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Antioxidant and antimicrobial activity of drumstick (*Moringa oleifera*) leaves in herbal chicken sausages



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ARTICLE INFO

Article history:

Received 30 March 2015

Received in revised form

3 July 2015

Accepted 12 July 2015

Available online 15 July 2015

Keywords:

Natural antioxidant

Natural antimicrobial preservative

Moringa oleifera

Chicken sausage

Deterioration

ABSTRACT

Drumstick (*Moringa oleifera*) leaves were evaluated for antioxidative capacity and antimicrobial activity when incorporated in chicken sausages. Different concentrations (0.25%, 0.5%, 0.75% and 1%) of *M. oleifera* leaves (MOL) incorporated sausages and two controls without MOL (one with added artificial antioxidant and other without any antioxidant) were prepared. TBARS value, pH, microbial analysis, sensory panel scores and instrumental color were assessed. Sausages with 0.5%, 0.75% and 1% MOL showed significantly lower ($p < 0.05$) TBARS value compared to 0.25% MOL and the two control samples. Sausages with 0.5%, 0.75% and 1% MOL showed significantly ($p < 0.05$) low pH values from the 2nd week to the 5th week of storage and significantly ($p < 0.05$) low Total Plate Count throughout the storage period, compared to 0.25% MOL and the two control samples. The sensory panel did not detect any difference in any sensory attribute in chicken sausages with 0.25% and 0.5% MOL compared to the controls. The study identifies the significant antioxidant and antimicrobial potential of Drumstick leaves in chicken sausages.

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1. Introduction

Chicken sausage is a minced meat product which has seen a dramatic increase in consumption throughout the world. Lipid oxidation and microbial growth are major causes of deterioration and reduced shelf life in minced meat products. Lipid oxidation may produce changes in meat quality parameters such as color, flavor, odor, texture and even nutritional value (Aguirrezábal, Mateo, Domínguez, & Zumalcáarregui, 2000) while microbial contamination can cause public health hazards and economic loss in terms of food poisoning and meat spoilage.

Previous research has indicated that lipid oxidation and microbial growth in meat products can be controlled or minimized by using either synthetic or natural food additives (Gray, Gomaa, & Buckley, 1996; Lee, Williams, Sloan, & Littell, 1997; Mielnik, Aaby, & Skrede, 2003). Synthetic antioxidants such as butylated

hydroxyanisole (BHA), butylated hydroxytoluene, (BHT) and tert-butyl hydroquinone (TBHQ) are commonly used in the food industry. However, some human health professionals and consumers (Decker & Mei, 1996) are concerned about the safety of such synthetic additives, which has led many meat processors to seek alternative “natural” antioxidants. Natural agents possessing both antioxidant and antimicrobial properties have the advantage of been readily accepted by both consumers and meat processors (Sallam, Ishioroshi, & Samejima, 2004).

Moringa oleifera is widely cultivated in Sri Lanka and other South Asian countries. It has both nutritional and medicinal value including some useful vitamins, minerals and amino acids, etc. (Sánchez-Machado, Núñez-Gastélum, Reyes-Moreno, Ramírez-Wong, & López-Cervantes, 2010). Immature pods (fruit) of *M. oleifera* is a very popular vegetable in Sri Lanka but almost all parts of this plant such as leaves, roots, bark, flowers and seeds are used to treat various ailments in indigenous medicine in Sri Lanka and other South Asian countries, including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, hematological and hepatorenal disorders. (Morimitsu et al., 2000). The leaves are an exceptionally good source of protein, provitamin A, vitamins B and C, minerals

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(particularly iron) and a rich source of essential amino acids such as methionine, cystine, tryptophan and lysine (Makkar & Becker, 1997; Sánchez-Machado et al., 2010).

