

# **Effects of natural antioxidants and thermal treatment on quality of meat from Bonsmara and non-descripts cattle**

**By**

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**University of Fort Hare**

*Together in Excellence*

**Alice, South Africa**

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## Declaration

I, Falowo Andrew Bamidele, hereby declare that this thesis is the result of my own investigation under the supervision of Prof. V. Muchenje. This thesis has not been previously submitted to another University for any degree. All citations and sources of information have been duly acknowledged in the text.

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## **Abstract**

### **Effects of natural antioxidants and thermal treatment on quality of meat from Bonsmara and non-descript cattle**

**By**

**A.B. Falowo**

The broad objective of this study was to determine the effects of natural antioxidants and thermal treatment on quality of meat from Bonsmara and non-descript cattle. A survey was conducted among 222 consumers in Alice (Fort Hare University community), Eastern Cape Province to determine the level of their awareness on application of antioxidants as preservatives in meat and meat products during storage. Representative samples of *Muscularis longissimuss thoracis et lumborum* and liver were collected from each carcass of Bonsmara (n=40) and non-descript (n=40) cattle reared on natural pasture to determine the effect of natural antioxidants and thermal treatment on their quality. The results from the survey revealed that 51.35% of the respondents had not heard about the use of antioxidant as preservatives in meat products. However, among the remaining respondents (48.65%) that were aware of antioxidant and its use as preservative, about 19% knew of natural antioxidants, 35% synthetic antioxidants while 46% had knowledge of both. The results further revealed that the majority of the respondents (82%) that had knowledge of natural antioxidants were concerned about the use of synthetic antioxidants in meat and meat products due to their health consequences. The *in vitro* antioxidant and antibacterial analyses of *Bidens pilosa* and *Moringa oleifera* leaf extracts revealed that they contain rich bioactive compounds. Furthermore, the addition of leaf extracts from *Moringa*

*oleifera* (ML, 0.05 and 0.1% w/w) and *Biden pilosa* (BP, 0.05 and 0.1% w/w) to ground raw beef as a natural antioxidant were found to improve the physicochemical, oxidative stability and microbiological qualities of meat compared to the control (meat without extract) and BHT treatment (0.02% w/w) during 6 days storage at 4<sup>0</sup> C. Cattle breed did not have much effect on colour parameters. However, the overall pH of ground beef treated with extracts showed lower values than control and BHT treated beef. Also, ground beef samples containing extracts exhibited ( $P < 0.05$ ) better colour stability, especially higher redness ( $a^*$  values), than the control. The formation of TBARS in beef samples treated with extracts was significantly ( $P < 0.05$ ) lower than the control and BHT treatment. The antibacterial assay of the extracts revealed an appreciable broad spectrum activity against the tested bacteria and microbial counts in ground beef samples compared to control and BHT group. Ground beef treated with plant extracts exhibited lower microbial and lactic acid bacterial counts ( $P < 0.05$ ) at day 0 and 3 than control samples. Moreover, it was observed that application of sous vides thermal method did not significantly affect the fatty acids and mineral loss in beef and liver across the treatments. However, the total concentration of MUFA was lower in raw liver ( $20.11 \pm 2.38 - 21.08 \pm 1.23\%$ ) than in beef ( $40.22 \pm 1.90 - 42.53 \pm 1.29\%$ ), while total PUFA content were higher in liver ( $30.73 \pm 2.60 - 31.11 \pm 1.19\%$ ) than in beef samples ( $10.13 \pm 3.36 - 11.02 \pm 2.74\%$ ) ( $p < 0.05$ ). The results also revealed that liver samples from Bonsmara and non-descript cattle had a higher percentage of intramuscular fat content of  $4.67 \pm 0.53\%$  and  $4.44 \pm 0.53\%$  respectively, and fat free dry matter of  $27.51 \pm 1.05\%$  and  $25.73 \pm 1.05\%$ , respectively, than the beef samples ( $p < 0.05$ ). The concentrations of Mg ( $52.80 \pm 0.22 - 53.70 \pm 0.02 \text{mg}/100\text{g}$ ) and Zn ( $8.90 \pm 0.15 - 19.60 \pm 0.15 \text{mg}/100\text{g}$ ) were higher in beef than liver samples. The level of Ca ( $17.00 \pm 0.17 - 17.50 \pm 0.17 \text{mg}/100\text{g}$ ) in liver was higher than that of beef samples. It was concluded that most

consumers preferred the use of natural antioxidants in meat products than synthetic antioxidants due to their health risk. The effectiveness of *M. oleifera* and *B. pilosa* leaf extracts on meat quality also revealed that these plants are promising candidates as natural preservatives and their application should be considered in the meat industry. Lastly, findings from this study showed that application of the sous vide technique could protect meat products from nutritional loss during thermal treatment.

**Key words:** Ground beef, liver, microbial counts, natural antioxidants, nutritional quality, oxidative stability, sous vide thermal treatment

## **Dedication**

I heartily dedicate this work to God Almighty and my family

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## List of Symbols

%	Percentage
μl	Microliters
°C	Degrees Celsius
G	Grams
g/L	Gram/liter
H	Hours
Kg	Kilograms
Log	Logarithms
mm	Millimeter
ml	Milliliter
Mg	Milligrams
Mg/ml	Milligram per milliliter
mM	Millimolar
nm	Nanometer
S	Seconds
ug	Micrograms
U/l	Units per liter
V	Volts
V/V	Volume per volume
W/W	Weight per weight

## List of abbreviations

a*	Redness of meat
Abs	Absorbance
ABTS	2,2 iazino- bis-3-ethylbenzothiazoline-6-sulfonic acid
AI	Atherogenicity Index
b*	Yellowness of meat
BHT	Butylated hydroxytoluene
BHA	Butylated hydroxyanisole
BP	<i>Biden pilosa</i>
CFU	Colony forming units
DI	Desaturase Index
DNP	Dinitrophenyl hydrazine
DPPH	2,2-Diphenyl-2-picrylhydrazyl
DW	Dry weight
FFD	Fat free dry matter
GAE	Gallic acid
GC-MS	Gas chromatography-mass spectrometry
GLM	General linear Model
L*	Lightness of meat
LAB	Lactic Acid Bacteria
MBC	Minimum bactericidal concentrations
MDA	Malonaldehyde
MIC	Minimum inhibitory concentrations

ML	<i>Moringa oleifera</i>
MUFA	monounsaturated fatty acids
PCA	Plate Count Agar
PDIFF	Probability difference
PUFA	polyunsaturated fatty acids
Ru	Rutin
SAS	Statistical Analysis System
SFA	Saturated fatty acid
TBHQ	Tert-butylhydro quinone
TBAS	Thiobarbituric acid reactive substances
TVC	Total viable counts
WBSF	Warner–Bratzler Shear Force

## **CHAPTER 1: Introduction**

### **1.1 Background information**

Meat and meat products play an important role in maintaining a healthy and balanced diet, because they provide energy, high-quality and readily digestible protein with all essential amino acids, and other absorbable micronutrients which are needed for human growth, cell functioning and sound health (De Smet, 2012; Mourouti et al., 2015). The average meat consumption worldwide is estimated at 42.9kg per capita, with industrial countries consuming about 76.1kg, twice the quantity in developing countries (33.6kg) (FAO, 2014). This estimate has been predicted to double by 2050 due to growing human population, rising incomes and urbanization (FAO, 2011). It is, therefore, important to increase production and reduce the level of meat wastage especially during processing, distribution and storage in order to meet the growing demand and ensure food security.

Approximately 3.5 billion kg of meat is lost at the consumer, retailer and food service levels annually with consequence of substantial economic loss and environmental impact (Kantor et al., 1997; Dave and Ghaly, 2011). It is also reported that if 5% of the meat loss is preserved, it can meet the daily needs of approximately 320,000 people in the world (Cervený et al., 2009; Dave and Ghaly, 2011). On many occasions, microbial, enzymatic and oxidative reactions cause most of these losses. Oxidation reaction is an inevitable and complex process that often occurs in meat and its products during processing and cold storage (Kumar et al., 2015). The rate of this reaction in meat products depends on the degree of unsaturation of the fatty acids, the level of antioxidants (internal or external) and the presence of prooxidants, such as metal iron (Morrissey et al., 1998; Kumar et al., 2015). Oxidation is believed to occur when a molecular oxygen reacts

with tissue biochemical substances (lipid, protein and pigments) through an auto-catalytic process to generate free radicals, mainly the reactive oxygen species (ROS) and reactive nitrogen species (RNS) in a chain reaction. As oxidation reaction progresses, the unsaturated fatty acid fraction of membrane phospholipids is oxidized and different hydroperoxides are formed (Kumar et al., 2015). The subsequent decomposition of hydroperoxides into secondary oxidation products such as aldehydes, ketones and other compounds (hexanal, pentanal, heptanal, and octanal) could adversely affect the acceptability and overall quality attributes of meat and meat products, thereby causing economic loss to the meat industry (Kumar et al., 2015). The process of oxidation according to Palmieri and Sblendorio (2007) and Contini et al. (2014) has been found to develop off-flavor; toxic compounds, rancid odour, poor shelf life, and nutrient losses during cold storage.

The growth of microorganisms on meat surface have been identified to cause slime formation, structural components degradation, decrease in water holding capacity, off odors, off-tastes, texture and appearance changes (Sanchez-Ortega et al., 2014). The extent of meat spoilage through microorganisms has been associated with the species and number of organisms, characteristics of the meat products (residual glucose, pH) and storage conditions (temperature, humidity and oxygen availability) surrounding the meat (Dave and Ghayl, 2011; Hernández-Macedo et al., 2011; Sun and Holley, 2012). Under storage conditions, various spoilage pathogenic organisms such as mesophiles, thermophiles, psychrophiles, have been discovered in meat and meat products causing economic loss and food-borne diseases in the meat industry (James and James, 2002; Ercolini et al., 2009).



In order to prevent these deteriorations and extend shelf life of meat, different synthetic preservatives such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiarybutyl hydroquinone (TBHQ) and propyl gallate have been used over the years. However, their impact on meat has recently been reported to be carcinogenic and injurious to health (Velasco and Williams, 2011). Plant-derived (natural) antioxidants are currently being explored as possible alternatives. Natural antioxidants can prevent oxidation by donating their hydrogen ions to break and terminate the oxidation cycle during the reaction and thereby preventing additional lipid and protein radicals from forming in muscle food (Allen and Cornforth, 2010). Many authors, for example Velasco and Williams (2011) and Shah et al. (2014) have also reported the use of natural antioxidants to be effective in preventing formation of free radicals, delay microbial activities and improve meat quality. Most of the natural antioxidants possess relatively high chemical nutrients and are recognized as functional or nutraceutical food products.

The occurrence of oxidation is not limited to meat during storage but also occur during thermal treatments. The use of conventional thermal method (boiling, grilling and frying) as treatment to improve meat palatability and increase shelf life has been reported to generate free radicals, polycyclic aromatic hydrocarbon, volatile (heterocyclic amines) compounds and cause loss of essential fatty acid, amino acid and minerals through oxidation reactions (Sanchez del Pulgar et al., 2011; Roldan et al., 2014; Khan et al., 2015). Any loss of lipids essential fatty acid and minerals will greatly impair the nutritional content and sensory quality of meat products.

Nowadays, many restaurants and homes are using the sous vide thermal technique to prepare their meat in order to improve quality and prolong shelf-life during storage (Oz and Zikirov, 2015). The mechanism of this technique has been linked to its ability to prevent oxidation by reducing the contact of free oxygen in the air with foods during cooking (Oz and Zikirov, 2015). Taking this into account, the use of natural antioxidants and sous vide thermal method in meat industry promises to provide a potential alternative in preventing oxidation and prolonging the shelf life of meat and its products during cooking and refrigeration storage.

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## **1.2 Justification**

Processing of meat and meat products through mechanical deboning, mincing/grinding and cooking usually damage the tissue membrane and allow the free radicals (catalysts) to react with unsaturated fatty acids to initiate lipid oxidation reaction (Nollet, 2012). In addition, this process also exposes the muscle membrane to air and microbial contamination after production (Alp and Aksu, 2010; Liu et al., 2015). The effect of lipid oxidation and microbial growth on meat products has been shown to shorten the shelf life of meat products by affecting the taste, odour, colour, texture and nutritional content. Application of synthetic preservatives such as BHT and BHA to prolong the meat shelf-life and overall safety/quality has is reported to be unsafe for consumer health (Race, 2009; Lobo et al., 2010; Ahmed et al., 2013). Due to this health concern, natural preservatives, especially from plant materials, are now being explored to improve meat quality and safety (Sánchez-Ortega et al., 2014). However, it is not fully known if most consumers are aware of its use as preservatives in meat and meat products.

Apart from what is articulated above, there is dearth of information regarding the potential of *Biden pilosa* L and *Moringa oleifera* L. leaf extracts as natural antioxidants to prevent meat quality deterioration during storage and processing. Both *Biden pilosa* L and *Moringa oleifera* L. leaf extracts have been reported in literature as edible and promising sources of natural antioxidants (Anwar et al., 2007; Sreelatha and Padma, 2009; Bartelome et al., 2013; Cortés-Rojas et al., 2013). Furthermore, the use of thermal treatment on meat products has been reported to induce free radical production and loss of essential fatty acids through lipid oxidation (Rodriguez-Estrada et al., 1997; Alfaia et al., 2010). In order to prevent the oxidation of lipid essential fatty acids and other nutritive compounds of meat during cooking, sous vide cooking method has been reported as the best alternative (Roldan et al., 2014). However, the effect of sous-vide thermal technique on oxidative stability of lipid, fatty acids and mineral composition of meat (beef and liver) from bovine species has not been fully studied. Recent studies on the use of this technique only focused on nutritional quality of meat from pig, fish and sheep (Picouet et al., 2011; Sanchez del Pulgar et al., 2012; Roldan et al., 2014). In the light of these issues, this study therefore attempts to fill the observed knowledge gaps.

### 1.3 Objective of the study

The broad objective of this study was to investigate the activities of natural antioxidants and thermal treatment on the quality of meat from Bonsmara and non-descript cattle.

Specific objectives of this study were:

1. To determine consumers' awareness on the use of antioxidants as preservative agents in meat products.
2. To determine the *in vitro* antioxidant and antibacterial activities of *Bidens pilosa* Lam. asteraceae and *Moringa oleifera* Lam. moringaceae plants
3. To determine the antioxidant and antibacterial activities of *B. pilosa* L and *M. oleifera* L. leaf extracts on pH, colour, lipid oxidation, spoilage microorganisms and sensory quality of meat from Bonsmara and non-descript cattle
4. To determine the effect of sous vide thermal techniques on lipid, fatty acid and mineral composition of meat from Bonsmara and non-descript cattle.

#### 1.4 Hypothesis of the study

The specific hypotheses tested were the following:

1. There are no differences in consumers' awareness on the use of antioxidants as preservatives in meat and meat products.
2. There are no differences in in-vitro antioxidant and antibacterial activities of *Bidens pilosa* and *Moringa oleifera* plants.
3. There are no differences in the antioxidant and antibacterial activities of *B. pilosa* and *M. oleifera* leaf extracts on pH, colour, lipid oxidation, spoilage microorganisms and sensory quality of meat from Bonsmara and non-descript cattle.
4. There are no differences in the effect of sous vide thermal techniques on fatty acid and mineral composition of beef and liver from Bonsmara and non-descript cattle.

## 1.5 References

- Ahmed, N., Mudasir, J. S., malik, A., Kour, H., Gupta, P., Chauhan, H. 2013. Naturally Occurring Preservatives in Food and their Role in Food Preservation. *International Journal of Pharmaceutical and Biological Archives* **4 (1)**: 22-30.
- Alfaia, C. M. M., Alves, S. P., Lopes, A. F., Fernandes, M. J. E., Costa, A S. H., Fontes, C.M.G.A., Castro, M. L.F., Bessa, R. J.B. and Prates, J. A.M. 2010. Effect of cooking methods on fatty acids, conjugated isomers of linoleic acid and nutritional quality of beef intramuscular fat. *Meat Science* **84**: 769–777.
- Allen, K., and Cornforth, D. 2010. Comparison of spice-derived antioxidants and metal chelators on fresh beef color stability. *Meat Science* **85**: 613–619.
- Alp, E. and Aksu, M. İ. 2010. Effects of water extract of *Urtica dioica* L. and modified atmosphere packaging on the shelf life of ground beef. *Meat Science* **86**: 468–473.
- Anwar, F., Latif, S., Ashraf, M. and Gilani, A. H. 2007. *Moringa oleifera*: a food plant with multiple medicinal uses. *Phytotherapy Research* **21**:17–25.
- Bartolome, A. P., Villaseñor, I. M. and Yang, W. 2013. *Bidens pilosa* L. (Asteraceae): botanical properties, traditional uses, phytochemistry, and pharmacology. *Evidence-Based Complementary and Alternative Medicine* **2013**: 51.
- Cervený, J., Meyer, J. D. and Hall, P. A. 2009. Microbiological Spoilage of Meat and Poultry Products. In Compendium of the microbiological spoilage of foods and beverages. Sperber, W.H. and Doyle, M. P. (Ed.). Springer Science and Business Media, New York, pp. 69-868.
- Contini, C., Alvarez, R., O'Sullivan, M., Dowling, D. P., Gargan, S. O. and Monahan, F. J.

2014. Effect of an active packaging with citrus extract on lipid oxidation and sensory quality of cooked turkey meat. *Meat Science* **96**: 1171–1176.

**Cortés-Rojas, D. F., Chagas-Paula, D. A., Da Costa, F. B., Souza, C. R. F. and Oliveira, W. P.** 2013. Bioactive compounds in *Bidens pilosa* L. populations: a key step in the standardization of phytopharmaceutical preparations. *Brazilian Journal of Pharmacognosy* **23 (1)**: 28-35.

**Dave, D. and Ghaly, A. E.** 2011. Meat spoilage mechanisms and preservation techniques: a critical review. *The American Journal of Agricultural and Biological Sciences* **6 (4)**: 486-510.

**De Smet, S.** 2012. Meat, poultry, and fish composition: Strategies for optimizing human intake of essential nutrients. *Animal Frontiers* **2 (4)**: 10-16.

**Ercolini, D., Russo, F., Nasi, A., Ferranti, P. and Villani, F.** 2009. Mesophilic and psychrotrophic bacteria from meat and their spoilage potential in vitro and in beef. *Applied and Environmental Microbiology* **75 (7)**: 1990–2001.

**FAO,** 2011. World livestock 2011. Livestock in food security. [www.fao.org/docrep](http://www.fao.org/docrep) Accessed 24/11/2014.

**FAO,** 2014. Food outlook - Biannual report on global food markets. [www.fao.org/docrep](http://www.fao.org/docrep) Accessed 24/11/2014.

**Hernandez-Macedo, M. L., Barancelli, G. V. and Contreras-Castillo, C. J.** 2011. Microbial deterioration of vacuum-packaged chilled beef cuts and techniques for microbiota detection and characterization: a review. *Brazilian Journal of Microbiology* **42**:1.

**James, S. J. and James, C.** 2002. Meat Refrigeration, Woodhead Publishing Limited, Cambridge: England and CRC Press, New York, Washington DC, USA pp 91- 94.

