Lipid Oxidation

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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.

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

1. Introduction

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 [1][2]. 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 [3]. 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) [4].

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 [5]. 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 [6]. 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 [7]. The *M. oleifera* plant has been extensively researched as a medicinal plant and animal feed additive in goats [8][9], cattle [10][11], chickens [12][13] and pigs [14], 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 [15]. The *M. oleifera* plant has earned names including “miracle tree”, tree of life, and God’s gift to man [16][17], 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) [16]. The *M. oleifera* plant is rich in minerals and nutrients, including amino acids, vitamin C, calcium and potassium [18][19][20]. Hence, it has been used to improve nutrition in some developing countries [18]. 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.

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

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 [21]. Secondary metabolites are involved in the protection of plants against biotic or abiotic stresses [22]. 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 [23]. 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. [7] also reported higher phenolic and flavonoid contents in the leaves and roots of *M. oleifera,* respectively. On the contrary, Amaglo et al. [24] found that the leaves had the highest and most complex flavonoid concentrations. This was also similar to other studies [25][26] 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 [26][27][28]. 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. [29], 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. [7], 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. [30], there are strong positive significant correlations between free radical scavenging and contents of phenolics and flavonoids. Chai and Wong [31] 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 [32], 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. [33], 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. [34] 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 [35]. 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 [36][37]. 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. [37], 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. [38], who reported lower L* values in cooked meatballs treated with *Laurus nobilis* and *Salvia officinalis*. Falowo et al. [39] 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. [33] 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. [29] 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) [40]. This is supported by Yin and Cheng [41], 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 [42], 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 [43], 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 [44]. 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) [45][46]. 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. [47], 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 [48], 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 [15].

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. [7], 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. [49] 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+}) [49]. 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 [50].

3. Conclusions

It 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.

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