M. oleifera leaves have been identified as having high antioxidant and antimicrobial activity (Das, Rajkumar, Verma, & Swarup, 2012) due to the presence of ascorbic acid, flavonoids, phenolics and carotenoids (Anwar, Latif, Ashraf, & Gilani, 2007; Makkar & Becker, 1996; Moyo, Oyedemi, Masika, & Muchenje, 2012). It has also been reported that Moringa leaves significantly increase the shelf life of ghee (Siddhuraju & Becker, 2003) and more recently it has been reported that mature *M. oleifera* leaf extracts protect goat meat patties against oxidative rancidity (Das et al., 2012). Dahot (1998) isolated small protein/peptides from the leaves of *M. oleifera* (Drumstick leaves) possessing antifungal and antimicrobial characteristics.

M. oleifera leaves, therefore, have an established history as a biopreservative in food applications and nutraceuticals. However, there have been very few attempts, so far, to investigate the antioxidant and antimicrobial effect of *M. oleifera* in meat products (Das et al., 2012; Hazra, Biswas, Bhattacharyya, Das, & Khan, 2012). The aim of this study was to evaluate the antioxidant and antimicrobial efficacies of *M. oleifera* leaves (MOL)¹ and to determine their effect on color and sensory attributes of chicken sausages.

2. Materials and methods

2.1. Preparation of *M. oleifera* leaf powder

M. oleifera leaves were obtained from the Kandy district in Sri Lanka. Leaves were then separated from stalks and all the immature and fully mature leaves were discarded. Selected leaves were thoroughly washed initially with drinking water followed by distilled water. Thereafter, leaves were spread in trays lined with tissue papers and allowed to air dry until the weight of the leaves became constant (moisture content 8%). Dried leaves were crushed in to small particles using mortar and pestle. Finally Moringa leaf particles were vacuum packed and stored in a cool dry location for future use. The moisture content of *M. oleifera* leaf particles was calculated according to the AOAC method, 1995.

Antioxidant activity was analyzed using the 1, 1-Diphenyl 1-2-picryl-hydrazyl (DPPH) method (Brand-Williams, Cuvelier, & Berset, 1995).

2.2. Preparation of chicken sausages for testing the antioxidant and antimicrobial efficacy of MOL

Chicken sausages were prepared to test the antioxidant and antimicrobial efficacy of MOL by measuring 2-thiobarbituric acid-reactive substances (TBARS)² and microbial count. Sausage processing was performed at Keels Food Products PLC, Sri Lanka under strict hygienic conditions. Frozen boneless chicken thigh meat was thawed at 10 °C just before the sausages were prepared. The thigh meat and subcutaneous fat, which was scraped from the outermost layer of skin, were ground together in a 0.4 cm grinder plate (Guangdong Henglian Food Machinery Co. Ltd, China). The ground meat was then formulated to contain four different concentrations of MOL (T1 = 0.25%, T2 = 0.50%, T3 = 0.75%, T4 = 1%) and two controls without MOL, Control (T5) with the synthetic antioxidant BHT (0.04%) and control (T6) without BHT. MOL and BHT were calculated based on the wet weight of the sausages. During the preparation of sausages, the

quantity of ice flakes was replaced by MOL. Three replicates were performed and measurements were made in duplicate. After stuffing and cooking, all the samples were stored at 4 °C overnight and the next day sensory evaluation was carried out. Instrumental color evaluation was carried out on the 1st, 3rd and 5th days of storage at 4 °C. pH, microbial analysis (Total Plate Count, *Escherichia coli*, *Staphylococcus aureus*) were analyzed during the 1st, 2nd, 3rd, 4th and 5th weeks of storage under refrigerated conditions (4 °C).

2.3. Antioxidant activity and polyphenol content

2.3.1. Determination of TBARS

Two grams of each sample was placed in a centrifuge tube to which 5 ml of a 10% (W/V) solution of trichloroacetic acid (TCA)³ was added and vortexed (K-550-GE, USA) for 2 min. Five milliliters of 0.02 M aqueous solution of 2-thiobarbituric acid were added to each centrifuge tube and was further vortexed for 30 s. The samples were then centrifuged at 3000 × g (MF6 Hitachi, Japan) for 10 min and the supernatants were filtered through a Whatman No.3 filter paper. Filtrates were heated in a boiling water bath for 45 min, cooled to room temperature in ice, and the absorbance of the resulting pigment was read at 532 nm using a UV spectrophotometer (D-17, Shimadzu, Japan). TBARS values were calculated by multiplying the absorbance reading by the factor, which was obtained from a standard line prepared using 1, 1, 3, 3-tetramethoxypropane as a precursor of malonaldehyde.