- Kantor, L.S., Lipton, K., Manchester, A. and Oliveira, V.** 1997. Estimating and addressing America's food losses. *Food Review* **20**: 3-11.
- Khan, M. I., Min, J., Lee, S., Yim, D. G., Seol, K., Lee, M. and Jo, C.** 2015. Cooking, storage, and reheating effect on the formation of cholesterol oxidation products in processed meat products. *Lipids in Health and Diseases* **14** (89): 1-9.
- Kumar, Y., Yadav, D. N., Ahmad, T. and Narsaiah, K.** 2015. Recent trends in the use of natural antioxidants for meat and meat products. *Comprehensive Reviews in Food Science and Food Safety* **00**: 1-14.
- Liu, F., Xu, Q., Dai, R. and Ni, Y.** 2015. Effects of natural antioxidants on colour stability, lipid oxidation and metmyoglobin reducing activity in raw beef patties. *CTA Scientiarum Polonorum Technologia Alimentaria* **14** (1): 37-44.
- Lobo, V., Patil, A., Phatak, A. and Chandra, N.** 2010. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Review* **4** (8): 118-126.
- Morrissey, P. A., Sheehy, P. J. A., Galvin, K., Kerry, J. P. and Buckley, D. J.** 1998. Lipid stability in meat and meat products. *Meat Science* **49** (1): 73-86.
- Mourouti, N., Kontogianni, M. D., Papavagelis, C., Plytzanopoulou, P., Vassilakou, T., Psaltopoulou, T., Malamos, N., Linos, A. and Panagiotakos, D. B.** 2015. Meat consumption and breast cancer: A case-control study in women. *Meat Science* **100**: 195-201.
- Nollet, L. M. L.** 2012. Handbook of meat, poultry and seafood quality. John Wiley and Son Inc, USA, Chapter 10.



- Oz, F. and Zikirov, E.** 2015. The effects of sous-vide cooking method on the formation of heterocyclic aromatic amines in beef chops. *LWT - Food Science and Technology* **64**: 120-125.
- Palmieri, B. and Sblendorio, V.** 2007. Oxidative stress tests: Overview on reliability and use Part II. *European Review for Medical and Pharmacological Sciences* **11**: 383–399.
- Picouet, P. A., Cofan-Carbo S., Vilaseca, H., Ballbè, L. C. and Castells, P.** 2011. Stability of sous-vide cooked salmon loins processed by high pressure. *Innovative Food Science and Emerging Technologies* **12**: 26–31.
- Race, S.** 2009. Antioxidants: The truth about BHA, BHT, TBHQ and other antioxidants used as food additives. Available on [www.foodcanmakeyouill.co.uk](http://www.foodcanmakeyouill.co.uk). Accessed 24/04/2015.
- Rodriguez-Estrada, M. T., Penazzi, G., Caboni, M. F., Bertacco, G. and Lercker, G.** 1997. Effect of different cooking methods on some lipid and protein components of hamburgers. *Meat Science* **45(3)**: 365–375.
- Roldan, M., Antequera, T., Armenteros, M. and Ruiz, J.** 2014. Effect of different temperature–time combinations on lipid and protein oxidation of sous-vide cooked lamb loins. *Food Chemistry* **149**: 129–136.
- Sanchez del Pulgar, J.S., Gázquez, A. and Ruiz-Carrascal, J.** 2012. Physico-chemical, textural and structural characteristics of sous-vide cooked pork cheeks as affected by vacuum, cooking temperature, and cooking time. *Meat Science* **90**: 828–835.
- Sánchez-Ortega, I., García-Almendárez, B. E., Santos-López, E., Amaro-Reyes, A., Barboza-Corona, J. E. and Regalado1, E.** 2014. Antimicrobial edible films and coatings for meat and meat products preservation. *Scientific World Journal* **2014**: 18.

- Shah, M. A., Don Bosco, S. J. and Mi, S. A.** 2014. Plant extracts as natural antioxidants in meat and meat products. *Meat Science* **98**: 21–33.
- Sreelatha, S. and Padma, P. R.** 2009. Antioxidant activity and total polyphenol content of *Moringa oleifera* leaves in two stages of maturity. *Plant Food Human Nutrition* **64**: 303-311.
- Sun, X. D and Holley, R. A.** 2012. Antimicrobial and Antioxidative Strategies to Reduce Pathogens and Extend the Shelf Life of Fresh Red Meats. *Comprehensive Reviews in Food Science and Food Safety* **11**.
- Velasco, V. and Williams, P.** 2011. Improving meat quality through natural antioxidants. *Chilean Journal of Agricultural research* **71**: 2.

## Chapter 2: Literature Review

(Part of this section has been published in the journal, *Food Research International*; see Appendix 2)

### 2.1 Introduction

Meat quality is very essential in maintaining consumer health. Factors that affect meat quality are enormous and complex, and they occur from farm to fork. In reality, these factors may present themselves as oxidative stress (from farm to abattoir) or as oxidative rancidity and microbial spoilage (from abattoir to the consumer table). Oxidative stress may occur due to succession of stimuli that disturb the homeostatic condition of an animal before slaughter (Cataldi, 2010). They can also initiate the generation of free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS) and cause severe damage to cell macromolecules including the lipid and protein fractions (Power and Jackson, 2008; Barbieri and Sestili, 2012).

The occurrence of oxidative rancidity and spoilage microorganisms is most noticeable during processing and storage conditions. Evidence has shown that oxidation affects virtually every muscle food irrespective of the protein and fat (lipid) contents (Velasco et al., 2010). Protein oxidation-induced changes and modify the digestibility of proteins which may reduce the bioavailability of amino acid residues and the nutritional values of meat proteins (Lund et al., 2011), while lipid oxidation results in rancid odour, development of off-flavour, discolouration, loss of nutrient value and decrease in shelf life (Hygreeva et al., 2014). The rate and extent of meat deterioration can be reduced through various means, such as freezing, application of antioxidant (natural/synthetic) etc. However, the use of natural antioxidant has been considered to be more beneficial to consumer health because they are rich bioactive compounds. The

addition of natural antioxidants had been found to stabilize cholesterol levels, inhibit the formation of cholesterol oxidized products, and reduce the formation and absorption of malondialdehyde, polycyclic aromatic hydrocarbon and heterocyclic amine (HCA) in cooked meat (Megan-Tempest, 2012; Kobus-Cisowska, et al., 2014). It has also been observed that the consumption of food (meat) rich in natural antioxidants can reinforce the activity of the endogenous antioxidants against degenerative diseases linked to oxidative stress and ROS-related tissue damage (Valenzuela et al., 2003). Therefore, in this chapter the application of natural antioxidants as meat preservatives and the effect of temperature on nutritional quality of meat are discussed.

## **2.2 Meat quality and preservatives**

### **2.2.1 Causes of meat quality deterioration: Oxidation in meat and meat products**

Since the discovery of oxygen in the early 18th century and its inevitable roles in plants and animals, the necessity to control its levels and impacts on meat and meat products, especially during processing, packaging and distribution, has been a major challenge in the meat industry. Oxidation involves the loss of at least one electron when chemicals in the food are exposed to oxygen in the air. In meat, oxidation has been demonstrated as the main, non-microbial cause of quality deterioration during processing. This is because lipids and proteins in meat are easily susceptible to oxidative damages due to rapid depletion of endogenous antioxidants after slaughter (Xiao et al., 2013). However, the susceptibility of meat to oxidation has also been found to be influenced by animal breed and species, muscle types and anatomical location (Min et al., 2008). The findings of Faustman and Cassens (1991) on two cattle breeds revealed that Holstein meat displays a higher lipid oxidation (TBA) than cross breed beef meat.

The authors also showed that meat from the gluteus medius muscles had a higher amount of thiobarbituric acid than the longissimus muscle type. Different studies have shown that the amount of metal ions that are present in enzymes and metalloproteins or those migrated from the processing machine, either by abrasion or due to acidic dissolving of metals from the surface factors could promote the rate of oxidation in meat (Lauritzsen et al., 1990; Rulisek and Vondrasek, 1998; Jacobsen et al., 2008). Moreover, the type of diet consumed by animals during the production phase has a big influence on the susceptibility of meat to oxidation postmortem. Zhang et al. (2011) reported an increase in lipid and protein oxidation in the breast muscles of birds that had been fed a dietary oxidized oil diet compared to antioxidant-supplemented and control diets. Exposure of meat to oxygen, light and temperature, as well as preservative and processing techniques, such as chilling, freezing, additives (salt, nitrate and spices), cooking, irradiation, high pressure and packaging, could influence the extent of oxidation. Currently, lipid oxidation is one of the biggest economic problems in the meat industry. It compromises the nutritional quality, reduces shelf life, increases toxicity and decreases the market value of meat and meat products (Sample, 2013). Oxidation in meat is usually assessed by measuring the amount of peroxide value (PV), thiobarbituric acid-reactive substances (TBARS), sulphhydryl and carbonyl group generated during the process. This analysis is carried out using spectrophotometric or chromatographic (head space gas chromatographic (GC), high-performance liquid chromatography (HPLC), liquid chromatographic mass spectrophotometer [(LC-MS) and 2,4 dinitrophenylhydrazine (DNPH)] methods.

### **2.2.1.1 Lipid oxidation in meat and meat products**

Lipids are widely distributed in both the intra and extra cellular space of meat as triacylglycerides, phospholipids and sterols. However, lipids are chemically unstable and, therefore, prone to oxidation, especially during post-mortem handling, and storage. Lipid oxidation results in rancid odour, off-flavour development, drip losses, discolouration, loss of nutrient value, decrease in shelf life, and the accumulation of toxic compounds, which may be detrimental to the health of consumers (Richards et al., 2002; Chaijan, 2008; Mapiye et al., 2012). Oxidation of lipids is a three-step radical chain reaction which consists of initiation, propagation, and termination with the production of free radicals (Figure 3). Initiation reaction produces the fatty acid (alkyl) radical ( $R\bullet$ ) which in turn reacts with oxygen to form peroxy radicals ( $ROO\bullet$ ) in the propagation reaction. The peroxy radicals react with unsaturated fatty acids and form hydroperoxides ( $ROOH$ ), which later decompose to produce the volatile aromatic compounds that give meat its perceived off-flavours and rancid odour (Gordon, 2001; Chaijan, 2008). The interaction of alkyl and peroxy radicals leads to the formation of non-radical products such as aldehydes, alkanes and conjugated dienes (Wsowicz et al., 2004). Formation of aldehydes has been found to be directly related to the deterioration of meat colour and flavour, protein stability and functionality (Lynch et al., 2001; Min and Ahn, 2005). The consequence of aldehydes has also been associated with atherosclerosis, putative mutagens and cancer formation in the body (Duthie et al., 2013).

Initiation:



Propagation:



Termination:



} Non radical products

**Figure 2.1: Radical-chain of processes involved in lipid oxidation in biological systems**

### **2.2.2 Causes of meat quality deterioration: Microbial activities in meat and meat products**

Beside oxidation, meat safety and shelf life are strongly related to the presence and activity of spoilage microorganisms (Paulsen and Smulders, 2014). The rate of spoilage is usually influenced by the species of microorganism and initially bacterial load present in the meat sample (James and James, 2002) coupled with physiological status of the animal at slaughter (Nychas et al., 2008). Most of these species are either gram positive, such as lactic acid bacteria, or gram negative bacteria, such as *Pseudomonas spp.* and *Enterobacteriaceae* (Ercolini et al., 2009). The gram negative bacteria have been reported to account for approximately 69% of the cases of bacterial food borne diseases (Clarence et al., 2009). The composition of microorganisms in meat depends on different factors which include pre-slaughter husbandry practices (extensive Vs intensive rearing), handling during slaughtering including evisceration, workers' health and hygiene, preservation technique and facilities, packaging materials, and handling and storage by consumers (Cervený et al., 2009; Dave and Ghayl, 2011; Hernández-Macedo et al., 2011). Other possible factors that can influence the composition of these pathogenic organisms include age and sex of the animal.

It has been reported that different bacteria, like psychrophile, psychrotrophic, mesophile and thermophile, can survive under different processing conditions to cause spoilage and wastage in the meat industry (Hernández-Macedo et al., 2011; Gadekar et al., 2014). It is believed that foodborne disease outbreaks involve agents such as *Escherichia coli*, *Salmonella* and chemical contaminants (FAO, 2003). Moreover, activity of microbes in meat products has been implicated in off-odours, off-flavours, discolouration and slime production (Borch et al., 1996; Nychas et al., 2008). The economic and public health consequences of spoilage microorganisms in meat



have been reported in literature (Adak et al., 2005; Ukut et al., 2010). However, meat spoilage bacteria can be reduced by applying natural antioxidants directly into the meat products.

### **2.2.3 Natural antioxidants as preservatives in meat products: Application and Consumer awareness**

In recent years, special attention has been paid to a number of plants that could be used as potential sources of natural antioxidants for muscle food preservation and nutritional quality improvement. Natural antioxidants can be applied either through dietary or technological strategies to reduce or prevent oxidative processes and microbial activities in muscle food. In dietary manipulations, antioxidants are introduced into the muscle via the animal feed or diet. The inclusion of natural antioxidants in animal diets has been reported by various authors to not only slow down oxidation, but also to greatly improve meat quality when compared to diets with no antioxidants (Moyo et al., 2012a; Nkukwana et al., 2014). Technological strategies involve the application of antioxidants directly into the meat and meat products or by coating packaging materials with plant extracts to improve the oxidative stability of the products.

Most natural antioxidants are obtained from plant resources, such as culinary herbs, spices, vegetables, as well as fruits and oilseed products (Shahidi and Zhong, 2010). Synthetic antioxidants, such as butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), and tertiary butyl hydroquinone (TBHQ,) have been used in inhibiting meat oxidation (Fasseas et al., 2007) but with side effects. The potential of synthetic antioxidants causing toxicological effects has created a demand for natural antioxidants by some consumers and meat industry (Karre et

al., 2013). Several authors have reported the efficacy of various natural antioxidants for reducing lipids and protein oxidation, discolouration and microbial growth in some types of meat (Table 2.1 and 2.2; Fasseas et al., 2007; Camo et al., 2008; Zinoviadou et al., 2009). Unfortunately, to date natural antioxidants have not been as widely applied as synthetic antioxidants in muscle food products in the food industry (Kobus-Cisowska et al., 2010). Public awareness on its application appears to be rather inadequate or sparse. Although the growing consumer interest in nutritious and novel meat products coupled with recent growing health concerns about synthetic antioxidants has been presumed to further encourage the global natural antioxidants market. It is clear that introduction of natural antioxidants to different groups of foodstuffs and meat products will be beneficial to both consumer and meat industry.

### **2.2.3.1 Preparation and extraction of antioxidant and antimicrobial compounds in plant materials**

Phenolic compounds are the major constituents of plant materials that contribute to their antioxidant capacity. Plants, fruits and their extracts that reflect concentrations of phenolic compounds are thus regarded as effective sources of antioxidants to inhibit oxidation in muscle foods (Pennington and Fisher, 2009). In order to determine the phenolic compounds, plant materials are subjected to different extraction processes. Basically, extractions are carried out to separate and recover the desired bioactive constituents (polyphenol) from plant matrices and to eliminate unwanted insoluble material (including inactive and potentially harmful substances) through selective solvents which are regarded as GRAS (Generally Recognized as Safe) (Kothari et al., 2012). It is also used to obtain the maximum yield of extracts from plant material. Most commonly used solvents include acetone, ethanol, methanol, hexane, ethyl acetate,

hydroalcoholic mixtures (mixture of alcohol and water in varying proportions) and water (Sultana et al., 2009). However, the use of aqueous solution (cold, distilled, de-ionized or hot water) for extraction of phenolics has been reported in literature; however, their efficacy in the extraction of phenolics compared to other alcoholic solvents has been very low. The type, concentration and polarity of the solvent used may affect the amount and rate of antioxidant-compounds extracted during the process (Anokwuru et al., 2011). Apart from this, other factors that could influence the recovery of antioxidant-compounds include chemical nature of the plant materials, method of extraction, extraction conditions (such as time, temperature and pressure), solid to solvent ratio and particle size (Dai and Mumper, 2010; Tan et al., 2011).

### **2.2.3.2 Prevention of oxidation in meat sample using natural antioxidants**

Some of the recent works on effects of natural antioxidants against lipid oxidation in meat during processing are presented in Table 2.1. The reaction of antioxidants with oxidation is believed to occur through two major pathways. Firstly, they can donate their electrons to break and terminate the oxidation cycle at the propagation step and thereby preventing additional lipid and protein radicals from forming (Dangles and Dufour, 2006; Allen and Cornforth, 2010). However, in the absence of antioxidants, the reaction becomes auto-propagative leading to the production of non-radical products. Secondly, by removing free radical (ROS) initiators in order to quench chain-initiating catalysts (radicals) (Antolovich et al., 2002) or limiting the radicals initiators by binding metals such as iron and copper as metal chelators to stabilize them in an inactive or insoluble form (Allen and Cornforth, 2010; Dai and Mumper, 2010). Moreover, the antioxidant free radical (oxidized antioxidant) formed in the first cycle may further interfere with chain propagation reactions by forming peroxy antioxidant compounds (Antolovich et al., 2002). The

metal chelating power of plant materials has been reported to be associated with chemical composition of the sample (Goncalves et al., 2009), including the presence of compounds, such as phytate and oxalates (Pokorny, 2007). Mirzaei and Khatami (2013) found that the extract of *Coriander sativum* possesses higher iron chelating activity than *Petroselinum crispum*, while the addition of *Mentha gentilis* L. showed higher chelating activity than other menthe species (Goncalves et al., 2009).

**Table 2. 1 Effect of dose concentration, storage temperature and time of technological natural antioxidants on lipid in meat.**

Natural sources	Dose in meat	Meat type	Storage (°C)	Storage duration	Effect on oxidation	References
Oregano + sage leaves	0.2% w/w each	Chicken breast and thigh	4°C	98 hours	SDL	(Sampaio et al., 2012)
Black currant extracts	5, 10 or 20g/kg	Pork patties	4°C	9 days	SDL	(Ji et al., 2012).
Rosemary extracts	250, 500, 750mg/kg	Porcine liver patties	-21°C	2 days	SDL in a dose dependent manner	(Doolaege et al., 2012)
Olive leaf extracts	100 and 200ug/g	Minced beef patties	4°C	9 & 12 days	SDL in a dose-dependent manner	(Hayes et al., 2010)
Herbal extracts (Marjoram, rosemary, sage)	0.04% v/w	Ground beef	5°C	41 & 48 days	SDL	(Mohameda et al., 2011)
Broccoli leaf extract Curry leaf extracts ( <i>Murrayakoenigii L.</i> ) Mint leaf extract ( <i>Menthaspicata</i> )	0.1% and 0.5% w/w 5ml extract/500g	Ground beef patties Pork meat	4 °C 4 °C	12 days 0-12days	SDL SDL	(Kim et al., 2013) 2012
Grape seed extracts	0.1%	Mutton slices	4°C	7 days	SDL	(Reddy et al., 2013)
Butterbur leaf extract	0.1% and 0.5% w/w	Ground beef patties	4°C	12 days	SDL	(Kim et al., 2013)
Grape seed extracts	1.0%	Cooked beef	4°C	9days	SDL	(Ahn et al., 2007)

Pine bark extracts	1.0%					
Oleoresin rosemary	1.0%					
Grape seed extracts	400 and 1000ug/g	Pork petties	4°C	12 days	SDL	(Carpenter et al., 2007)
Bearberry extracts	80 and 1000u/g					
Broccoli powder Extracts	1.5 and 2%	Goat meat nugget	4°C	4-16 days	SDL	(Banerjee et al., 2012)
Cocoa leaf extract	200 mg/kg	Deboned	4°C	21 days	SDL	(Hassan and Fan, 2005)
Green tea leaf extract	200 mg/kg	Chicken meat				
Ginkgo <i>biloba</i> leaf extract	0.05%	Meat dumplings	-18°C	180days	SDL	(Kobus-Cisowska et al., 2010)
<i>Hypericum perforatum L.</i> extract	500ppm	Meat ball	4°C	21 days	SDL	(Kobus-Cisowska et al., 2014)
	0.0005% 0.001%	Pork meat	2°C±2	50 days	SDL	(Sanchez-Muniz et al., 2012)

SDL= significantly decrease lipid oxidation

### **2.2.3.3 Anti-microbial activities of natural antioxidants on meat safety and quality preservation**

Globally, approximately 1.3 billion tons of food, including meat, is spoiled or wasted per year throughout the supply chain, from production down to final household consumption (FAO, 2011). The massive spoilage and wastage of meat products which has become a major concern to consumers, governments and food industries is also associated with the outbreak of foodborne diseases (Sant'Ana, 2012). Almost 50% of the total meat spoilage and wastage occurs at the household consumption level due to poor preservative techniques and facilities. Meat wastages are caused through microbial and chemical spoilage with the consequence of foodborne illnesses, economic loss and food insecurity. However, meat spoilage bacteria can be reduced by applying natural antioxidants directly into the meat products. The antimicrobial activities of the plant-derived antioxidants for example: *Artemisia absinthium*, *Hypericum perforatum*, *Oleoresin rosemary*, *Origanum vulgare*, *Satureja horvatii*, *Syzygium aromaticum*, *Fatsia spp*, and olive among others, against microbial growth in meat and meat products have been reported in several studies (Table 2.2; Sanchez-Muniz et al., 2012; Kim et al., 2013; Kurcubic et al., 2014). Some interesting results were however found by combining different plants together to test their efficacy against food borne organisms that are prevalent in meat and meat products. Krishnan et al. (2014) found a stronger antimicrobial effect of the combination of *Syzygium aromaticum*, *Cinnmomum cassia* and *Origanum vulgare* extracts in chicken meat than individual spices, and they attributed this to synergistic actions of each specific compounds present in the mixed spices.

