract and its effect on storage stability of cooked meat model system of Indian mackerel (*Rastrelliger kanagurta*) stored at 4±2 C. *Biochem. Cell. Arch.* **2017**, *17*, 183–1837.
52. Rahman, M.H.; Hoissain, M.M.; Rahman, S.M.E.; Amin, M.R.; Oh, D.H. Evaluation of physicochemical deterioration and lipid oxidation of beef muscle affected by freeze-thaw cycles. *Korean J. Food Sci. Anim. Resour.* **2015**, *35*, 772. [CrossRef]
53. Dominguez, R.; Pateiro, M.; Gagaoua, M.; Barba, F.J.; Zhang, W.; Lorenzo, J.M. A comprehensive Review on Lipid Oxidation in Meat and Meat Products. *Antioxidants* **2019**, *10*, 429. [CrossRef]
54. Jayawardana, B.C.; Liyanage, R.; Lalantha, N.; Iddamalgoda, S.; Weththasinghe, P. Antioxidant and antimicrobial activity of drumstick (*Moringa oleifera*) leaves in herbal chicken sausages. *LWT-Food Sci. Technol.* **2015**, *64*, 1204–1208. [CrossRef]
55. Mukumbo, F.E. *Moringa oleifera* Leaf Powder as a Functional Antioxidant Additive in Pork droëwors. Ph.D. Thesis, University of Fort Hare, Alice, South Africa, 2016.
56. Kong, B.; Zhang, H.; Xiong, Y.L. Antioxidant activity of spice extracts in a liposome system and in cooked pork patties and the possible mode of action. *Meat Sci.* **2010**, *85*, 772–778. [CrossRef] [PubMed]
57. Chen, X.; Ahn, D.U. Antioxidant activities of six natural phenolics against lipid oxidation induced by Fe^{2+} or ultraviolet light. *J. Am. Oil Chem. Soc.* **1998**, *75*, 1717. [CrossRef]