2.3.2. Determination of total polyphenol content

The total phenolic content was determined using the Folin-Ciocalteu method (Singleton, Joseph, & Rossi, 1965) with a few modifications. One gram of dried Moringa leaf powder was extracted into 80% methanol. A volume of 125 µl of the sample solution and 125 µl of the Folin-Ciocalteu reagent was mixed and vortexed for 1 min and allowed to stand for 5 min at room temperature. 1.75 ml of 5% Sodium carbonate in water was added and allowed to react for 1.5 h at room temperature. The absorbance was measured at 765 nm on a UV-visible spectrophotometer. Gallic acid was used as the standard solution.

2.4. pH and instrumental color evaluation

For determination of the pH, 10 g of sample was homogenized with 50 ml distilled water and the pH value was measured using a digital pH-meter (HM-5S; TOA Electric Industrial Co. Ltd., Tokyo, Japan). The effect of MOL on color properties (L*, a* and b*) of cooked sausages was evaluated using a chroma meter (5104, No: 453, WPA Linton Cambridge UK).

2.5. Microbial analysis

Frozen samples were thawed to room temperature (28 °C) and 10 g of each sample was homogenized with 90 mL buffered peptone and was properly blended. 1 mL dilutions were inoculated onto TPC petrifilm (Petrifilm^R 3M) to obtain Total Plate Count and incubated at 30 °C for 48 h. Furthermore, 1 ml dilutions were inoculated on to *E coli* petrifilm (3MTM Petrifilm *E coli*/Coliform count plates) and Baird Parker medium (Oxoid, UK) to obtain *E. coli* and *S. aureus* count respectively at 37 °C for 24 h.

¹ MOL: *Moringa oleifera* leaves.

² TBARS: 2-thiobarbituric acid-reactive substances.

³ TCA: Trichloroacetic acid.

2.6. Sensory analysis

The sensory evaluation was performed by 30 randomly selected panelists – 15 were trained and 15 untrained. Thirty gram sausage samples from each batch were served to each panelist after 2 min of frying. A hedonic test was carried out to select the most consumer preferred sausage among the four MOL incorporated sausage types (T1–T4) and the positive control (T5). Different sensory attributes, such as color, odor, texture, juiciness, taste and overall acceptability was determined by using a five point Hedonic scale.

2.7. Statistical analysis

Five replications of the study were performed and measurements of all parameters were made in duplicate. Data were analyzed by one-way analysis of variance (ANOVA)⁴ using the General Linear Model (GLM)⁵ procedure of the SAS (SAS Institute Inc., 2000) software. Sensory analysis data were analyzed using the MINITAB software package. Significant differences among means were separated by the Least Significance Difference (LSD).⁶ Differences at $P < 0.05$ were considered significant.

3. Results and discussion

3.1. Antioxidant activity and polyphenolic content in *M. oleifera* leaves

3.1.1. Total polyphenolic content

The total phenolic content of *M. oleifera* leaves (MOL) was 24 mg garlic acid equivalents (GAE)/g of dry weight. MOL showed a concentration dependent DPPH radical-scavenging activity with IC₅₀ of around 100 ppm. Similar results were reported by Sreelatha and Padma (2009) who found that MOL extract significantly reduced DPPH radicals with an increase in concentration.

3.1.2. Thiobarbituric acid reactive substances (TBARS)

Fig. 1 shows TBARS values of four different concentrations of MOL incorporated chicken sausage samples when compared with BHT added samples and samples without MOL or BHT.