The use of natural compounds such as organic acids and essential oils has been identified for decontamination of beef, pork and poultry products against *Salmonella* (Mani-Lopez et al., 2012;

Sant'Ana et al., 2014). The effectiveness of the essential oils and/or crude extracts from most of the natural antioxidant plants in Tables 2.2 have been reported in several studies. The presence and level of concentration of different phytochemical compounds such as phenolic, flavonoid, alkaloids, saponins, tannins, carvacrol, terpenes, thymol among others, have been recognized as the potential source of antimicrobial activities in plant materials (Sharma et al., 2012). Although the potential mechanism of action of plant phytochemicals against microbial growth in muscle food has not been fully aimed, however, some authors have reported their ability to disrupt or degrade the cytoplasmic membrane and cell wall of spoilage microorganisms to inhibit their growth (Kim et al., 2013; Krishnan et al., 2014).

#### **2.2.3.4 Preservative effect of natural antioxidants on pH, colour and sensory properties of meat**

Reports on the use of plant-derived antioxidants revealed that they can regulate and improve colour, pH, flavor and taste of meat and meat products compared to control or synthetic antioxidant (ST) treated meat samples (Velasco and Williams, 2011; Shah et al., 2014). Colour is one of the most important quality attributes of meat that attract the preference of the consumers (Lorenzo et al. 2013). In fresh meat, consumers evaluate the freshness and wholesomeness of meat by observing the colour, while in cooked meat; colour is exploited as an indicator of doneness and freshness at the point of consumption (Suman and Joseph, 2013). Moreover, the intensity of meat colour is dependent on species, age and muscle type, and the colour differences are affected by different content of myoglobin in muscle (Joo et al., 2013).



**Table 2. 2 Antimicrobial activities of medicinal plants on meat and meat products compared to control.**

Plant materials	Meat type	Effect on foodborne pathogenic organisms	Dosages	Storage degrees	Storage time	References
<i>Syzygium aromaticum</i> extracts <i>Cinnmorum cassia</i> extracts <i>Origanum vulgare</i> extracts <i>Brassica nigra</i> extracts	Raw chicken meat	It reduces the growth of <i>Psueodomonus</i> species <i>Enterobacteriaceae</i> (psychrotrophic) Lactic acid bacteria	1% v/w	4 °C	0-15 days	(Krishnan et al., 2014)
<i>Kitaibeliavitifolia</i> extract Rosemary extracts	Fermented dry sausage chicken meat model	It reduces the growth of <i>Escherichia coli</i> It reduces the growth of <i>Campylobacter jejuni</i>	12.5 g/kg of meat dough 0.20 mg/mL	4 °C 8 °C	0-60 days	(Kurcubic et al., 2014) (Piskernik et al., 2011)
Saturejahorvatii essential oil	pork meat	It reduces the growth of <i>Listeria monocytogenes</i>	0.16–20 mg/mL.	25 °C	4 days	(Bukvicki et al., 2014)
<i>Moringa oleifera</i> leaf extract	Ground pork patties	It reduces the growth of aerobic plate count and psychrophilic microorganisms	300, 450, 600 ppm	4 °C	0-9 days	Muthukumar et al, 2012
Chamnamul leave extract Fatsia leaf extract	Ground beef patties	It greatly reduces the count of Lactic acid bacteria, Coliform bacteria, Yeast and mold	0.1% and 0.5% (w/w)	4 °C	0-12days	(Kim et al., 2013)

<i>Artemisia absinthium</i> <i>L</i> extract	Raw beef meat	It reduces the multiplication of <i>Salmonella typhimurium</i> , <i>Escherichia coli</i> <i>Listeria</i> <i>monocytogenes</i> <i>Staphylococ-</i> <i>us aureus</i>	$5 \pm 2$ °C	7 days	(Cruz-Galvez et al., 2013)
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The addition of plant extracts in fresh and cooked meat has been reported to inhibit degradation of heme pigments, delay metmyoglobin formation and stabilize the redness (Fernandez-Lopez et al., 2003; Rababah et al., 2011; Wojciak et al., 2011; Muthukumar et al., 2012). Although in some cases, little or no significant changes was found in colour parameters of meat treated with plant-derived antioxidants compared to synthetic antioxidants. Furthermore, Krishnan et al. (2014) found in their study that raw meat treated with antioxidants had lower pH values than control and other treatments. They revealed that any increase in pH value during refrigeration storage could be due to the utilization of amino acids by bacteria during the protein break down when the stored glucose is depleted (Krishnan et al., 2014). Consequently, the degradation of amino acid coupled with accumulation of ammonia results in pH increase. However, some plant extracts have been noted to exert positive effect on sensory properties of meat. Ozvural and Vural (2012) and Reddy et al. (2013) in their studies found that meat products treated with plant-derived antioxidants had higher scores of color, flavor, juiciness and overall palatability than control and BHA treated meat. It is generally known that incorporation of plant extracts in meat products will promote preference and overall acceptability of meat.

#### **2.2.3.5 *Moringa oleifera* and *Biden pilosa* plants as sources of natural antioxidants**

*Moringa oleifera* L. (Moringaceae), commonly known as drumstick, is a fast-growing, annual or perennial plant. It is widely cultivated in many tropical and subtropical countries of Asia and Africa. The plant contains a variety of bioactive substances, which are of considerable interest in the food industry. Conventionally, almost all parts of these plants (the whole plant, the aerial parts and/or the roots) have been used on an extensive basis, either as food or medicinal components (Verma et al., 2009). The leaves are rich source of protein, essential amino acids and

other micronutrients (Sanchez-Machado et al., 2010; Moyo et al., 2012b). *Moringa oleifera* L. leaves have been identified as having high antioxidants, hypoglycemic, hypotensive, antidyslipidemic, anticancer, antibacterial and anti-inflammatory properties (Anwar et al., 2007; Sreelatha and Padma, 2009). Similarly, *Bidens pilosa* L. (Asteraceae), common known as black-jack, is a fast-growing, annual herb which formally is a native to the Americans but nowadays widely cultivated in Africa and Australia. In some parts of the world, its leaves and shoot are commonly utilized as an ingredient in foods and medicines, especially in times of food scarcity (Yang, 2014). According to Bartolome et al. (2013) about two hundred bioactive compounds have been isolated from *B. pilosa*, including aliphatics, flavonoids, terpenoids, phenylpropanoids, aromatics and others. Studies on biological activities have shown that its roots, leaves, and seeds possess antibacterial, antidiarrheal, anti-inflammatory, antimicrobial, antimalarial, diuretic, hepatoprotective, and hypotensive properties (Cortés-Rojas et al., 2013). Because of the above mentioned activities, the Food and Agriculture Organization of the United Nations has advocated the mass cultivation of *B. pilosa* in Africa for food and treatment of diseases (Young et al., 2010).

## **2.5 Effect of cooking temperature on meat nutritional quality**

Cooking is an essential process to transform fresh meat to edible, palatable and ready-to-eat products (Tornberg, 2005; Baldwin, 2012). However, cooking often influences the changes in colour, juiciness, tenderness and essential micronutrients including lipid, protein, minerals and vitamins of meat (Kondjoyan et al., 2013; Roldan et al., 2014). The rate and extent of these changes is dependent on type of cooking methods, durations and core temperatures that are used during the process. According to Tornberg (2005), cooking process can cause protein

denaturation, shrinkage of muscle fibres, destruction of cell membrane and myofibrillar protein, solubilization of connective tissue and loss of essential fatty acids. The higher and longer the cooking temperature applied on the meat, the greater the denaturation and nutrient loss. Moreover, the extent of changes in connective tissue and myofibrillar proteins as a result of cooking effect contribute greatly to the degree of moisture loss and toughness of meat (Baldwin, 2012). Many studies have shown that different cooking methods such as roasting, boiling, stewing, grilling, microwaving, and pan-frying can initiate the production of free radicals, causing oxidation of meat lipid and protein (Roldan et al., 2014; Dominguez et al., 2014). In addition, Alfaia et al. (2010) and Zhang et al. (2014) found that use of these cooking methods caused modification of polyunsaturated fatty acid (PUFA) and a severe reduction in essential amino acids, and mineral contents of cooked meat.

But in recent time, the technique of vacuum-sealing of meat and submerging them in a temperature-time controlled water bath (also known as sous vide techniques) has been found to greatly minimize nutrient losses in cooked meat (Sanchez del Pulgar et al., 2012). The method allows heat to be efficiently transferred from the water (or steam) to the meat sample. It also allows greater control over degree of doneness compared to other cooking methods (Baldwin, 2012). Specifically, sous vide cooking techniques can reduce heat damage to proteins and lipids, diminish heat-sensitive nutrients loss and prolong the shelf life of cooked products (Diaz et al., 2008). It can also increase tenderness, improves colour retention, reduces moisture loss and provides a superior flavour profile (Vaudagna et al., 2008; Roldan et al., 2014). The finding of Roldan et al. (2014) also revealed that the combination of different low cooking temperature and long cooking time cause a decrease in thiobarbituric reactive substances (TBARS) values and

volatile compound of cooked mutton. Additionally, occurrence of intense collagen solubilization has been noted using this technique, but changes in myofibrillar proteins which are responsible for toughening has been very mild (Sanchez del Pulgar et al., 2012; Roldan et al., 2014). Awareness on the appropriateness and ease of application of this cooking technique has encouraged its wide-used in restaurants, catering and industrial food processing (Roldan et al., 2014).

## **2.6 Consumption and utilization of liver meat in human diet**

Liver is an important internal organ that is essential for maintaining optimum function of other organs in live animals. It is described as the largest and almost complex organ in the body (Sundari et al., 2013). Biologically, the liver plays a significant role in the protein and lipid biosynthesis, storage and distribution of nutrients including glucose, minerals and vitamins in the body (Rhodes and Bell, 2013; da Costa et al., 2014). Beside these, it plays a crucial role in maintaining the internal environments through detoxification and excretion of many endogenous and exogenous compounds in the body (Sundari et al., 2013). On weight basis, liver accounts for 1-2% of whole-body tissue mass in bovine species (Li et al., 2014) indicating the largest organ in the body. Nutritionally, liver contain a wide range of important macro and micro nutrients which are comparable with lean meat tissue. Li et al. (2014) revealed that beef liver contains 17-19% protein, 3-5% fat 1.5% ash, 2-4% carbohydrate and 103-140 kcal total energy. In comparison to fresh lean meat, beef liver contains higher amounts of leucine, threonine, serine, glutamic acid, aspartic acid and phenylalanine, among other amino acids (Li et al., 2014).

Liver meat contains iron (Fe) content that is five times higher than beef steak when it comes to mineral composition, (Li et al., 2014). Moreso, the vitamin content of liver has been found to be higher than that of lean meat issue. Jayathilakan et al. (2012) reported that liver meat contains large amounts of riboflavin (1.697– 3.630 mg/100 g) which is 5–10 times higher than lean meat. In addition, liver meat has been described as the excellent source of niacin, vitamin B12, B6, folacin, carnosine, L-carnitine ascorbic acid and vitamin A (Ercan and El, 2011; Purchas and Busboom, 2005; Jayathilakan et al., 2012). Many studies have also showed that liver contains lower levels of monounsaturated fatty acids and higher levels of polyunsaturated fatty acids than lean tissue (Enser et al., 1998; Liu 2002; Jayathilakan et al., 2012). Based on the aforementioned nutritional quality, consumption of liver meat offers enormous opportunities to meet daily nutritional requirements of the populace. Studies have showed that liver is traditionally consumed in a number of countries around the world especially in South East Asia, Australia and Africa (Fatma and Mahdey, 2010). In some cookery, liver meat is considered as primal food that has health benefits and is consumed by people of all ages (Fayemi and Muchenje, 2014). Liver meat is usually consumed boiled, fried or processed into products such as liver pate, foiegras, leverpastej and liver sausages. The liver sausage is popular with most consumers, because of its beneficial fatty acid profile, high oxidative stability, and a balanced volatile profile (Estevez et al., 2005).

## **2.7 Summary of Review**

It can be concluded from this review that the occurrence of lipid oxidation and microbial spoilage in processed meat during processing and storage are inevitable and has a tremendous effect on product quality. However, this can be prevented through the application of antioxidants. Treatment of meat products with natural antioxidants will reduce rate of decoloration and rancidity during storage. It is also revealed that the use of natural antioxidants has a great antimicrobial potential to preserve meat from oxidative deterioration. In addition, the consumption of meat treated with natural antioxidants will help to boost consumer health and reduce economic loss in the meat industry. Lastly, it was also revealed that application of thermal treatments can induce oxidation of lipid through the loss of essential fatty acids and other bioactive components of meat. However, Sous vide cooking technique can be used to minimize this loss and optimize the nutritional content of meat during cooking.



## 2.8 References

- Adak, G. K., Meakins, S. M., Yip, H., Lopman, B. A. and O'Brien, S. J.** 2005. Disease risks from foods, England and Wales, 1996– 2000. *Emerging Infectious Diseases* **11 (3)**: 365- 372.
- Ahn, J., Grun, I. U., and Mustaphab, A.** 2007. Effects of plant extracts on microbial growth, color change, and lipid oxidation in cooked beef. *Food Microbiology* **24**: 7–14.
- Alfaia, C. M. M., Alves, S. P., Lopes, A. F., Fernandes, M. J. E., Costa, A S. H., Fontes, C.M.G.A., Castro, M. L.F., Bessa, R. J.B. and Prates, J. A.M.** 2010. Effect of cooking methods on fatty acids, conjugated isomers of linoleic acid and nutritional quality of beef intramuscular fat. *Meat Science* **84**: 769–777.
- Allen, K. and Cornforth, D.** 2010. Comparison of spice-derived antioxidants and metal chelators on fresh beef color stability. *Meat Science* **85**: 613–619.
- Anokwuru, C. P. Anyasor, G. N. Ajibaye, O., Fakoya, O. and Okebugwu, P.** 2011. Effect of Extraction Solvents on Phenolic, Flavonoid and Antioxidant activities of Three Nigerian Medicinal Plants. *Nature and Science* **9**: 7.
- Anwar, F., Latif, S., Ashraf, M. and Gilani, A. H.** 2007. Moringa oleifera: a food plant with multiple medicinal uses. *Phytotherapy Research* **21**:17–25.
- Antolovich, M., Prenzler, P.D., Patsalides, E., Mc Donald, S. and Robards, K.** 2002. Methods for testing antioxidant activity. *The Analyst* **127**: 183 -198.
- Baldwin, D. E.** 2012. Sous vide cooking: A review. *International Journal of Gastronomy and Food Science* **1 (1)**: 15–30.

- Banerjee, R., Verma, A. K., Das, A. K., Rajkumar, V., Shewalkar, A. A. and Narkhede, H. P.** 2012. Antioxidant effects of broccoli powder extract in goat meat nuggets. *Meat Science* **91**: 179-184.
- Barbieri, E. and Sestili, P.** 2012. Reactive oxygen species in skeletal muscle signaling. *Journal of Signal Transduction* **2012** 1–17.
- Bartolome, A. P., Villaseñor, I. M. and Yang, W.** 2013. *Bidens pilosa* L. (Asteraceae): Botanical Properties, Traditional Uses, Phytochemistry, and Pharmacology. *Evidence-Based Complementary and Alternative Medicine* **2013**: 51
- Biswas, A. K., Chatli, M. K. and Sahoo, J.** 2012. Antioxidant potential of curry (*Murrayakoenigii* L.) and mint (*Menthaspicata*) leaf extracts and their effect on colour and oxidative stability of raw ground pork meat during refrigeration storage. *Food Chemistry* **133**: 467–472.
- Borch, E., Kant-Muemans, M and Blixt, Y.** 1996. Bacterial spoilage of meat products and cured meat. *International Journal of Food Microbiology* **33**: 103-120.
- Bukvicki, D., Stojkovic, D., Sokovic , M., Vannini, L., Montanari , C., Pejcin , B., Savic, A., Veljic, M., Grujic , S. and Marin, P.D.** 2014. Satureja horvatii essential oil: In vitro antimicrobial and antiradical properties and in situ control of *Listeria monocytogenes* in pork meat. *Meat Science* **96**: 1355–1360.
- Camo, J., Beltran, J. A. and Roncales, P.** 2008. Extension of the display life of lamb with an antioxidant active packaging. *Meat Science* **80**: 1086-109.
- Carpenter, R., O’Grady, M. N., O’Callaghan, Y. C., O’Brien, N. M. and Kerry, J. P.** 2007. Evaluation of the antioxidant potential of grape seed and bearberry extracts in raw and cooked pork. *Meat Science* **76**: 604–610.

- Cataldi, A.** 2010. Cell responses to oxidative stressors. *Current Pharmaceutical Design* **16(12)**: 1387–1395.
- Cervený, J., Meyer, J.D. and Hall, P.A.** 2009. Microbiological spoilage of meat and poultry products In: Compendium of the microbiological spoilage, of foods and beverages. Food Microbiology and Food Safety, W.H. Sperber and M.P. Doyle (Ed.). Springer Science and Business Media, New York, pp 69-868.
- Chaijan, M.** 2008. Review: Lipid and myoglobin oxidations in muscle foods. *Songklanakarin Journal of Science and Technology* **30 (1)**: 47-53.
- Clarence, S. Y., Obinna, C. N. and Shalom, N. C.** 2009. Assessment of bacteriological quality of ready to eat food (Meat pie) in Benin City metropolis, Nigeria. *African Journal of Microbiology Research* **3(6)**: 390-395.
- Cortés-Rojas, D. F., Chagas-Paula, D. A., Da Costa, F. B., Souza, C. R. F. and Oliveira, W. P.** 2013. Bioactive compounds in *Bidens pilosa* L. populations: a key step in the standardization of phytopharmaceutical preparations. *Brazilian Journal of Pharmacognosy* **23(1)**: 28-35.
- Cruz-Galvez, A. M., Gómez-Aldapa, C.A., Villagomez-Ibarra, J. A., Chavarria-Hernández, N.,Rodriguez-Banos, J., Rangel-Vargas, E. and Castro-Rosas, E.** 2013. Antibacterial effect against foodborne bacteria of plants used in traditional medicine in central Mexico: Studies in vitro and in raw beef. *Food Control* **32**: 289-295.
- da Costa, A. S. H.,Bessa, R. J. B., Pires, V. M. R., Rolo, E. A., Pinto, R. M. A., Fontes, C. M. G. A. and Prates, J. A. M.** 2014. Is hepatic lipid metabolism of beef cattle influenced by breed and dietary silage level? *BMC Veterinary Research* **10**: 65.