TBARS values were significantly ($p < 0.05$) low in 0.50%, 0.75% and 1.00% MOL incorporated sausage samples compared to the negative control (T6) throughout the storage period. In addition, 0.75% and 1.00% MOL incorporated sausage samples showed significantly ($p < 0.05$) lower TBARS values throughout the storage period compared to the BHT added sample (T5). Reduction of TBARS values may be due to inhibition of lipid peroxidation by *M. oleifera* leaves which contain polyphenols that have antioxidant effects. Antioxidant activity of polyphenols are mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet oxygen or decomposing peroxides (Cao, Sofic, & Prior, 1997). Hazra et al., (2012) also observed that the thiobarbituric acid (TBA) value of cooked ground buffalo meat (GBM)⁷ treated with 1.5% crude extract of *M. oleifera* leaves was significantly lower than that of the control. Moreover, Das et al., (2012) found that 0.1% MOL extract retarded lipid oxidation and significantly reduced TBARS values in cooked goat meat patties compared to the control.

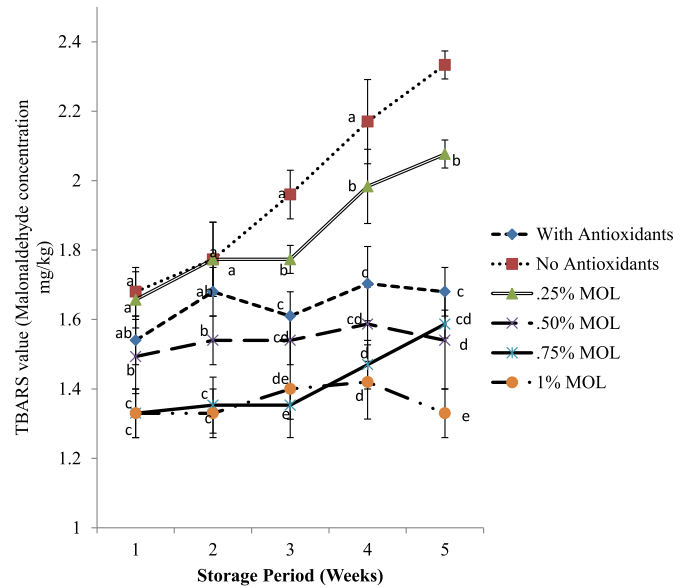


Fig. 1. Effect of different concentrations of *Moringa oleifera* leaves (MOL), 0.04% butylated hydroxytoluene (BHT) and on TBARS values (malonaldehyde mg/kg) in chicken sausages stored at 4 °C. Values are expressed as the mean \pm SD (n = 5).

3.2. pH changes in chicken sausages

Changes of pH values are given in Fig. 2. During the first week pH values were not significantly ($p < 0.05$) different among treatments. However, pH was significantly ($p < 0.05$) higher in the 0.25% MOL incorporated sausage sample, and in both controls (control with BHT and without any antioxidant) compared with the other treatments during the 2nd to 5th weeks of storage. This was in line with the total plate count results showed in Fig. 3.

Reduction in pH was observed in all treatments with storage time. Similar observations were reported by Wang, Ren, Liu, Zhu, & Wang, 2013, where accumulation of lactic acid due to the growth of

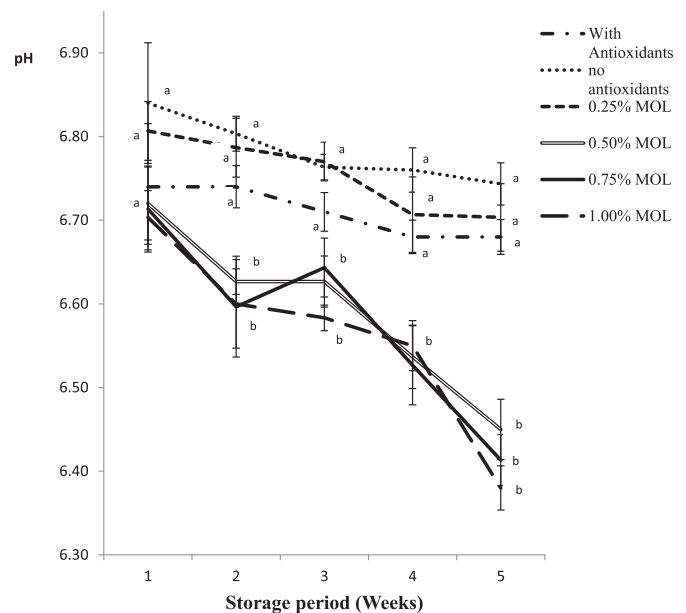


Fig. 2. Effect of different concentrations of *Moringa oleifera* leaves (MOL) and 0.04% butylated hydroxytoluene (BHT) on pH in chicken sausages stored at 4 °C. Values are expressed as the mean \pm SD (n = 5).

⁴ ANOVA: One-way analysis of variance.

⁵ GLM: General Linear Model.

⁶ LSD: Least Significance Difference.

⁷ GBM: Ground buffalo meat.

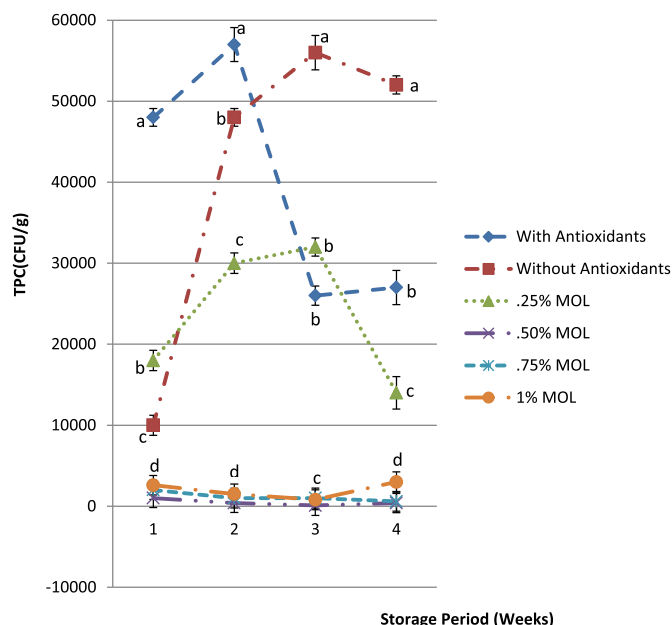


Fig. 3. Effect of different concentrations of *Moringa oleifera* leaves (MOL) and 0.04% butylated hydroxytoluene (BHT) on Total Plate Count (TPC) in chicken sausages stored at 4 °C. Significant differences ($P < 0.05$), between different treatments within a week of storage are denoted by different letters.

lactic acid bacteria resulted in decreases of pH values in sausages samples. The results of the present study were also in agreement with those of Karabacak and Bozkurt (2012), and Wenjiao, Yunchuan, Junxiu and Yongkui (2014) who reported a decrease in the pH value with storage time, in Turkish dry fermented sausage at 30 °C for 36 days and 0.03% tea polyphenol treated sausage at 42 °C for 42 days. However, low pH value is a positive character in sausage production because microorganism growth is reduced in low pH conditions (Osterlie & Lerfall, 2005). Therefore, shelf life of the product will increase with low pH values.

3.3. Microbial analysis of chicken sausages

According to the data for microbial analysis *E. coli* were absent in all treatments and *S. aureus* was less than 10^2 CFU per gram. This may be due to the strict hygienic practices followed during the production process at Keels Food Products PLC, Sri Lanka.

According to Fig. 3 total plate count (TPC)⁸ of sausages formulated with 0.5%, 0.75% and 1% MOL was significantly ($p < 0.05$) lower than that of either the 0.25% MOL sample or the two controls during the 5-week storage period.

Several low weight proteins and peptides are responsible for the antibacterial and antifungal activity of *M. oleifera* leaves (Dahot, 1998). *M. oleifera* leaves contain chemical compounds called pter-ygospermin, which readily dissociate into two molecules of benzyl isothiocyanate. Benzyl isothiocyanate was already understood to have antimicrobial properties (Fahey, 2005). Bukar, Uba, and Oyeyi (2010) have shown the potential of *M. oleifera* leaves as a sanitizer/preservative that inhibits the growth of the *E. coli*, *S. aureus*, *Pseudomonas aeruginosa* and *Enterobacter aerogenes*, which range from food-borne pathogens to spoilage causing organisms in food.