- Dai, J and Mumper, R. J.** 2010. Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. *Molecules* **15**: 7313-7352.
- Dangles, O. and Dufour, C.** 2006. Flavonoid-protein interactions. In: Flavonoids: chemistry, biochemistry and applications, O. M. Andersen and K. R. Markham (Ed), pp 443-464. CRC Press, Washington.
- Dave, D. and Ghaly, A. E.** 2011. Meat Spoilage Mechanisms and Preservation Techniques: A Critical Review. *American Journal of Agricultural and Biological Sciences* **6 (4)**: 486-510.
- Diaz, P., Nieto, G., Garrido, M. D. and Banon, S.** 2008. Microbial, physical–chemical and sensory spoilage during the refrigerated storage of cooked pork loin processed by the sous vide method. *Meat Science* **80**: 287–292.
- Domínguez, R., Gomez, M., Fonseca, S. and Lorenzo. J. M.** 2014. Influence of thermal treatment on formation of volatile compounds, cooking loss and lipid oxidation in foal meat. *LWT - Food Science and Technology* **58**: 439-445.
- Doolaee, E. H. A., Vossen, E., Raes, K., Meulenaer, B. D., Verhé, R., Paelinck, H. and Smet, S. D.** 2012. Effect of rosemary extract dose on lipid oxidation, colour stability and antioxidant concentrations, in reduced nitrite liver pâtés. *Meat Science* **90**: 925-931.
- Duthie, G., Campbell, F., Bestwick, C., Stephen, S. and Russell, W.** 2013. Antioxidant effectiveness of vegetable powders on the lipid and protein oxidative stability of cooked turkey meat patties: Implications for health. *Nutrients* **5 (4)**: 1241–1252.
- Enser, M., Hallett, K. G., Hewett, B., Fursey, G. A. J., Wood, J. D. and Harrington, G.** 1998. The polyunsaturated fatty acid composition of beef and lamb liver. *Meat Science* **49 (3)**: 321-327, 1998.

- Ercan, P and El, S. F.** 2011. Changes in content of coenzyme Q10 in beef muscle, beef liver and beef heart with cooking and in vitro digestion. *Journal of Food Composition and Analysis* **24**: 1136–1140.
- Ercolini, D., Russo, F., Nasi, A., Ferranti, V. and Villani, V.** 2009. Mesophilic and psychrotrophic bacteria from meat and their spoilage potential in vitro and in beef *Applied Environmental Microbiology* **75**:1990–2001.
- Estevez, M., Ventanas, S., and Cava, R.** 2005. Physico-chemical properties and oxidation stability of liver pa<sup>te</sup> as affected by fat content. *Food Chemistry* **92**: 449–457.
- FAO,** 2003. Assuring food safety and quality: guidelines for strengthening national food control systems. Available on: [http:// www.fao.org](http://www.fao.org). Accessed on 12/12/2014.
- FAO,** 2011. Global food losses and food waste. Rome. [Hpp:// www.fao.org/docrep](http://www.fao.org/docrep). Accessed on 12/12/2014.
- Fasseas, M. K., Mountzouris, K. C., Tarantilis, P. A., Polissiou, M. and Zervas, G.** 2007. Antioxidant activity in meat treated with oregano and sage essential oils. *Food Chemistry* **106**: 1188-1194.
- Fatma, H. M. and Mahdey, E. A.** 2010, ‘Incidence of *Brucella* species in slaughtered food animals and its edible offal at Beni-suef, Egypt’, *Global Veterinaria* **5**: 248–254.
- Fayemi, P. O. and Muchenje, V.** 2014. Farmers’ preference for mutton and lamb from natural velds in the Eastern Cape Province of South Africa. *Journal of Food, Agriculture and Environment* **12 (2)**: 15-19.
- Faustman, C. and Cassens, R .G.** 1991. The effect of cattle breed and muscle type on discoloration and various biochemical parameters in fresh beef. *Journal of Animal Science* **69**:184-193.

- Fernandez-Lopez, J., Sevilla, L., Sayas-Barbera, E., Navarro, C., Marin, F. and Perez-Alvarez, J. A.** 2003. Evaluation of the antioxidant potential of hyssop (*Hyssopus officinalis* L.) and rosemary (*Rosmarinus officinalis* L.) extracts in cooked pork meat. *Journal of Food Science* **68**: 660–664.
- Gadekar , Y. P., Sharma, B. D., Shinde, A. K., Verma, A.K. and Mendiratta, S. K.** 2014. Effect of natural antioxidants on the quality of cured, restructured goat meat product during refrigerated storage ( $4 \pm 1$  °C). *Small Ruminant Research* **119**: 72–80.
- Goncalves, R.S.I, Battistin, A., Pauletti, G., Rota, L. and Serafini, L. A.** 2009. Antioxidant properties of essential oils from *Mentha* species evidenced by electrochemical methods. *Brazilian Journal of Medicinal Plant* **11**: 4.
- Gordon M.H.** 2001. The development of oxidative rancidity: Antioxidants in Food – Practical Applications, J. Pokorny, N. Yanishlieva and M. H.Gordon (Ed.), CRC Press, Washington, pp. 7–22.
- Hassan, O. and Fan, S.** 2005. The anti-oxidation potential of polyphenol extract from cocoa leaves. *LWT-Food Science and Technology* **315**: 321.
- Hayes, J. E., Stepanyana, V., Allena, P., O’Grady, M. N. and Kerry, J. P.** 2010. Effect of lutein, sesamol, ellagic acid and olive leaf extract on the quality and shelf-life stability of packaged raw minced beef patties. *Meat Science* **84**: 613–620.
- Hernandez-Macedo, M. L., Barancelli, G. V. and Contreras-Castillo, C. J.** 2011. Microbial deterioration of vacuum-packaged chilled beef cuts and techniques for microbiota detection and characterization: a review. *Brazilian Journal of Microbiology* **42**:1.

- Hygreeva, D., Pandey, M.C. and Radhakrishna, K.** 2014. Potential applications of plant based derivatives as fat replacers, antioxidants and antimicrobials in fresh and processed meat products. *Meat Science* **98**: 47–57.
- Jacobsen, C., Undeland, I., Storrø, I., Rustad, T., Hedges, N. and Medina, I.** 2008. Preventing lipid oxidation in seafood, in *Improving Seafood Products for the Consumer: Improving seafood products for the consumer*, T. Borresen (Ed), Cambridge, Woodhead Publishing. pp 426-460.
- James, S. J. and James, C.** 2002. *Meat Refrigeration*, Woodhead Publishing Limited, Cambridge: England and CRC Press, New York, Washington DC, USA. pp 91- 94.
- Jayathilakan, K., Sultana, K., Radhakrishna, K. and Bawa, A. S.** 2012. Utilization of byproducts and waste materials from meat, poultry and fish processing industries: a review. *Journal of Food Science and Technology* **49 (3)**: 278–293.
- Ji, N., Kong, B., Liu, Q., Diao, X. and Xia, X.** 2012. Antioxidant activity of black currant (*Ribesnigrum* L.) extract and its inhibitory effect on lipid and protein oxidation of pork patties during chilled storage. *Meat Science* **91**:533–539.
- Joo, S.T., Kim, G.D., Hwang, Y.H. and Ryu, Y. C.** 2013. Control of fresh meat quality through manipulation of muscle fibre characteristics. *Meat Science* **95**: 828-836.
- Karre, L., Lopez, K. and Getty, K. J.** 2013. Natural antioxidants in meat and poultry products. *Meat Science* **94 (2)**: 220-227.
- Kim, S., Min, S. C., Shin, H., Lee, Y., Cho, A. R., Kim, S. Y. and Han, J.** 2013. Evaluation of the antioxidant activities and nutritional properties of ten edible plant extracts and their application to fresh ground beef. *Meat Science* **93**: 715–722.

- Kobus-Cisowska, J. Flaczyk, E. and Jeszka, M.** 2010. Antioxidant activities of ginkgo biloba extracts: application in freeze stored meat dumplings. *ACTA Scientiarum Polonorum Technologia Alimentaria* **9 (2)**: 161-170.
- Kobus-Cisowska, J., Flaczyk, E., Rudzinska, M. and Kmiecik, D.** 2014. Antioxidant properties of extracts from Ginkgo biloba leaves in meatballs. *Meat Science* **97**: 174–180.
- Kondjoyan, A., Oilic, S., Portanguen, S. and Gros, J.** 2013. Combined heat transfer and kinetic models to predict cooking loss during heat treatment of beef meat. *Meat Science* **95**: 336–344.
- Kothari, V., Gupta, A. and Naraniwal, M.** 2012. Comparative study of various methods for extraction of antioxidant and antibacterial compounds from plant seeds. *Journal of Natural Remedies* **12 (2)**: 162 – 173.
- Krishnan, K. R., Babuskina, S., AzhaguSaravanaBabu, P., Sasikala, M., Sabina, K., Archana, G. Sivarajan, M. and Sukumar, M.** 2014. Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. *International Journal of Food Microbiology* **171**: 32–40.
- Kurcubic, V.S., Maskovic, P. Z., Vujic, M., Vranic, D. V., Veskovic-Moracanin, S. M., Okanovic, D. G. and Lilic, V.** 2014. Antioxidant and antimicrobial activity of Kitaibelia vitifolia extract as alternative to the added nitrite in fermented dry sausage. *Meat Science* **97**: 459–467.
- Lauritzsen, K., Martinsen, G. and Olsen, R. L.** 1990. Copper induced lipid oxidation during salting of COD (*Gadus morhua* L.). *Journal of Food Lipids* **6 (4)**: 299-315.



- Li, R., Yu, Q., Han, L. and Cao, H.** 2014. Nutritional characteristics and active components in liver from Wagyu×Qinchuan cattle. *Korean Journal for Food Science of Animal Resources* **34 (2)**: 214~220.
- Liu, D. C.** 2002. Better utilization of by-products from the meat industry. *EFTC Extension Bulletins* **304**:2462.
- Lorenzo, J. M., González-Rodríguez, R. M., Sánchez, M., Amado, I. R. and Franco, D.** 2013. Effects of natural (grape seed and chestnut extract) and synthetic antioxidants (butylatedhydroxytoluene, BHT) on the physical, chemical, microbiological and sensory characteristics of dry cured sausage “chorizo” *Food Research International* **54**: 611–620.
- Lund, M. N., Heinonen, M., Baron, C. P. and Estevez, M.** 2011. Protein oxidation in muscle foods: A review. *Molecular, Nutrition and Food Research* **55**: 83–95.
- Lynch, M. P., Faustman, C., Silbart, L. K., Rood, D. and Furr, H. C.** 2001. Detection of lipid derived aldehydes and aldehyde: Protein adducts in vitro and in beef. *Journal of Food Science* **66**: 1093–1099.
- Mani-Lopez, E., Garcia, H.S. and Lopez-Malo, A.** 2012. Organic acids as antimicrobials to control Salmonella in meat and poultry products. *Food Research International* **45 (2)**: 713-721.
- Mapiye, C., Aldai, N., Turner, T.D., Aalhus, J. L., Rolland, D.C., Kramer, J. K. G. and Dugan, M. E. R.** 2012. The labile lipid fraction of meat: From perceived disease and waste to health and opportunity. *Meat Science* **92**: 210-220.
- Megan-Tempest, R. D.** 2012. Adding spice for a healthier life — Evidence shows antioxidant-rich herbs and spices may cut chronic disease risk. *Today's Dietitian* **14 (3)**: 40.

- Min, B. and Ahn, D. U.** 2005. Mechanism of lipid peroxidation in meat and meat products -a review. *Food Science Biotechnology* **14 (1)**: 152- 163.
- Min, B., Nam, K.C., Cordray, J. and Ahn, D.U.** 2008. Endogenous factors affecting oxidative stability of beef loin, pork loin, and chicken breast and thigh meats. *Journal of Food Science* **73**: 6.
- Mirzaei, A. and Khatami, R.** 2013. Antioxidant and iron chelating activity of coriander sativum and petroselinum crispum. *Bulletin of Environment, Pharmacology and Life Sciences* **2 (7)**: 27-31.
- Mohamed, H. M. H., Mansour, H. A. and Farag, M. D.** 2011. The use of natural herbal extracts for improving the lipid stability and sensory characteristics of irradiated ground beef. *Meat Science* **87**: 33–39.
- Moyo, B., Oyedemi, S., Masika, P. J. and Muchenje, V.** 2012a. Polyphenolic content and antioxidant properties of *Moringa oleifera* leaf extracts and enzymatic activity of liver from goats supplemented with *Moringa oleifera* leaves/sunflower seed cake. *Meat Science* **91**: 441–447.
- Moyo, B., Masika, P. J., Hugo, A. and Muchenje, V.** 2012b. Nutritional characterization of *Moringa* (*Moringa oleifera* Lam.) leaves. *African Journal of Biotechnology* **10 (60)**: 12925-12933.
- Muthukumar, M., Naveena, B. M., Vaithyanathan, S., Sen, A. R., and Sureshkumar, K.** 2012. Effect of incorporation of *Moringa oleifera* leaves extract on quality of ground pork patties. *Journal of Food Science and Technology* **51 (11)**: 3172-3180.

- Nkukwana, T.T., Muchenje, V., Masika, P.J., Hoffman, L.C., Dzama, K. and Descalzo, A.M.** 2014. Fatty acid composition and oxidative stability of breast meat from broiler chickens supplemented with *Moringa oleifera* leaf meal over a period of refrigeration. *Food Chemistry* **142**: 255–261.
- Nychas, G. E., Skandamis, P. N., Tassou, C. C. and Koutsoumanis, K. P.** 2008. Meat spoilage during distribution. *Meat Science* **78**: 77–89.
- Ozvural, E. B. and Vural, H.** 2012. The effects of grape seed extract on quality characteristics of frankfurters. *Journal of Food Processing and Preservation* **36**: 291–297.
- Paulsen, P. and Smulders, F. J. M.** 2014. Microbiology. In Encyclopedia of Meat Science: 3-volume set. Dikeman, M and Devine, C (Ed.). Academic Press, London, UK, pp 430-450.
- Pennington, J. A. T. and Fisher, R. A.** 2009. Classification of fruits and vegetables. *Journal of Food Composition and Analysis* **22**: 23-31.
- Piskernik, S., Klancnik, A., Riedel, C. T., Brondsted, L. and Mozina, S.S.** 2011. Reduction of *Campylobacter jejuni* by natural antimicrobials in chicken meat-related conditions. *Food Control* **22**: 718-724.
- Pokorny, J.** 2007. Antioxidant in food preservation. In Handbook in food preservation, Rahman, S. (Ed). CRC Press, USA, pp 260-281.
- Power, S. T. and Jackson, M. J.** 2008. Exercise-induced oxidative stress: Cellular mechanisms and impact on muscle force production. *Physiology Reviews* **88 (4)**: 1243–1276.
- Purchas, R. W. and Busboom, J. R.** 2005. The effect of production system and age on levels of iron, taurine, carnosine, coenzyme Q10, and creatine in beef muscles and liver. *Meat Science* **70**: 589–596.

- Rababah, T. M., Ereifej, K. I., Alhamad, M. N., Al-Qudah, K. M., Rousan, L. M., Al-Mahasneh, M.A., Al-u'datt, M. H. and Yang, W.** 2011. Effects of green tea and grape seed and TBHQ on physicochemical properties of Baladi goat meats. *International Journal of Food Properties* **14**: 1208–1216.
- Reddy, G. V. B., Sen, A. R., Nair, P. N., Reddy, K. S., Reddy, K. K. and Kondaiah, N.** 2013. Effects of grape seed extract on the oxidative and microbial stability of restructured mutton slices. *Meat Science* **95**: 288–294.
- Rhodes, R. and Bell, D. R.** 2013. Medical Physiology- Principles for Clinical Medicine Chapter: The physiology of Liver, Lippincott Williams and Wilkins, New York, pp 536- 565.
- Richards, M. P., Modra, A.M., and Li, R.** 2002. Role of deoxyhemoglobin in lipid oxidation of washed cod muscle mediated by trout, poultry and beef hemoglobin. *Meat Science* **62**: 157–163.
- Roldan, M., Antequera, T., Armenteros, M. and Ruiz, J.** 2014. Effect of different temperature–time combinations on lipid and protein oxidation of sous-vide cooked lamb loins. *Food Chemistry* **149**:129–136.
- Rulisek, L. and Vondrasek, J.** 1998. Coordination geometries of selected transition metal ions ( $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Hg}^{2+}$ ) in metalloproteins. *Journal of Inorganic Biochemistry* **71** (3–4): 115-127.
- Sampaio, G. R., Saldanha, T., Soares, R. A. M. and Torres, E. A. F. S.** 2012. Effect of natural antioxidant combinations on lipid oxidation in cooked chicken meat during refrigerated storage. *Food Chemistry* **135**: 1383–1390.

- Sample, S.** 2013. Oxidation and Antioxidants in Fish and Meat from Farm to Fork. In Food industry. Muzzalupo, I. (Ed.), InTech Publishing, Croatia, pp 114-144.
- Sanchez del Pulgar, J., Gazquez, A. and Ruiz-Carrascal, J.** 2012. Physico-chemical, textural and structural characteristics of sous-vide cooked pork cheeks as affected by vacuum, cooking temperature, and cooking time. *Meat Science* **90**: 828–835.
- Sanchez del Pulgar, J.S., Gázquez, A. and Ruiz-Carrascal, J.** 2012. Physico-chemical, textural and structural characteristics of sous-vide cooked pork cheeks as affected by vacuum, cooking temperature, and cooking time. *Meat Science* **90**: 828–835.
- Sanchez-Machado, D. I., Núñez-Gastelum, J. A., Reyes-Moreno, C., Ramírez-Wong, B. and Lopez-Cervantes, J.** 2010. Nutritional quality of edible parts of *Moringa oleifera*. *Food Analytical Methods* **3**:175-180.
- Sanchez-Muniz, F. J., Olivero-David, R., Triki, M., Salcedo, L., Gonzalez-Munoz, M. J., Cofrades, S., Ruiz-Capillas, C., Jiménez-Colmenero, F. and Benedi, J.** 2012. Antioxidant activity of *Hypericum perforatum* L. extract in enriched n-3 PUFA pork meat systems during chilled storage, *Food Research International* **48** (2): 909-915.
- Sant'Ana, A. S.** 2012. Introduction to the special issue: Salmonella in foods: Evolution, strategies and challenges. *Food Research International* **45** (2): 451–454.
- Sant'Ana, A. S., Franco, B. D. G. M. and Schaffner, D. W.** 2014. Risk of infection with Salmonella and *Listeria monocytogenes* due to consumption of ready-to-eat leafy vegetables in Brazil. *Food Control* **42**: 1-8.
- Shah, M. A., Don Bosco, S. J. and Mi, S. A.** 2014. Plant extracts as natural antioxidants in meat and meat products. *Meat Science* **98**: 21–33.

- Shahidi, F. and Zhong, Y.** 2010. Novel antioxidants in food quality preservation and health promotion. *European Journal of Lipid Science and Technology* **112**: 930–940.
- Sharma, A. K., Gangwar, M., Tilak, R., Nath, G., Sinha, A. S. K., Tripathi, Y. B. and Kumar, D.** 2012. Comparative in vitro antimicrobial and phytochemical evaluation of methanolic extract of root, stem and leaf of *Jatropha curcas* Linn. *Pharmacognosy Journal* **4 (30)**: 34–40.
- Sreelatha, S. and Padma, P. R.** 2009. Antioxidant activity and total polyphenol content of *Moringa oleifera* leaves in two stages of maturity. *Plant Food Human Nutrition* **64**: 303-311.
- Sultana, B, Anwar, F. and Ashraf, M.** 2009. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules* **14**: 2167-2180.
- Suman, S. P. and Joseph, P.** 2013. Myoglobin Chemistry and Meat Color. *Annual Review of Food Science and Technology* **4**: 79-99.
- Sundari, K., Karthik, D., Ilavenil, S., Kaleeswaran, S., Srigopalram, S. and Ravikumar, S.** 2013. Hepatoprotective and proteomic mechanism of *Sphaeranthus indicus* in paracetamol induced hepatotoxicity in wistar rats. *Food Bioscience* **1**: 57–65.
- Tan, P. W., Tan, C. P. and Ho, C. W.** 2011. Antioxidant properties: Effects of solid-to-solvent ratio on antioxidant compounds and capacities of Pegaga (*Centella asiatica*). *International Food Research Journal* **18**: 557-562.
- Tornberg, E.** 2005. Effect of heat on meat proteins—implications on structure and quality of meat products. *Meat Science* **70**: 493–508.
- Ukut, I. O. E., Okonko, I. O. and Ikpoh, I. S.** 2010. Assessment of bacteriological quality of fresh meats sold in Calabar metropolis, Nigeria. *EJEAF Chemistry* **9 (1)**: 89-100.