Furthermore, lower pH values in 0.5%, 0.75% and 1% MOL incorporated sausage samples from the 2nd to the 5th week of storage may also discourage the development of undesirable

microorganisms, compared to the two control samples and the 0.25% MOL incorporated sample which had a significantly higher pH value from the 2nd week to the 5th week of storage.

3.4. Instrumental color evaluation

There was significantly higher ($P < 0.05$) redness (a^* value) in the two control samples and 0.25% MOL incorporated samples in the 4th and 5th week of storage. There was no significant differences ($p < 0.05$) in L^* and b^* values in any of the treatments. Changes in meat color depend on the state of myoglobin. The formation of metmyoglobin leads to unfavorable color changes due to the predominant action of free radicals (Renerre & Labas, 1987) and partly due to the presence of aerobic bacteria (Robach & Costilow, 1962). Sreelatha and Padma (2009) observed that crude extracts of *M. oleifera* leaves can considerably scavenge free radicals and therefore retain color.

3.5. Sensory evaluation of chicken sausages

Sensory panel results are shown in Fig. 4. Highest consumer preference for appearance, color, odor and taste were observed in the control (0.04% BHT) and 0.25% and 0.50% MOL incorporated sausage samples. Furthermore, sensory panel results of the texture did not significantly differ for any of the sausage samples. Increasing MOL percentages above 0.50% had a significant negative effect on sensory attributes except on texture. MOL 0.75% and 1% additions may have changed the characteristic sensory attributes associated with chicken sausages. Das et al., (2012) observed that treating with 0.1% MOL did not have any negative effect on sensory attributes of cooked goat patties. Furthermore, Hazra et al., (2012) also showed a significant ($p < 0.05$) improvement in flavor of GBM treated with 1.5% crude extract of MOL.

4. Conclusions

This study concludes that *M. oleifera* leaves provide antioxidant and antimicrobial benefits to chicken sausages during cold storage (4 °C) and that the effects are concentration dependent. Addition of *M. oleifera* leaves at 0.50% concentration significantly retards lipid oxidation as well as reduces microbial presence without altering

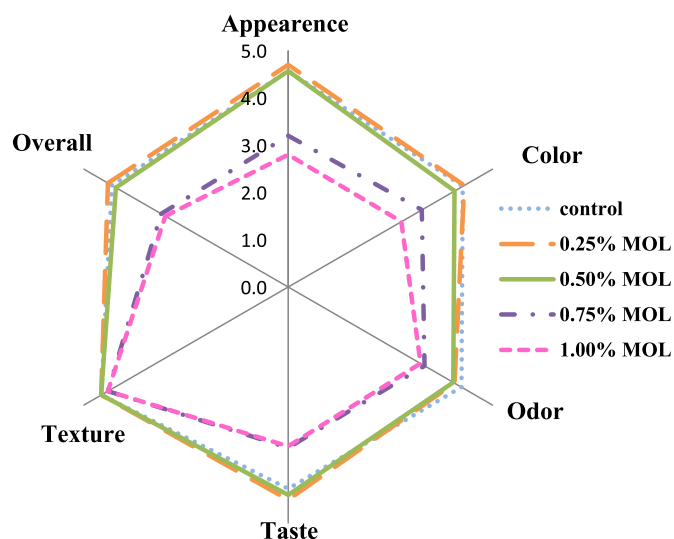


Fig. 4. Spider web analyses of sensory characteristics of chicken sausages formulated with different concentrations of *Moringa oleifera* leaves (MOL) and 0.04% BHT.

⁸ TPC: Total plate count.

the color and sensory attributes of chicken sausages. Therefore, it is suggested that drumstick leaves – a natural plant material, could be used to extend the shelf-life of meat products, providing consumers food containing natural as opposed to artificial additives, which are seen as healthier than those of synthetic origin by consumers and manufacturers alike.

Acknowledgment

We are grateful to Dr. Harshana Rambukwella, Senior Lecturer, Postgraduate Institute of English, The Open University of Sri Lanka, Sri Lanka commenting on our manuscript.

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