- Valenzuela, A.B., Sanhueza, J. and Nieto, S.** 2003. Natural antioxidants in functional foods: From food safety to health benefits. *Grasas y Aceites* **54 (3)**: 295–303.
- Vaudagna, S. R., Pazos, A.A., Guidi, S.M., Sanchez, G., Carp, D. J. and Gonzalez, C. B.** 2008. Effect of salt addition on sous vides cooked whole beef muscles from Argentina. *Meat Science* **79**: 470–482.
- Velasco, J., Dobarganes, C. and Márquez-Ruiz, G.** 2010. Oxidative rancidity in foods and food quality: Chemical deterioration and physical instability of food and beverages. CRC Press New York, pp 3–32.
- Velasco, V. and Williams, P.** 2011. Improving meat quality through natural antioxidants. *Chilean Journal of Agricultural Research* **71**: 2.
- Verma, A. R., Vijayakumar, M., Mathela, C .S. and Rao, C. V.** 2009. In vitro and in vivo antioxidant properties of different fractions of Moringa oleifera leaves. *Food Chemistry and Toxicology* **47**: 2196–2201.
- Wojciak, K. M., Dolatowski, Z. J. and Okon, A.** 2011. The effect of water plant extracts addition on the oxidative stability of meat products. *Acta Scientiarum Polonorum. Technologia Alimentaria* **10**: 175–188.
- Wsowicz, E., Gramza, A., Hêoe, M., Jelen, H. H., Korczak, J., Maecka, M., Mildner-Szkudlarz, S., Rudzinska, M., Samotyja, U. and Zawirska-Wojtasiak, R.** 2004. Oxidation of lipids in food. *Polish Journal of Food and Nutrition Sciences* **13/54 (1)**: 87–100.
- Xiao, S., Zhang, W. G., Lee, E. J. and Ahn, D. U.** 2013. Effects of diet, packaging and irradiation on protein oxidation, lipid oxidation of raw broiler thigh meat. *Animal Industry Report* 659-2761.

- Yang, W.** 2014. Botanical, pharmacological, phytochemical, and toxicological aspects of the antidiabetic plant *Bidens pilosa* L. *Evidence-Based Complementary and Alternative Medicine* **2014**: 14.
- Young, P. H., Hsu, Y. J. and Yang, W. C.** 2010. *Bidens pilosa* and its medicinal use. *Recent Progress in Medicinal Plants/Drug Plants II* **28**: 411–426.
- Zhang, W., Xiao, S., Lee, E. J. and Ahn, D. U.** 2011. Consumption of oxidized oil increases oxidative stress in broilers and affects the quality of breast meat. *Journal of Agricultural and Food Chemistry* **59 (3)**: 969-674.
- Zhang, Y., Wang, X., Wang, W. and Zhang, J.** 2014. Effect of boiling and frying on nutritional value and in vitro digestibility of rabbit meat. *African Journal of Food Science* **.8 (2)**: 92-103.
- Zinoviadou, K. G., Koutsoumanis, K. P. and Biliaderis, C. G.** 2009. Physico-chemical properties of whey protein isolate films containing oregano oil and their antimicrobial action against spoilage flora of fresh beef. *Meat Science* **82**: 338-345.



### **Chapter 3: Consumers' awareness of the use of natural antioxidants as preservatives in meat and meat products**

*(Submitted to Journal of New Generation Sciences)*

#### **Abstract**

The objective of the study was to assess the level of consumers' awareness on the use of natural antioxidants in meat and meat products in University of Fort Hare community, Eastern Cape Province, South Africa. Data was collected randomly from a total of 222 respondents and analyzed using descriptive statistics. The  $X^2$  test was used to determine associations between consumer demographic characteristics and their awareness on the use of natural antioxidants to preserve meat products. Results from the study revealed that approximately 53% and 47% of the respondents were male and female, respectively. Majority of the respondents (98.65%) indicated that they ate meat. In addition, 51.35% of the respondents indicated that they had not heard about the use of antioxidants as preservatives in meat products. However, among the remaining respondents (48.65%) were aware of antioxidants and their use as preservatives, about 19% knew of natural antioxidants, 35% synthetic antioxidants and 46% both natural and synthetic antioxidants. The results further revealed that the majority of the respondents (82%) that had knowledge of natural antioxidants were greatly concerned about the use of synthetic antioxidants in meat and meat products due to their health consequences. In conclusion, this study revealed that not all consumers were aware of application of natural antioxidants as preservatives in meat and meat products.

**Key words:** Antioxidants, consumers, meat spoilage, storage

### 3.1 Introduction

The use of antioxidants as preservatives in muscle foods has been instrumental in increasing shelf life and food security over the years (Metsovas, 2013). It is well known that meat and meat products are easily susceptible to oxidation and microbial contamination due to their high chemical composition and rapid depletion of endogenous antioxidants after postmortem (Xiao et al., 2013). Evidence has shown that live muscle contains relative amount of endogenous antioxidants, including alpha-tocopherol, histidine-containing dipeptides, ubiquinone, glutathione, carnosine and anserine, which are capable of scavenging free radicals and disrupting oxidative processes *in vivo* (Decker et al., 2000; Williams, 2007; Xiao et al., 2013). However, after slaughtering, this muscle tissue begins to lose its antioxidative potential due to various post-slaughter conditions such as anaerobic environment, presence of free radicals (reactive oxygen and nitrogen species) and lack of enzymatic mechanisms (Carlsen et al., 2005; Kumar et al., 2015 ).

As *postmortem* time increases, the activities of endogenous antioxidants continue to diminish (Xiao et al., 2013; Kumar et al., 2015), thereby exposing the lipid and protein component of muscle to rapid deterioration during processing and storage. In an attempt to boost meat antioxidant content and increase shelf life, different antioxidants (natural or synthetic) are used in the meat industry. Antioxidants are compounds or systems that can safely interact with free radicals to prevent oxidation of pigment, lipid and proteins in muscle food during processing and cold storage (Embuscado, 2015; Oroian and Escriche, 2015).

Recently, the use of synthetic antioxidants in food/meat products has been reported in causing negative health effect on consumers (Sarafian et al., 2002; Faine et al., 2006; Hazra et al., 2012; Kumar et al., 2015). Consequently, there is high preference for meat products containing natural antioxidants. Natural antioxidants are found in abundance in a wide range of natural sources including fruits, herbs, grains, spices, nuts, seeds, leaves and roots (Kumar et al., 2015). The consumption of meat products containing antioxidant-rich spices has been reported to reduce *in vivo* formation of malondialdehyde and lower the risk of cancer and cardiovascular disease (Li et al., 2010).

Despite the health benefits of natural antioxidants, there is a paucity of information on studies that specifically address the level of consumer awareness on antioxidant application in meat products across countries. Presently, synthetic antioxidants are incorporated into a variety of foods, including meat patties, processed burgers, hot dogs, cereal and chewing gum, among others, to extend their shelf life (Metsovas, 2013). Report has shown that continuous production and distribution of food products with synthetic compounds could be detrimental to public health over time (Metsovas, 2013). Synthetic antioxidants are not advertised prominently on food labels including meat, and many people may not be aware of their implication on health. Moreso, it is known that the choice of products that consumers purchase and consume could be influenced by the level of their awareness (Chi et al., 2009). Therefore, this study was designed to assess the level of consumer awareness on application of antioxidants in meat and meat products.

## **3.2 Materials and Methods**

### **3.2.1 Study site**

The study was conducted at University of Fort Hare, Alice, Eastern Cape Province of South Africa. The University community has a population of well over 13,000 students comprising of undergraduate and postgraduate, local and international students. The University is located within a latitude of 32<sup>0</sup> 47' South and longitude of 26<sup>0</sup> 50` East of the Equator, with a mean altitude of 524 meters (1,720 feet) above sea level. The site receives a mean annual rainfall of 480 meters and annual temperature of 18.7 degrees centigrade.

### **3.2.2 Data Collection**

A total of two hundred and twenty two (222) respondents were sampled using simple random sampling techniques and interviewed through structured questionnaires. The questionnaires were organized into different sections comprising of questions on demographic characteristics (such as age group, gender and education), consumer knowledge on causes of meat spoilage during storage, the use of antioxidants as meat preservatives, and negative effects of synthetic antioxidants. Prior to data collection, a group of personnel was recruited and trained on how to administer the questionnaire and guide each respondent to answer the questions raised correctly.

### **3.2.3 Statistical analysis**

Data were analyzed using PROC FREQ and PROC CHISQ procedures of the Statistical Analysis System (SAS, version 1.9.3 of 2007). PROC FREQ was computed on the age groups and gender of the respondents. PROC CHISQ was used to determine the association between demographic characteristics of the respondents and their knowledge on the use of natural antioxidants as preservatives in meat and meat products. The significance was tested at  $P < 0.05$ .

## **3.3 Results**

### **3.3.1 Demographic characteristics of Consumer**

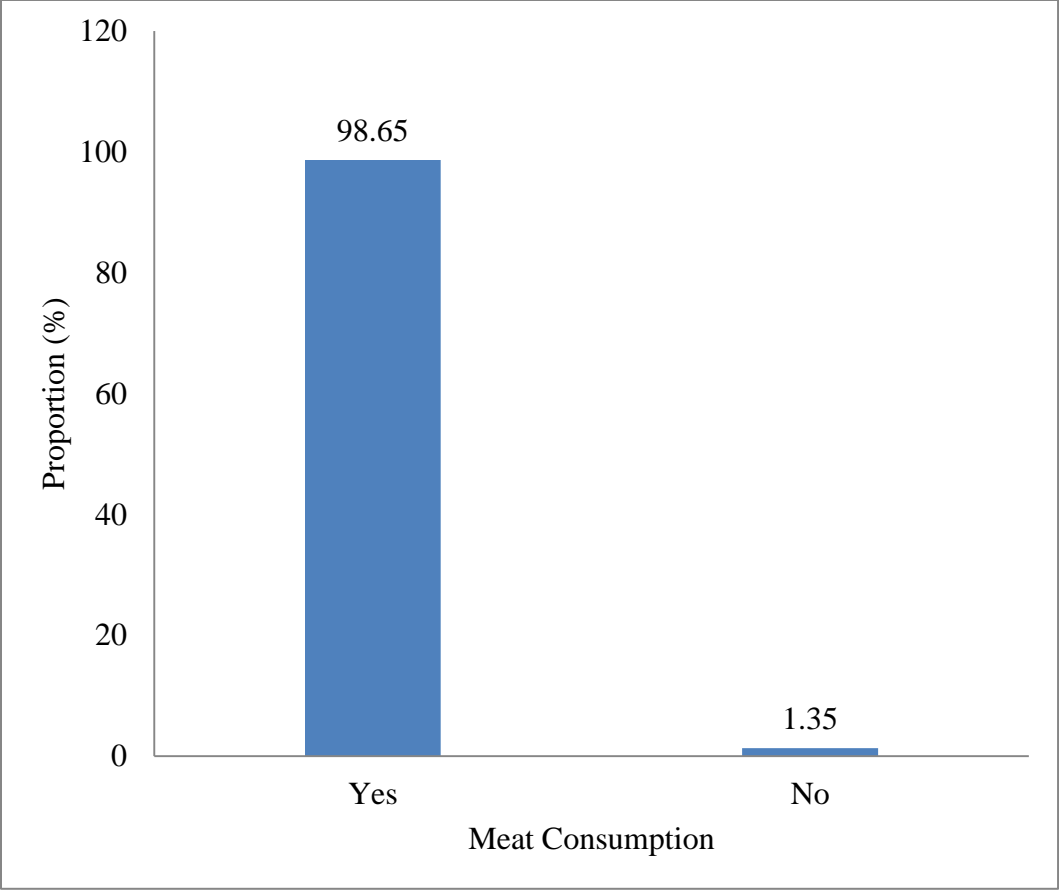
Results on demographic characteristics of the respondents are presented in Table 3.1. The results showed that 52.70% were male and 47.35% were female. More than half of the respondents (62.16%) were aged between 20 and 25 years while others were within the age categories of 19 or below (7.66%), 26-30 (17.57%), 31-40 (10.81%) and 1.80% were 41 or above. Overall, most of the interviewees were below the age of 40 years.

**Table 3. 1 Demographic characteristics of the respondents (n=222).**

<b>Variable</b>	<b>Group</b>	<b>Frequency</b>	<b>Proportion (%)</b>
Gender	Male	117	52.70
	Female	105	47.30
Age	< 20	17	7.66
	20-25	138	62.16
	26-30	39	17.57
	31-40	24	10.81
	41-50	4	1.80

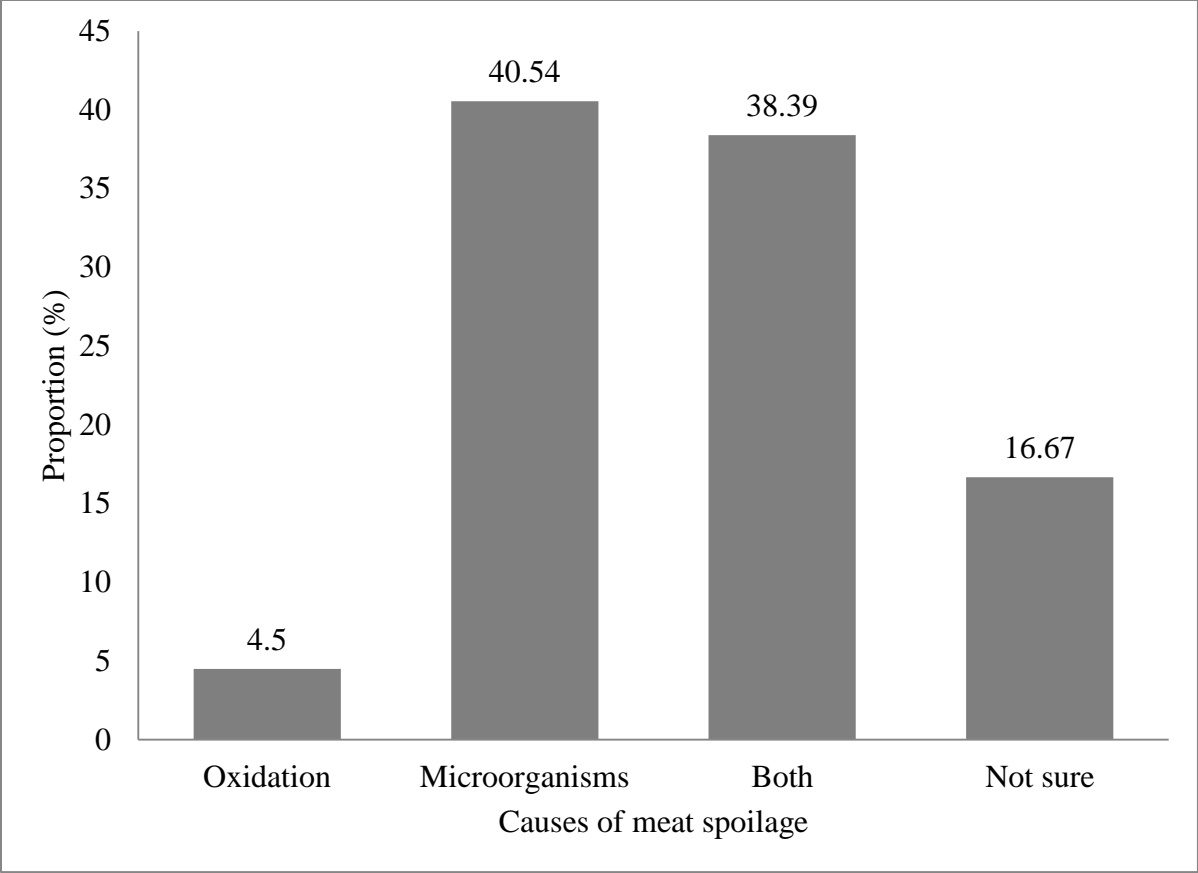
### **3.3.2 Consumers' perceptions on meat consumption and causes of spoilage in meat**

Results on respondent's meat consumption are showed on Figure 3.1. Majority of the respondents (98.65%) ate meat while 1.35% did not. However, concerning the causes of meat spoilage, 40.54% of the respondents indicated that microorganisms are the main causes of meat spoilage while 4.5% attributed it to occurrence of oxidation reaction (Figure 3.2). Furthermore, 39.39% of the respondents believed that both oxidation and microorganisms can cause spoilage in meat products during storage. Approximately 17% of the respondents did not know the possible causes of spoilage in meat products during storage.



**Figure 3. 1 Proportion of respondents that consume meat and meat products.**

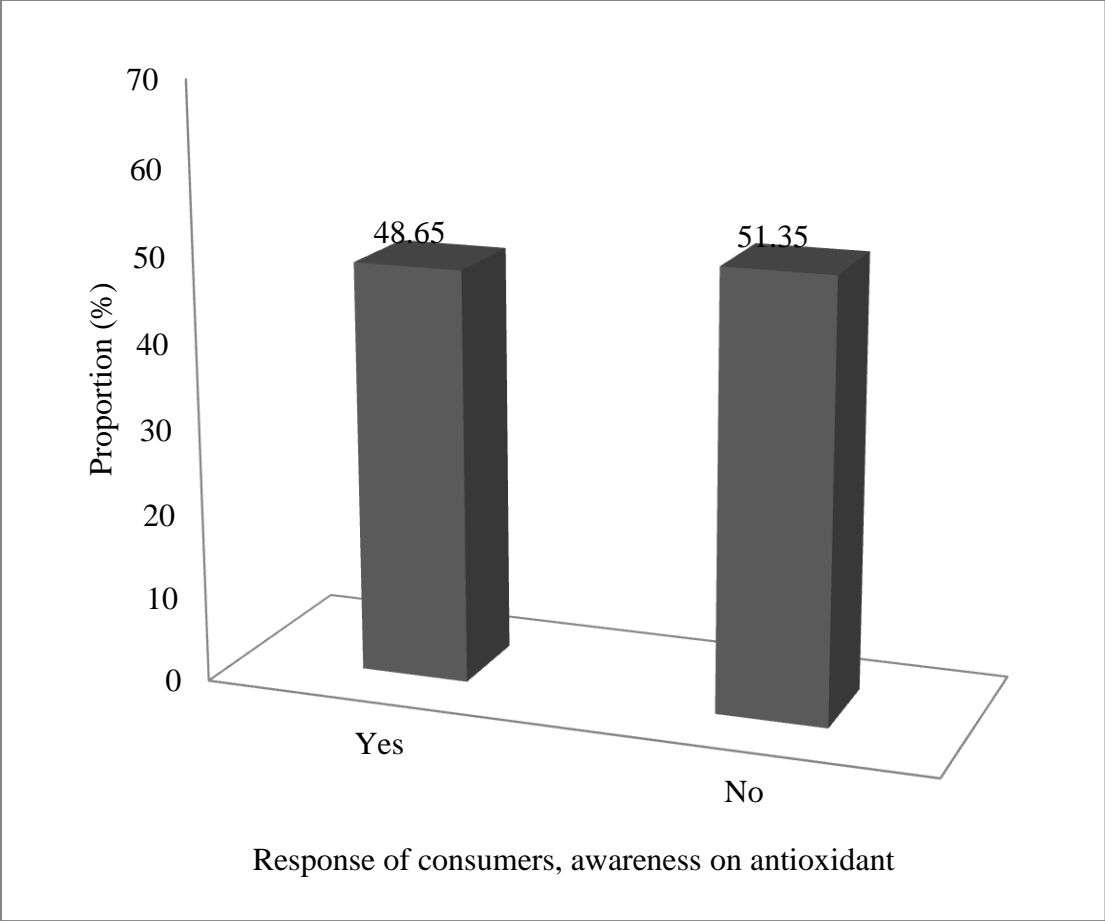




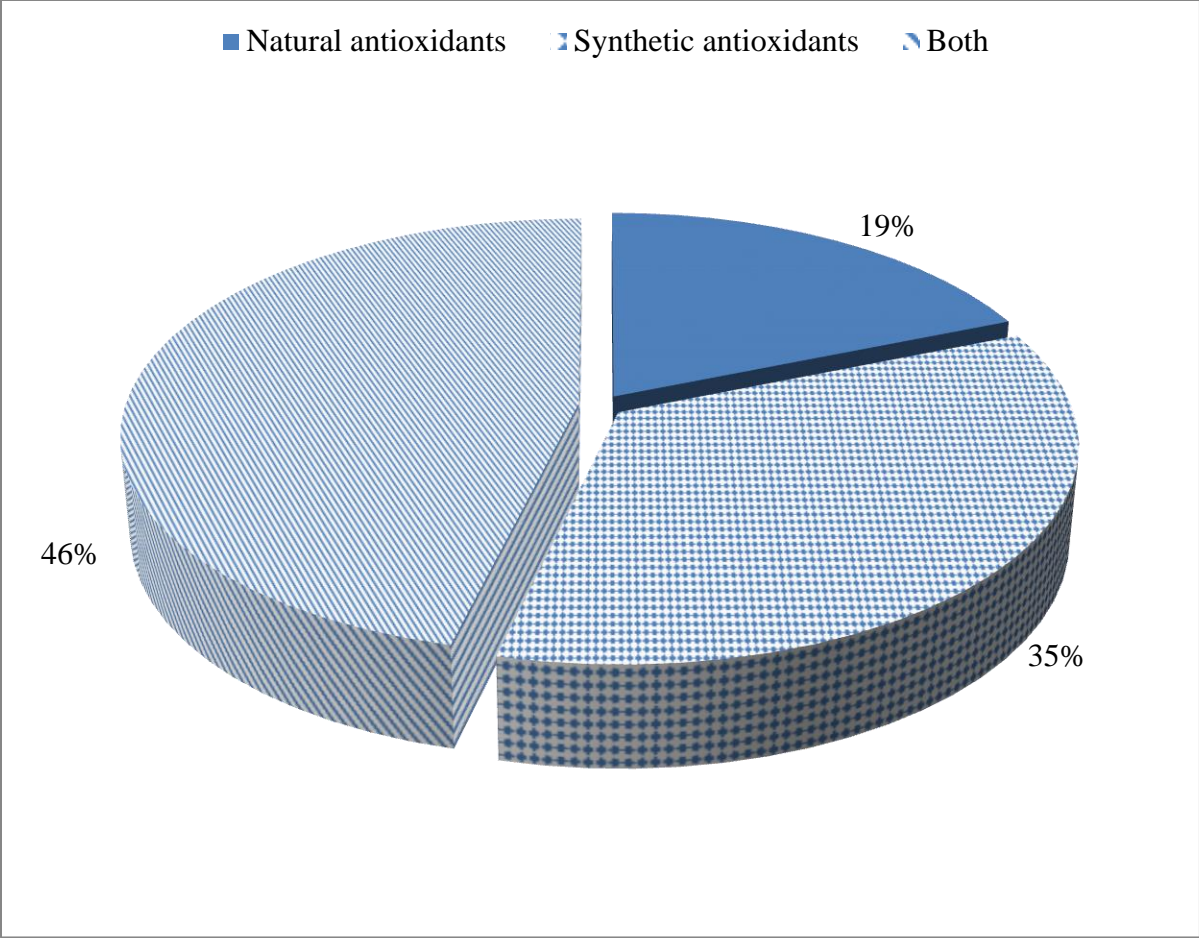
**Figure 3. 2 Consumers perception on the causes of spoilage in meat and meat products during storage.**

### **3.3.3 Consumer awareness on application of antioxidants in meat and meat products**

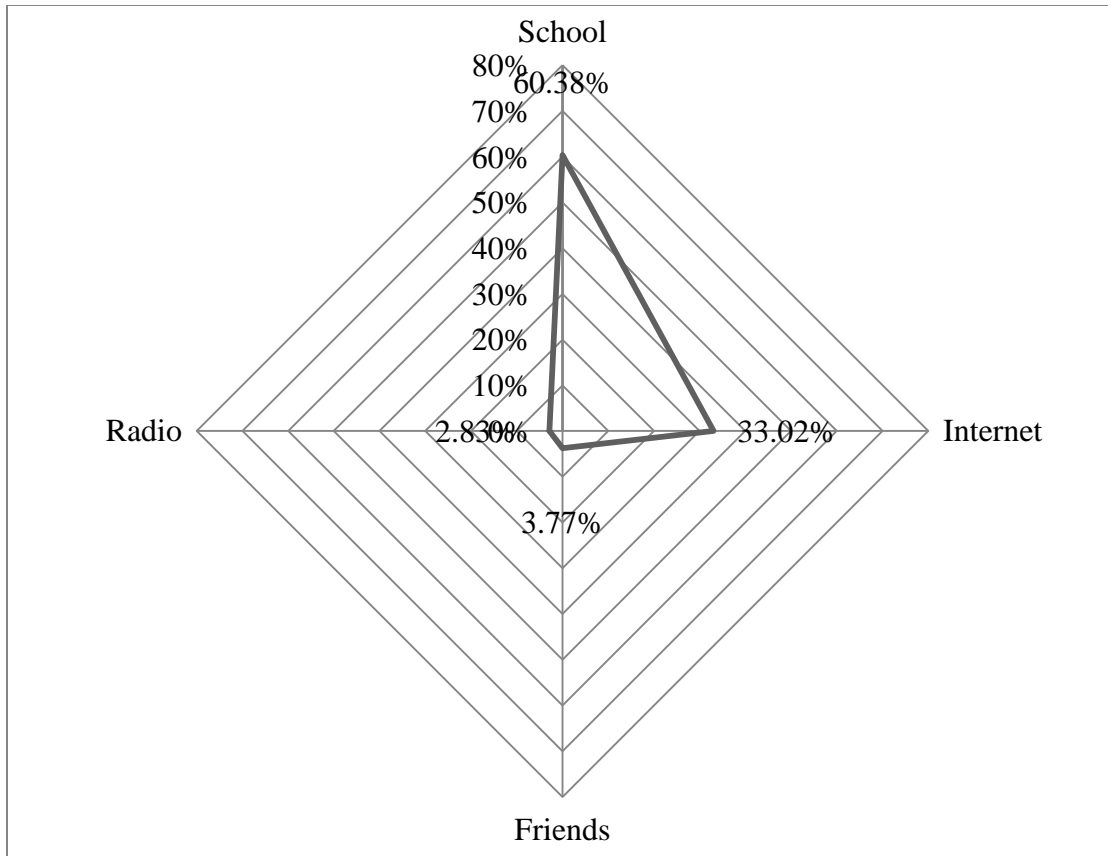
Results on consumer awareness on the use of antioxidants as preservatives are shown on Figure 3.3. More than half of the respondents (51.35%) indicated that they have not heard about antioxidants and their use as preservatives in meat and meat products. However, among the remaining 108 respondents (48.65%) who were aware of antioxidant's application in meat preservation, only 19% knew of natural antioxidants, 35% synthetic antioxidant while 46% had the knowledge of both (Figure 3.4). The results further showed that 64 (60.38%) of the respondents that were aware of the use of antioxidants as preservatives got the information from School/University, 35 (33.02%) from internet, 4 (3.77%) from friends and 3 (2.83%) from media (radio program) (Figure 3.5). Additionally, majority of the respondents 86 (81.13%) indicated that they were aware of the possible health consequences of synthetic antioxidants while 20 (18.87%) did not (Figure 3.6). The group of respondents that consented that they were aware of the health consequences of synthetic antioxidants also expressed their great concern about their use as preservatives in meat and meat products. Among this group of respondents, 49.06% had a strong dislike for the use of synthetic antioxidants as preservatives, 32% slightly dislike, 2.08% somehow dislike it, while 4.2% were unconcerned of the use of synthetic antioxidants as preservatives (Figure 3.7). Overall, a strong significant association was observed between consumers' age and health consequences of synthetic antioxidants while other parameters were not significantly associated with consumers' demographic characteristics (Table 3.2).



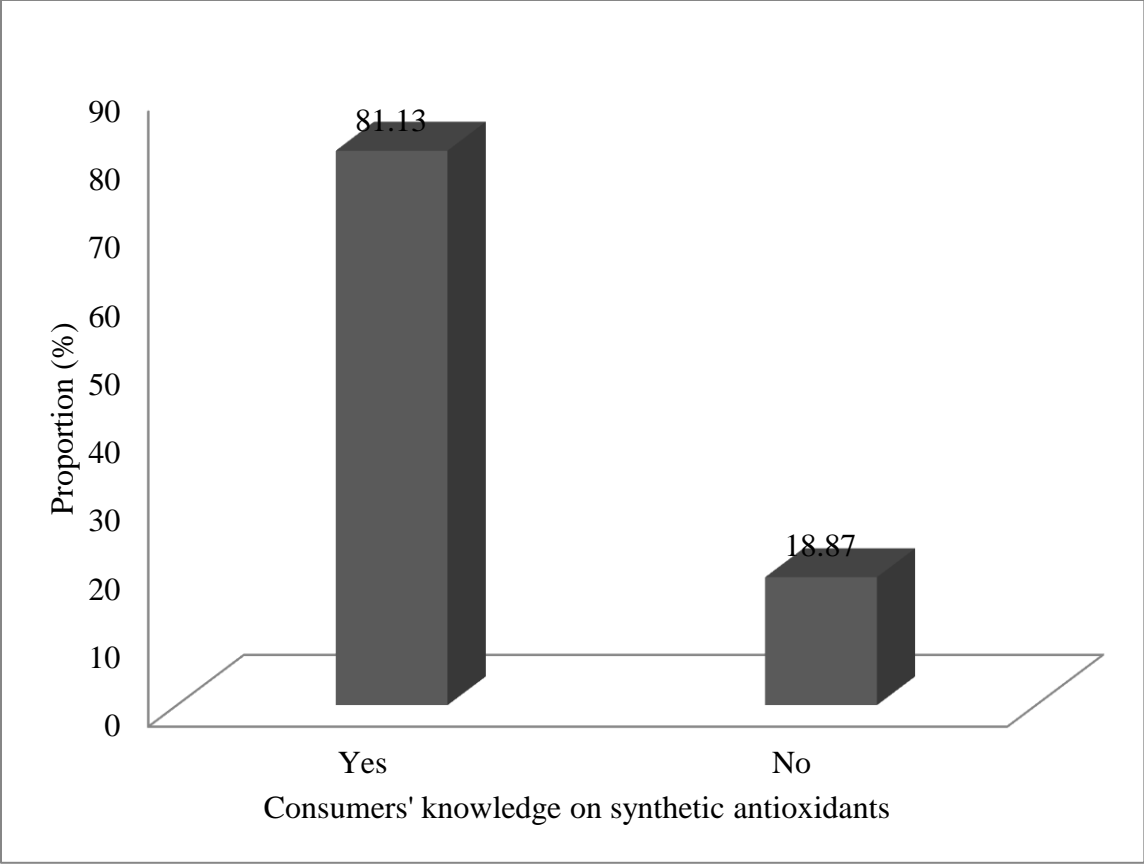
**Figure 3. 3 Consumers' awareness on the use of antioxidants as preservatives in meat and meat products.**



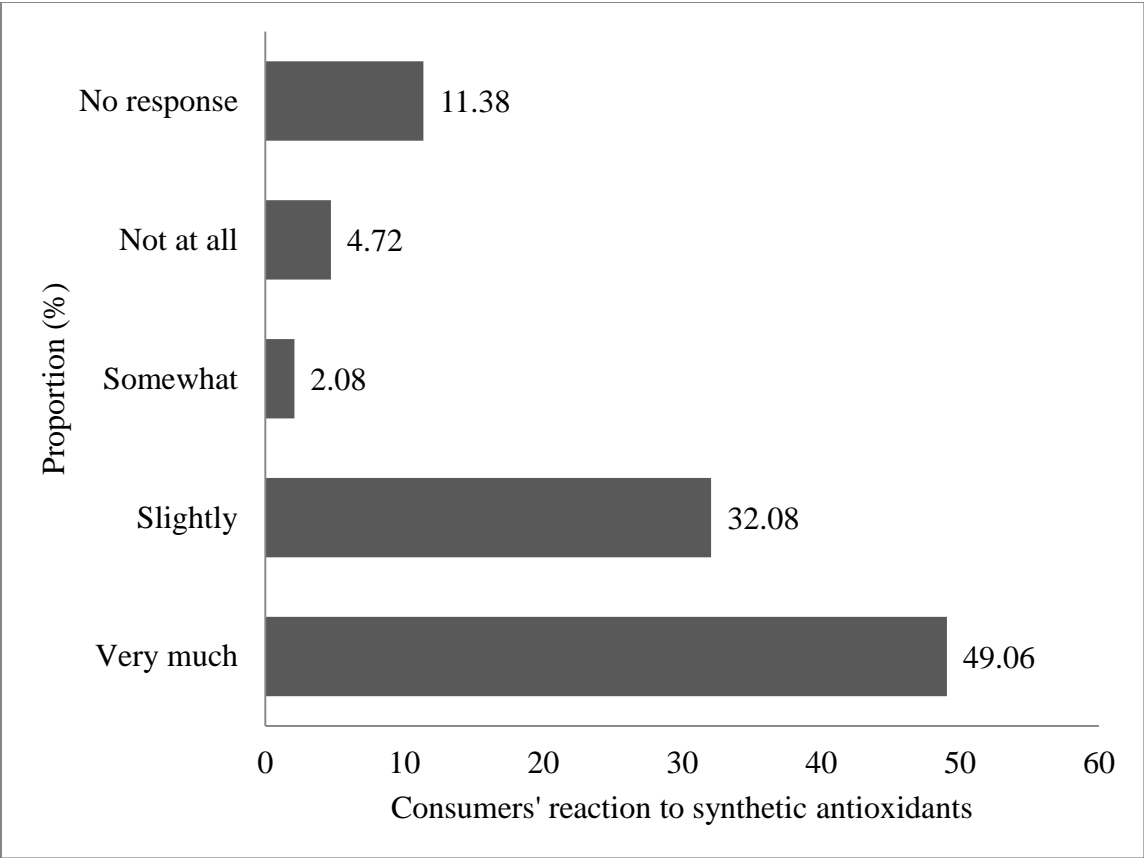
**Figure 3. 4 Consumers' knowledge on type of antioxidants for meat preservation.**



**Figure 3. 5 Effect of information source on consumers' awareness on the use of natural antioxidants as preservatives in meat and meat products.**



**Figure 3. 6 Consumers' knowledge on the health consequences of synthetic antioxidants**



**Figure 3. 7 Consumers' reaction to the use of synthetic antioxidants as preservatives in meat and meat products.**

**Table 3. 2 Effect of demographic factor on consumers' awareness of natural antioxidants as a meat preservative agent.**

Variable	X <sup>2</sup> - value	<sup>1</sup> Sig
Consumer awareness on antioxidants		
Gender	0.69	ns
Age	4.08	ns
Consumer awareness on type of antioxidants		
Gender	2.30	ns
Age	3.22	ns
Consumer awareness on health consequences of synthetic antioxidants		
Gender	3.29	ns
Age	24.18	*

<sup>1</sup>Significant at \*P < 0.05 but NS not significant at P > 0.05.



### **3.4 Discussion**

Findings from the current study revealed that the majority of the respondents recognized that oxidation and microbial contamination were the main causes of meat spoilage or deterioration during storage. This is in line with other studies that reported that oxidation and microbial contamination were major causes of discoloration, rancidity and nutritional value in meat products (Hernández-Macedo et al., 2011; Kumar et al., 2015). In most cases, occurrence of oxidation reaction is inevitable under postmortem conditions and they usually cause adverse effects on flavor and sensory quality of meat products (Bekhit et al., 2013). In order to reduce or prevent meat spoilage during storage, antioxidants are explored in the meat industry. However, our results on consumer knowledge about antioxidants showed that more than half of the respondents were unaware of their use as preservatives in meat products. This could be due to the fact that antioxidants are not predominantly advertised or mentioned on meat labels (Venkatesh, 2011). In addition, information regarding their application in meat and meat products and benefits are rarely disseminated or relayed on public media.

Most of the respondents that were aware of the use of antioxidants as preservatives indicated that they got the information from School/University and website/internet. This is suggesting that the majority of consumers who do not have access to internet or academic environment might be unaware of the use of antioxidants as preservatives in meat and meat products and its consequences on human health. Thus, a proactive communication scheme on the application of antioxidants would be necessary to create more awareness on the importance for consumer benefits.

Our results further revealed that the majority of respondents were aware of the use of either natural or synthetic antioxidants in meat products. Both natural and synthetic antioxidants have been reportedly used as preservatives to extend the shelf life of meat during storage (Chen et al., 1992; Karakaya et al., 2011; Kumar et al., 2015). However, applications of synthetic antioxidants have been suspected to cause health problems in consumers. In agreement with this assertion, our results showed that the majority of the respondents were aware of the health risks involved in the use of synthetic antioxidants as meat preservative agents. This group of respondents also expressed concerns about their application in meat products. It is interesting to note that several studies have shown that the application of synthetic antioxidants in meat and meat products can cause cancers and cardiovascular diseases (Faine et al., 2006; Kumar et al., 2015). On the contrary, natural antioxidants in meat and meat products have been found to be safe, healthy and easily accessible.

Evidence has shown that the inclusion of spice mixture (Cloves, Cinnamon, Oregano, Rosemary, Ginger, Black pepper, Paprika and Garlic) in cooked hamburgers significantly decrease the formation and concentration of malondialdehyde after consumption (Li et al., 2010). The consequence of malondialdehyde has been associated with several diseases including atherosclerosis and cancer formation in the body (Marnett, 1999; Li et al., 2010). This finding from Li et al. (2010) demonstrates that natural antioxidants have potential health benefits against atherogenesis and carcinogenesis. Presently, food market and consumers (those that are aware of antioxidants) are increasingly demanding for meat products containing natural antioxidants (Karre et al., 2013).

### **3.5 Conclusions**

Findings from this study revealed that most consumers believed that natural antioxidants can be used to inhibit the rate of oxidation and microbial spoilage in meat products. The use of natural antioxidants as preservatives would be more acceptable than synthetic antioxidant due to their health consequences. Thus, a further study aiming at creating and increasing consumer awareness on application of natural antioxidants would be necessary in developing more natural antioxidants products for commercial uses.

### 3.6 References

- Bekhit, A. A., Hopkins, D. L., Fahri, F. T. and Ponnampalam, E. N.** 2013. Oxidative processes in muscle systems and fresh meat: sources, markers, and remedies. *Comprehensive Reviews in Food Science and Food Safety* **12 (5):** 565–597
- Carlsen, C. U., Møller, J. K. S. and Skibsted, L. H.** 2005. Heme-iron in lipid oxidation. *Coordination Chemistry Reviews* **249:**485–98.
- Chen, C. H.; Pearson, A. M. and Gray, J. I.** 1992. Effects of synthetic antioxidants (BHA, BHT and PG) on the mutagenicity of IQ-like compounds. *Food Chemistry* **45:** 177–183.
- Chi, H. K., Yeh, H. R. and Yang, Y. T.** 2009. The impact of brand awareness on consumer purchase intention: the mediating effect of perceived quality and brand loyalty. *The Journal of International Management Studies* **4 (1):** 135- 144.
- Decker, E., Livisay, S. and Zhou, S.** 2000. Mechanisms of endogenous skeletal muscle antioxidants: chemical and physical aspects. In *Antioxidants in muscle foods*, E. Decker, C. Faustman and C. Lopez-Bote (Editors). New York: WileyInterscience, pp 25-60.
- Embuscado, M. E.** 2015. Spices and herbs: Natural sources of antioxidants – a mini review. *Journal of Functional Foods* **18:** 811–819.
- Faine, L. A., Rodrigues, H. G., Galhardi, C. M., Ebaid, G. M. X., Diniz, Y. S., Fernandes, A. A. H. and Novelli, E. L. B.** 2006. Butyl hydroxytoluene (BHT)-induced oxidative

- stress: Effects on serum lipids and cardiac energy metabolism in rats. *Experimental and Toxicological Pathology* **57**:221–226.
- Hazra, S., Biswas, S., Bhattacharyya, D., Das, S. K. and Khan, A.** 2012. Quality of cooked ground buffalo meat treated with the crude extracts of *Moringa oleifera* (Lam.) leaves. *Journal of Food Science and Technology* **49 (2)**: 240–245.
- Hernández-Macedo, M. L., Barancelli, G. V. and Contreras-Castillo, C. J.** 2011. Microbial deterioration of vacuum-packaged chilled beef cuts and techniques for microbiota detection and characterization: a review. *Brazilian Journal of Microbiology* **42:1**.
- Karakaya, M., Bayrak, E. and Ulusoy, K.** 2011. Use of Natural Antioxidants in Meat and Meat Products. *Journal of Food Science and Engineering* **1**: 1-10.
- Karre, L., Lopez, K. and Getty, K. J.** 2013. Natural antioxidants in meat and poultry products. *Meat Science* **94 (2)**: 220-227.
- Kumar, Y., Yadav, D. N., Ahmad, T. and Narsaiah, K.** 2015. Recent Trends in the Use of Natural Antioxidants for Meat and Meat Products. *Comprehensive Reviews in Food Science and Food Safety* **00**: 1-14.
- Li, Z., Henning, S. M., Zhang, Y., Zerlin, A., Li, L., Gao, K., Lee, R. P., Karp, H., Thames, G., Bowerman, S. and Heber, D.** 2010 Antioxidant-rich spice added to hamburger meat during cooking results in reduced meat, plasma, and urine malondialdehyde concentrations. *American Journal of Clinical Nutrition* **91**:1180 – 1184.
- Marnett, L. J.** 1999. Lipid peroxidation-DNA damage by malondialdehyde. *Mutation Research* **424**:83–95.

- Metsovas, S.** 2013. Synthetic Antioxidants: Longer Shelf-Life May Lead to Shorter Lifespan.  
<http://pontoniere.blogautore.espresso.repubblica.it/2013/05/20/synthetic-antioxidants-longer-shelf-life-may-lead-to-shorter-lifespan> Accessed on 20/09/2015.
- Oroian, M. and Escriche, I.** 2015. Antioxidants: Characterization, natural sources, extraction and analysis. *Food Research International* **74**: 10–36.
- Sarafian, T. A., Kouyoumjian, S., Tashkin, D. and Roth, M. D.** 2002. Synergistic cytotoxicity of 9-tetrahydrocannabinol and butylated hydroxyanisole. *Toxicology Letter* **133**:171–9.
- Statistical Analysis System (SAS), 2007.** In: *SAS/STAT Guide to Personal Computers, Version 9.1.3, Statistical Analysis System Institute Inc. Cary, NC*”.
- Venkatesh, R.** 2011. A Review of the Physiological Implications of Antioxidants in Food.  
<https://www.wpi.edu> Accessed on 20/09/2015.
- Williams, P. G.** 2007. Nutritional composition of red meat. *Nutrition and Dietetics* **64 (4)**: 113-119.
- Xiao, S., Zhang, W. G., Lee, E. J. and Ahn, D. U.** 2013. Effects of diet, packaging and irradiation on protein oxidation, lipid oxidation of raw broiler thigh meat. *Animal Industry Report AS 659*: ASL R2761.

**Chapter 4: *In vitro* evaluation of antioxidant and antibacterial activities of *Bidens pilosa* Lam. asteraceae and *Moringa oleifera* Lam. moringaceae plants**

(Submitted to *CyTA - Journal of Food*)

**Abstract**

The objective of this study was to evaluate the *in vitro* antioxidant and antibacterial activities of aqueous ethanolic extract of *Bidens pilosa* Lam. Asteraceae (*B. pilosa*) and *Moringa oleifera* Lam. Moringaceae (*M. oleifera*) leaf plants. The plants' extracts were screened for the presence of phytochemicals and for antimicrobial activities. The phytoconstituents of the extracts were identified using gas chromatography-mass spectrometry (GC-MS) while the free radical scavenging activities of the extracts were determined using 2,2-diphenyl-2-picryl hydrazyl (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radical assays. The result of GC-MS analysis revealed higher amount of phytoconstituents in *B. pilosa* (20) than *M. oleifera* (13) extracts. The antioxidant activity of the plants' extracts ( $P < 0.05$ ) showed that *M. oleifera* extracts had higher phenolic ( $77.5 \pm 0.94$  mg Ru/g DW) and flavonoid ( $17.4 \pm 0.15$  mg Ru/g DW) contents than the *B. pilosa* extract with phenolic ( $75.9 \pm 0.53$  mg GAE/g DW) and flavonoid ( $14.9 \pm 0.05$  mg Ru/g DW) equivalents. The extracts also demonstrated high DPPH and ABTS radical scavenging activities which were comparable to those of rutin and butylated hydroxytoluene (BHT). Moreover, *B. pilosa* was found to possess higher total chlorophyll, carotenoid and lower vitamin C levels of  $3.60 \pm 0.04$  mg/g DW,  $0.73 \pm 0.00$  mg/g DW and  $0.03$  mg/g DW, respectively than those of *M. oleifera* which were  $1.46 \pm 0.01$  mg/g DW,  $0.39 \pm 0.00$  mg/g DW and  $0.35$  mg/g DW, respectively. The antibacterial assay of the extracts revealed an appreciable broad spectrum activity against tested bacteria with minimum inhibitory concentrations (MICs) range of between 0.6 and 10.0 mg/ml. The best antibacterial activities of the extracts were against *E. faecalis* and *S. epidermidis* and the lowest activity was against *E.*

*coli*. In conclusion, this study revealed *B. pilosa* and *M. oleifera* plants as potential sources of natural antioxidants.

**Keywords:** *in vitro*, antioxidant, antimicrobial, *Biden pilosa*, *Moringa oleifera*



## 4.1 Introduction

Meat preservatives are essential and they play an important role in delivery of high quality and safe meat and meat products to consumers. They prevent oxidation, enzymatic reaction and growth of foodborne and pathogenic microorganisms that cause deterioration, nutritional and economic losses to meat industries (Williams et al., 1999; Yadav and Singh, 2004; Sanchez-Ortega et al., 2014). Various synthetic antioxidants such as butylated hydroxytoluene (BHT), tertiary-butylhydro-quinone (TBHQ) and butylated hydroxyl-anisole (BHA) have been widely used in a variety of food industry. The addition of BHT, TBHQ and BHA in meat products has recently been reported to be carcinogenic and toxigenic, hence putting consumer's health associated risks (NTP, 2011; Lobo et al., 2010). Conversely, the use of synthetic antioxidants have also been found to be either ineffective to completely delay microbial spoilage or eliminate important pathogens such as *Listeria monocytogenes* in meat products (Gutierrez et al., 2009; Tajkarimi et al., 2010).

In response to this, consumers and food markets are demanding high quality meat and meat products with extended shelf life and without chemical preservatives (Sanchez-Ortega et al., 2014; Kumar et al., 2015). Thus meat industries are now searching for alternative meat preservatives to meet the consumer demand (Karre et al., 2013; Kumar et al., 2015). Numerous edible plant materials have been identified as alternative to chemical preservatives; these plant materials are known to exhibit secondary metabolites including antioxidant and antimicrobial compounds that promote good health (Jaberian et al., 2013). Studies on the use of plant materials as natural antioxidant and preservatives in meat and meat products have shown that they are able to extend meat shelf-life and improve nutritional quality (Mohameda et al., 2011; Doolaege et

al., 2012; Kobus-Cisowska, et al., 2014). Additionally, the use of natural antioxidants (plant-derived antioxidants) has been found to retard lipid oxidative rancidity in food and also protect the body from free radicals and chronic diseases by limiting oxidation of low-density lipoprotein cholesterol (Daniells, 2006; Koolen et al., 2013). The *in vitro* activity of plant extracts have also been reported against several foodborne and human pathogenic microorganisms including *Klebsiella*, *Bacillus*, *Pseudomonas*, *Staphylococcus*, *Salmonella* and *Escherichia coli* (Khan et al., 2001; Rahman et al., 2010).

*Biden pilosa* and *Moringa oleifera* are plants with a lot of potential in this regard; both plants are considered as potential functional ingredients and promising sources of natural antioxidants (Sreelatha and Padma, 2009; Bartolome et al., 2013). *Biden pilosa* and *M. oleifera* are fast-growing perennial plants which are widely cultivated across the temperate and tropical regions including in South Africa. Conventionally, almost all parts of these plants (the whole plant, the aerial parts and/or the roots) have been used extensively, either as food or medicinal components (Yang, 2014). Their leaves are especially good source of protein, vitamins, minerals and amino acids (Adedapo et al., 2012; Jayawardana et al., 2015). However, the effect of medicinal plants are apparently related to the presence of polyphenol, alkaloids, saponins, tannins, ascorbic acid, chlorophyll and carotenoid compounds in the plants (Kim et al., 2013). In order to ascertain the efficacy of medicinal plants in biological system, they are usually subjected to *in vitro* antioxidant and antimicrobial assays. Therefore, this study was conducted to evaluate the antioxidant and antimicrobial properties of *M. oleifera* and *B pilosa* plants.

## 4.2 Materials and Methods

### 4.2.1 Plant sample and extract preparation

*Biden pilosa* and *M. oleifera* leaf were obtained from the University of Fort Hare farm (South Africa) and Moringa South Africa Ltd, respectively. The dry plant samples (200 g) were exhaustively macerated with 800 ml of ethanol-water solution (7:3) at room temperature for 2 days. Each extract was separated from the residue by filtration, using Whatmann no.1 filter paper and then concentrated under reduced pressure at 55 °C using a rotary evaporator. The extract lyophilized with a freeze-drier and the dried extracts were used for the determination of the antioxidant activity at concentration of 1 mg/ml. Determination of the nutritive values of the plants were carried out on the dry samples. All analyses were done in triplicate. The dried powder of plant extracts were then stored at 20<sup>0</sup> C for further analysis.

### 4.2.2 Chemicals

Gallic acid, 2, 2-Diphenyl-2-picrylhydrazyl (DPPH), 2, 2 áazino- bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 3- (2-pyridyl) -5, 6-diphenyl-1,2,4-triazine-4',4'-disulfonic acid, sodium carbonate, butylated hydroxytoluene (BHT), rutin and were purchased from Sigma Chemical Co. (St. Louis, MO, USA)., n-haxaneAluminium chloride (AlCl<sub>3</sub>), Folin-Ciocalteu phenol reagent and sodium carbonate were from Merck (Damstadt, Germany). All other chemicals used including the solvents, were of analytical grade.

### 4.2.3 GC-MS analysis of *Biden pilosa* and *Moringa oleifera* leaf extracts

The GC-MS analysis of crude extract of *B. pilosa* and *M. oleifera* plant were quantitatively performed using an Agilent 7890B GC system coupled with an Agilent 5977A MSD with a Zebron-5MS column (ZB-5MS 30 m x 0.25 mm x 0.025µm) (5% -phenylmethylpolysiloxane).

GC-grade helium was used as carrier gas at a constant flow rate of 2 mL/min. The crude extracts were dissolved with appropriate solvent, filtered and diluted in *n*-hexane. The samples were diluted at a ratio of 1:50 and injection was achieved through an auto-sampler. The column temperature was maintained at 50°C and gradually increased at 10°C per minute until a final temperature of 250°C was reached. The time taken for the GC-MS analysis was calculated automatically as 18.23 min. The identification of the components was based on computer matching of the mass spectra with the National Institute of Standards and Technology library (NIST 11 MS library).

#### **4.2.4 Determination of antioxidant contents and activities of the plant extracts**

##### **4.2.4.1 Total phenolic content**

Total phenol contents of the extracts were determined as described by Wolfe et al. (2003). The extract was mixed with 5 mL of Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 mL (75 g/L) of sodium carbonate. The tubes were vortexed for 15 s and allowed to stand for 30 min at 40°C for color development. Absorbance was then measured at 765<sub>nm</sub> using the Hewlett Packard UV-VS spectrophotometer. Total phenolic contents were expressed as mg/g Gallic Acid Equivalent (GAE) using the following equation based on the calibration curve:  $y = 0.181x$ ,  $r^2 = 0.993$ , where X was the absorbance and Y was the GAE (mg/g).

##### **4.2.4.2 Total flavonoids content**

Total flavonoids were determined using the method described by Ordonez et al. (2006). An aliquot of 0.5 mL of 2% AlCl<sub>3</sub> ethanol solution was added to 0.5 mL of sample solution. The samples were incubated for one hour at room temperature, followed by measuring the absorbance at 420<sub>nm</sub>. A yellow color indicated the presence of flavonoids. Total flavonoid

contents were calculated as rutin (RU, mg/g) using the following equation based on the calibration curve:  $y = 0.2645x$ ,  $r^2 = 0.992$ , where X was the absorbance and Y was the RU equivalent (mg/g).

#### **4.2.4.3 DPPH radical scavenging assay**

The free radical scavenging activity of extracts on 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical was estimated using the method described by Liyana-Pathiranan et al. (2006). A solution of 0.135 mM DPPH in ethanol was prepared and 1.0 mL of this radical solution was mixed with 1.0 mL of sample solution. The reaction mixture was incubated in the dark for 30 minutes at room temperature and then the absorbance was measured at 517<sub>nm</sub> using spectrophotometer. Rutin and BHT were used as reference standards. The ability of the extract to scavenge DPPH radical was calculated by the following equation:

DPPH radical scavenging activity (%) =  $[(\text{Abs control} - \text{Abs sample}) / (\text{Abs control})] \times 100$   
where Abs control is the absorbance of DPPH radical + ethanol; Abs sample is the absorbance of DPPH radical + sample extract /standard.

#### **4.2.4.4 ABTS radical scavenging assay**

ABTS radical cation decolourisation assay to determine the free radical scavenging activity of plant extracts was carried out as described by Re et al. (1999). Stock solutions (ABTS<sup>•+</sup>) were prepared by reacting a 7 mM ABTS solution with 2.4 mM potassium persulphate solution in equal quantities and the mixture was allowed to stand in the dark at room temperature for 16 -18 hours before use. The stock solution was then diluted by mixing 1 mL ABTS solution with 53 ml of ethanol to obtain an absorbance of 0.705 units at 734nm. One millimeter of diluted ABTS working standard solution was mixed with 1ml of plant extract/standard and the absorbance was

measured after 7 min at 734<sub>nm</sub> using the spectrophotometer. The ABTS scavenging capacity of the extract was compared with that of rutin and BHT as reference standards. The percentage inhibition was calculated as ABTS radical scavenging activity (%) = [(Abs control – Abs sample)]/ (Abs control)] x 100, where Abs control is the absorbance of ABTS radical + ethanol; Abs sample is the absorbance of ABTS radical + sample extract /standard.

#### **4.2.5 Determination of nutritional value**

##### **4.2.5.1 Total chlorophyll and total carotenoid contents**

The content of chlorophylls a and b, and as well as total carotenoids, was determined using the method of Lichtenthaler (1987). Approximately 1 g of dry plant samples (1 g) was extracted with 50 mL of 80% acetone (v/v) solution after incubation in the dark for 24 h at room temperature. After filtration (Whatman no. 1 filter paper), the filtrate volume was adjusted to 100 mL with 80% acetone (v/v). Absorbance was read at 662<sub>nm</sub>, 644<sub>nm</sub>, and 470<sub>nm</sub> using spectrophotometer to measure the content of chlorophyll a, chlorophyll b, and carotenoids, respectively. Total chlorophyll was calculated as the sum of chlorophylls a and b. Total chlorophyll and total carotenoid contents were expressed as mg/g on a dry weight basis

##### **4.2.5.2 Vitamin C content**

Vitamin C content was determined according to the method described in the Association of Official Analytical Chemists (AOAC, 1995). Dried plant powder (2 g) was dissolved in 25 mL of extract solution (metaphosphoric acid: acetic acid=1:5). The homogenate was centrifuged at 12,000 rpm for 5 min, and the supernatant was filtered through Whatman no. 1 filter paper. Two microliters from the supernatant were then placed in test tubes, after which 200 µL of indophenol and 2 mL of thiourea metaphosphoric solution were added. After that, 1 mL of 2,4-dinitrophenyl

hydrazine (DNP) solution was mixed with the sample solution and the mixture was incubated at 37 °C for 3 hours and then cooled on ice. Thereafter, 5 mL of 85% H<sub>2</sub>SO<sub>4</sub> solution was added, and the resulting mixture was left in the dark for 30 minutes at room temperature before determining the absorbance at 520<sub>nm</sub>. Vitamin C content was expressed as mg ascorbic acid (AAE) equivalent/g of plant on a dry weight basis.

#### **4.2.6 *In vitro* antimicrobial activity of plant extracts**

##### **4.2.6.1 Bacterial strains and growth conditions**

Bacterial isolates used in this study were reference strains obtained from GI Microbiology and Biotechnology Laboratory, Agricultural Research Council-Animal Production Institute, Irene, South Africa. These strains were chosen for their histories in pathological effects on humans and deterioration of food products. The bacteria include four Gram-positive (*Staphylococcus aureus*, *Bacillus cereus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*) and four Gram-negative (*Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella flexinerii*, *Serratia marcescens*) strains. The test organisms were inoculated in 10 mL previously sterilized nutrient agar media, mixed thoroughly and transferred immediately to the sterile petri dish in an aseptic condition using a sterile loop. The bacterial strains were incubated at 37 °C overnight. After incubation, the test organisms were maintained in nutrient broth, and then standardized at 560<sub>nm</sub> to achieve 10<sup>5</sup> colony forming units per mL (CFU/mL).

##### **4.2.6.2 Agar well diffusion test**

The inhibitory effects of the plant extracts on test bacteria were determined by the agar well diffusion method. Twenty milliliters of Müller-Hinton agar solution in McCartney bottle was autoclaved and cooled in water bath at 50°C. One hundred microliters of each standardized

bacteria strain were added to the suspension and poured into a sterile Petri dish (90 mm diameter), and then allowed to stand at room temperature to reach solidification. Two wells per Petri dish were bored on agar plate under aseptic condition and 20 mg/ml of each plant extract was dispensed into each agar well. Plates were left for 30 minutes at room temperature to allow the extracts to diffuse into the agar before incubation at 37<sup>0</sup>C for 24 h. After incubation, clear zone of inhibitions were measured and expressed in millimeters.

#### **4.2.6.3 Determination of minimum inhibitory concentration (MIC) of the plant extracts**

The MIC of the extract was determined by a broth micro-dilution method as described by Jorgensen and Turnidge (2007). Ninety-six-well culture plates were prepared, and serial two-fold dilutions of the extracts were dispensed into the plate wells. The volume of dispensed extract was 100 µl per well in the concentration range of 20 mg/ml to 0.625 mg/ ml. The same volume (100 µl) of bacterial culture at a density of 10<sup>5</sup> CFU/ml was added to the wells, and the culture plates were incubated at 37<sup>0</sup>C for 24 h. The lowest concentration of the plant extract required to inhibit visible growth of the tested microorganism was designated as the MIC. The MBC was determined by streaking the suspension in the well with concentrations greater than the MIC. After that, the sub-cultured agar plates were incubated overnight at 37<sup>0</sup>C. The MBC was defined as the lowest concentration of extract at which no viable microorganism was detected by subculture.

#### **4.2.7 Statistical analysis**

Data obtained on antioxidant and antimicrobial contents of the plant extracts were analyzed using PROC MEANS and PROC ANOVA procedures of the Statistical Analysis System (SAS, version 1.9.3 of 2007). The effect of plant type on concentration of phenolic, flavonoid, chlorophyll, carotenoid and Vitamin C contents as a response variable was determined. Post-hoc



analysis was performed on DPPH and ABTS scavenging activity of plant extracts using Duncan Multiple Range test.

## 4.3 Results

### 4.3.1 Identification and quantification of phytochemicals in ethanolic extract of *Moringa oleifera* and *Biden pilosa*

The composition of phytochemical constituents of the extract with their retention time (RT) as revealed by GC-MS analysis were presented in Table 4.1 and 4.2. The extract of *B. pilosa* exhibited more volatile compounds (20 compounds) than *M. oleifera* (13 compounds) during maximum run time of 18.23 minutes (Figure 4.1 and 4.2). The results also revealed that the ethanol extract of *B. pilosa* and *M. oleifera* contain both antioxidant and antimicrobial compounds such as Tetradecanoic acid, n-Hexadecanoic acid, Hexadecanoic acid ethyl ester, Phytol, Octasiloxane 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15 -hexadecamethyl, Linoleic acid ethyl ester, DL-alpha-Tocopherol, 9,12,15-Octadecatrienoic acid ethyl ester (Z,Z,Z), 9,12-Octadecadienoic acid (Z,Z), Phenol, 2,2'-methylenebis [6-(1,1-dimethylethyl)]-4-methyl and 4-Methyl-2-trimethylsilyloxy-acetophenone (Table 4.3).

**Table 4. 1 Chemical composition of leaf extracts of *Moringa oleifera*.**

<b>No</b>	<b>Compound</b>	<b>Retention time</b>	<b>% Peak area</b>
<b>1</b>	Tetradecanoic acid	10.677	7.54
<b>2</b>	n-Hexadecanoic acid	10.734	2.23
<b>3</b>	Hexadecanoic acid, ethyl ester	10.827	20.59
<b>4</b>	Phytol	11.425	6.05
<b>5</b>	9,12-Octadecadienoic acid (Z,Z)-	11.581	6.44
<b>6</b>	Linoleic acid ethyl ester	11.650	8.39
<b>7</b>	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)	11.696	36.36
<b>8</b>	Octadecanoic acid, ethyl ester	11.756	3.91
<b>9</b>	1,5-Cyclodecadiene	12.650	2.77
<b>10</b>	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl	12.832	0.42
<b>11</b>	4-Dehydroxy-N-(4,5-methylenedioxy 2-nitrobenzylidene) tyramine	12.867	1.40
<b>12</b>	DL-alpha-Tocopherol	16.305	2.67
<b>13</b>	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	18.113	1.24

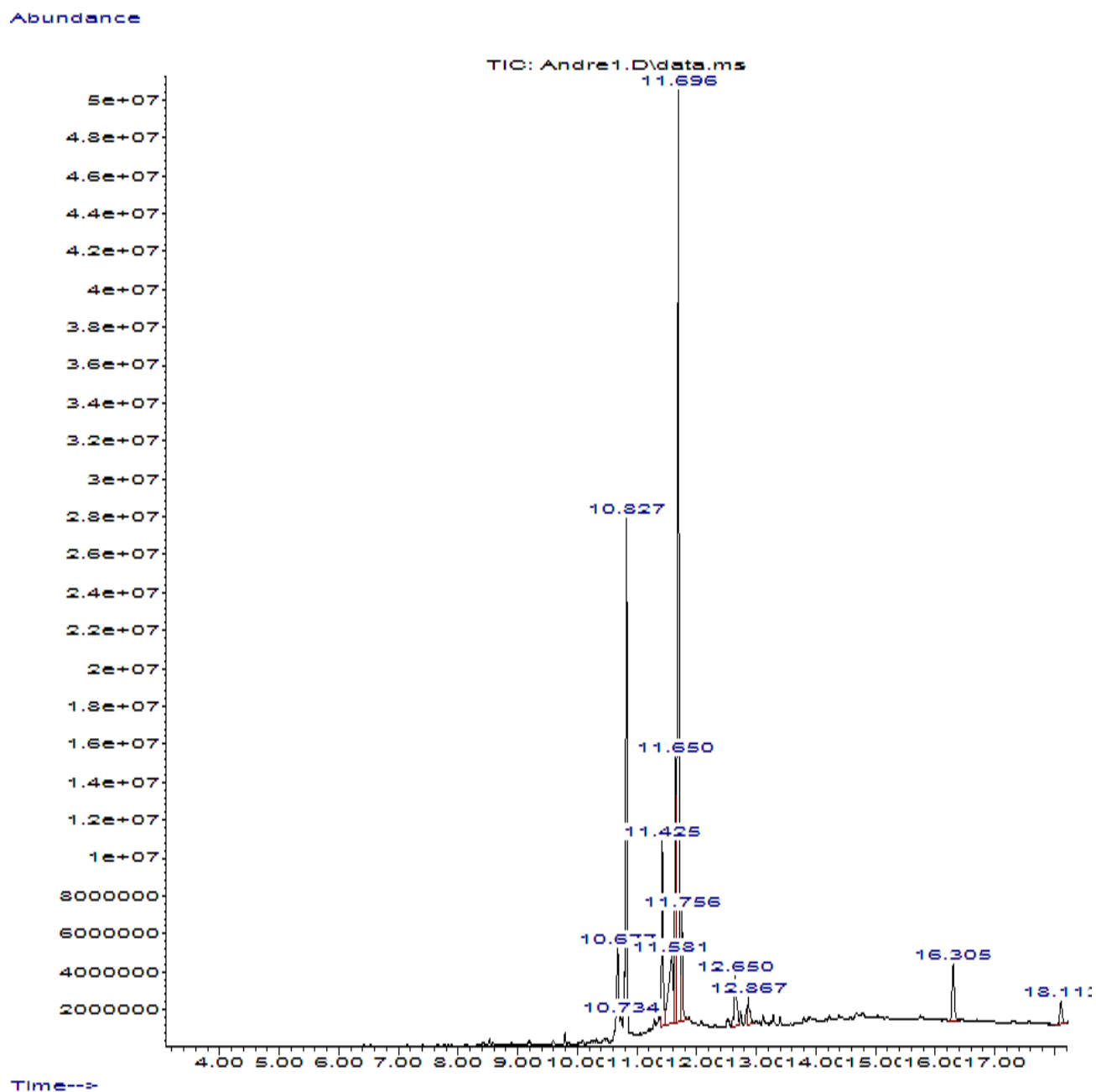
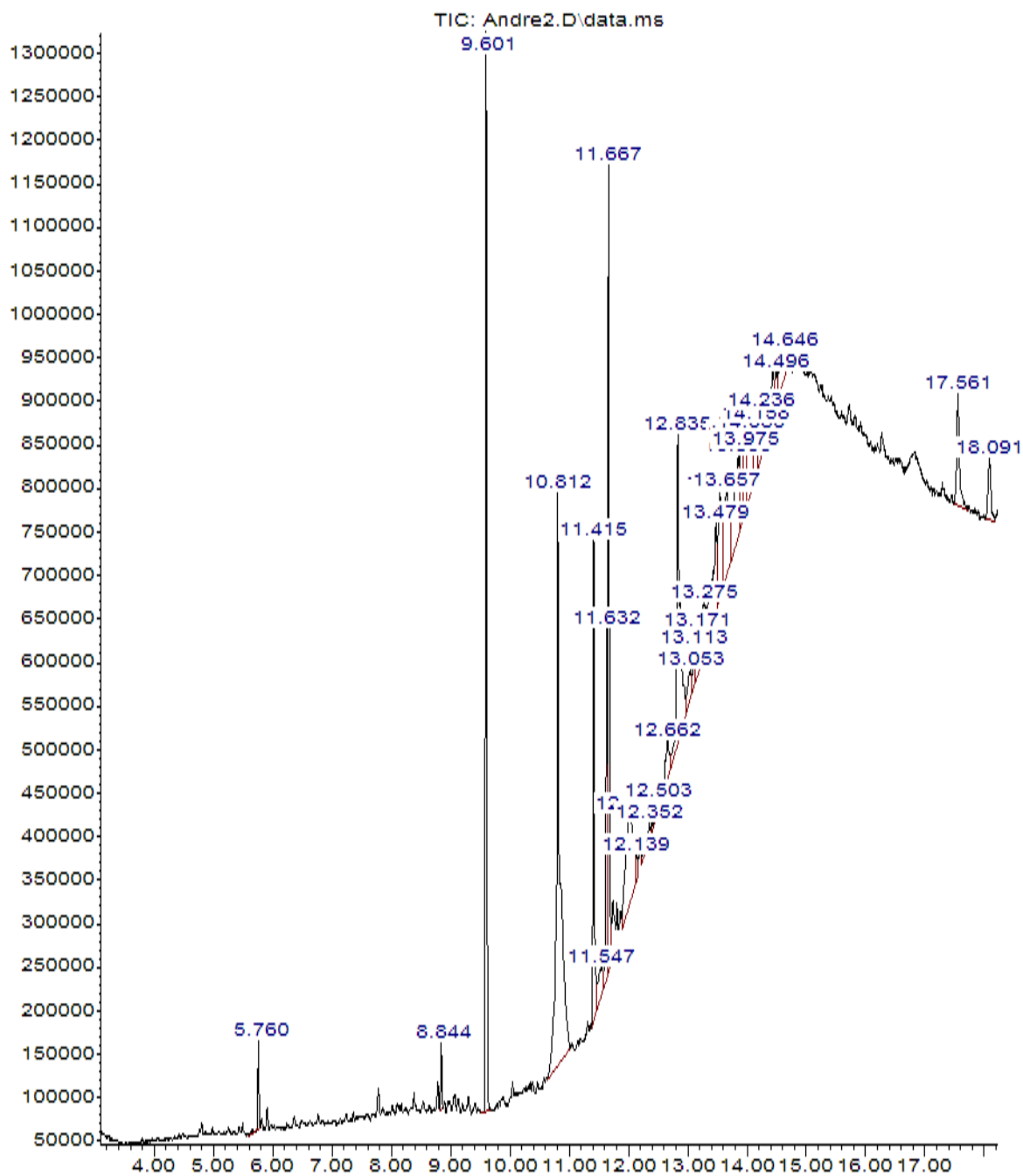


Figure 4. 1 GC-MS chromatogram of *Moringa oleifera* leaf extracts.

**Table 4. 2 Chemical composition of leaf extracts of *Biden pilosa*.**

No	Compound	Retention time	% Peak area
1	Bicyclo[2.2.1] heptan-2-one, 1,7,7-trimethyl, (1S)	5.760	0.94
2	Benzamide, 4-methoxy-N-[2-(1-methylcyclopropyl) phenyl	8.844	0.71
3	9H-Fluorene, 9-diazo	9.601	11.34
4	Hexadecanoic acid, ethyl ester	10.812	15.98
5	Phytol	11.415	5.58
6	Benzo[h]quinoline, 2,4-dimethyl	11.547	1.14
7	Linoleic acid ethyl ester	11.632	3.64
8	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	11.667	8.80
9	5-Acetamido-4,7-dioxo-4,7-dihydro benzofurazan	12.022	5.60
10	Trimethyl [4-(2-methyl-4-oxo-2-pentyl) phenoxy] silane	12.139	0.29
11	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	12.352	0.51
12	1,2-Bis (trimethylsilyl) benzene	12.503	0.23
13	5-Methyl-2-trimethyl silyloxy-acetophenone	12.662	1.02
14	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl-4-methyl	12.835	7.67
15	Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15-hexadecamethyl	13.053	1.01
16	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13-tetradecamethyl	13.113	0.64
17	Tris (tert-butyl dimethyl silyloxy) arsane	13.171	0.95
18	Cyclotrisiloxane, hexamethyl	14.646	1.65
19	4-Methyl-2-trimethylsilyloxy-acetophenone	17.561	2.51
20	Arsenous acid, tris (trimethylsilyl) ester	18.091	1.39

Abundance



Time-->

Figure 4. 2 GC-MS chromatogram of *Biden pilosa* leaf extracts.

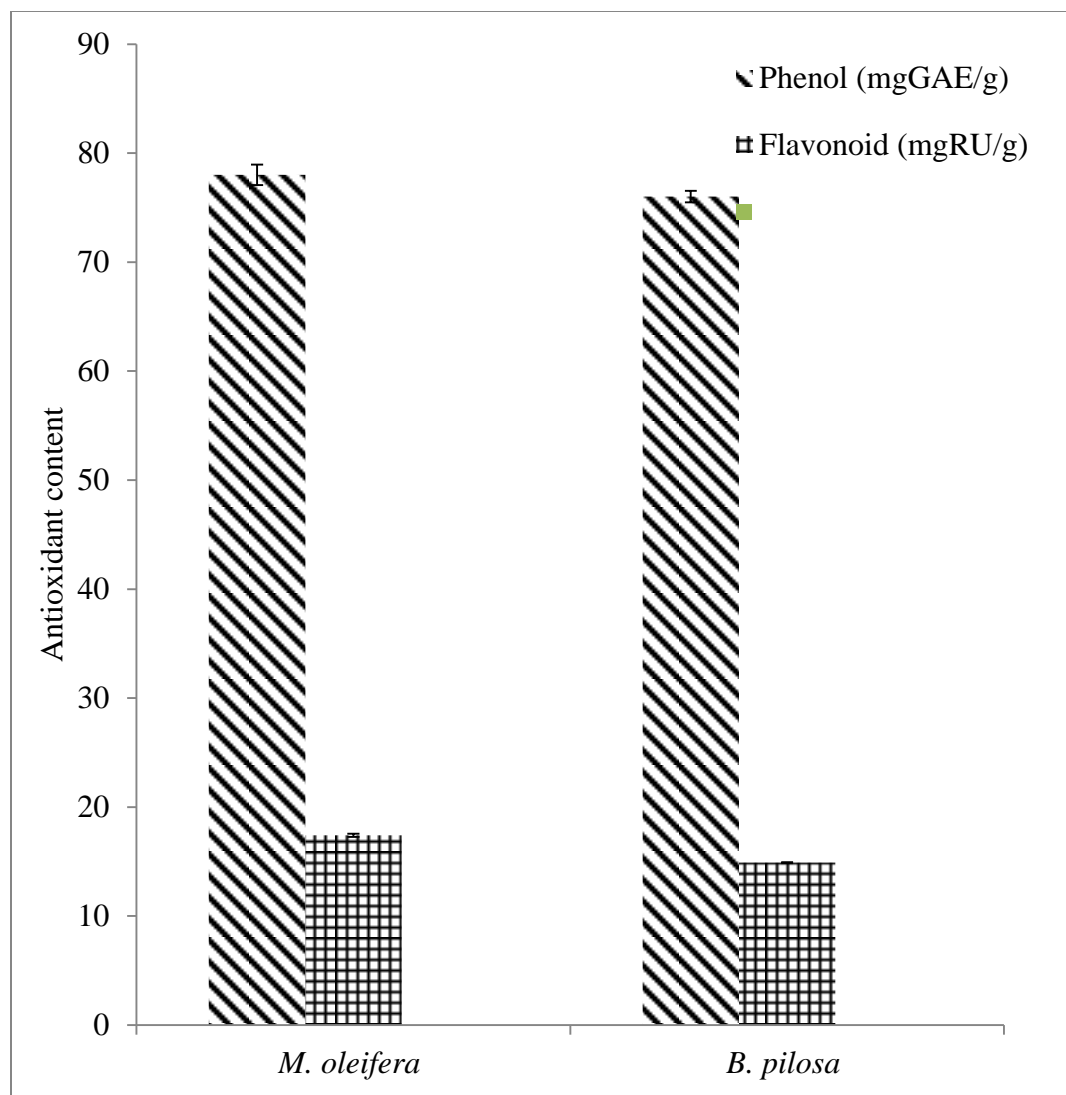
**Table 4. 3 Bioactivity of phytochemicals identified in the leaf extracts of *Moringa oleifera* and *Biden pilosa* by GC-MS.**

Compound	Extract	Compound Structure	Molecular Weight	Activity
Tetradecanoic acid	<i>M. oleifera</i>	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	228	Antimicrobial Antioxidant
n-Hexadecanoic acid	<i>M. oleifera</i>	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	Antioxidant
Hexadecanoic acid, ethyl ester	<i>M. oleifera</i> <i>B. pilosa</i>	C <sub>16</sub> H <sub>36</sub> O <sub>2</sub>	284	Antioxidant
Phytol	<i>M. oleifera</i> , <i>B. pilosa</i>	C <sub>20</sub> H <sub>40</sub> O	296	Antimicrobial Antioxidant
Octasiloxane, 1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13, 15, 15 - hexadecamethyl	<i>M. oleifera</i> , <i>B. pilosa</i>	C <sub>16</sub> H <sub>50</sub> O <sup>7</sup> Si <sub>8</sub>	578	Antimicrobial
Linoleic acid ethyl ester	<i>M. oleifera</i>	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>	308	Antimicrobial
DL-alpha-Tocopherol	<i>M. oleifera</i>	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>	430	Antioxidant
9, 12-Octadecadienoic acid (Z,Z)	<i>M. oleifera</i>	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	280	Antimicrobial
Linoleic acid ethyl ester	<i>B. pilosa</i>	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>	308	Antimicrobial
Phenol, 2, 2'-methylenebis [6-(1, 1-dimethylethyl)]-4-methyl	<i>B. pilosa</i>	C <sub>15</sub> H <sub>24</sub> O	220	Antimicrobial antioxidant
4-Methyl-2-trimethylsilyloxy-acetophenone	<i>B. pilosa</i>	C <sub>12</sub> H <sub>18</sub> O <sub>2</sub> Si		Antimicrobial
9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)	<i>M. oleifera</i> , <i>B. pilosa</i>	C <sub>20</sub> H <sub>34</sub> O <sub>2</sub>	306	Antimicrobial Antioxidant
Bicyclo[2.2.1] heptan-2-one, 1,7,7-trimethyl, (1S)	<i>B. pilosa</i>	C <sub>10</sub> H <sub>16</sub> O	152	Antimicrobial

### 4.3.2 Antioxidant capacity of the plant extracts

The results on antioxidant contents of plant extracts are presented in Figure 4.3. The extracts of *M. oleifera* revealed higher amount of antioxidant content than the extract of *B. pilosa*. The concentration of phenol and flavonoids in *M. oleifera* plant extracts were  $77.5 \pm 0.94$  mg GAE/g DW and  $17.4 \pm 0.15$  mg Ru/g DW while that of *B. pilosa* were  $75.9 \pm 0.53$  mg GAE/g DW and  $14.9 \pm 0.05$  mg Ru/g DW, respectively. The antioxidant activities of the extracts are illustrated in Table 4.4. There were significant differences ( $P < 0.05$ ) in DPPH and ABTS radical scavenging activities of *B. pilosa*, *M. oleifera* extracts and reference standard. The percentage inhibition of DPPH radicals for *M. oleifera* extracts, *B. pilosa* extracts, rutin, and BHT were 75.9, 77.1, 73.8 and 70.6% while that of ABTS radicals were 82.8, 83.24, 79.3 and 85.0%, respectively.

The total chlorophyll, vitamin C and carotenoid contents of the plant samples are presented in Figure 4.4 and 4.5. The chlorophyll contents a ( $2.62 \pm 0.05$  mg/g DW) and b ( $0.98 \pm 0.01$  mg/g DW) in *B. pilosa* were, respectively, more than twice and thrice those in *M. oleifera* leaves. The total chlorophyll values were  $3.60 \pm 0.04$  and  $1.46 \pm 0.01$  mg/g DW for *B. pilosa* and *M. oleifera* respectively. The total carotenoid content was higher for *B. pilosa* ( $0.73 \pm 0.00$  mg/g DW) and lower for *M. oleifera* ( $0.39 \pm 0.00$  mg/g DW) while vitamin C content in *M. oleifera* was about 12 times higher than those in *B. pilosa*.



**Figure 4. 3** Antioxidant content of *Moringa oleifera* and *Biden pilosa* leaf extracts.

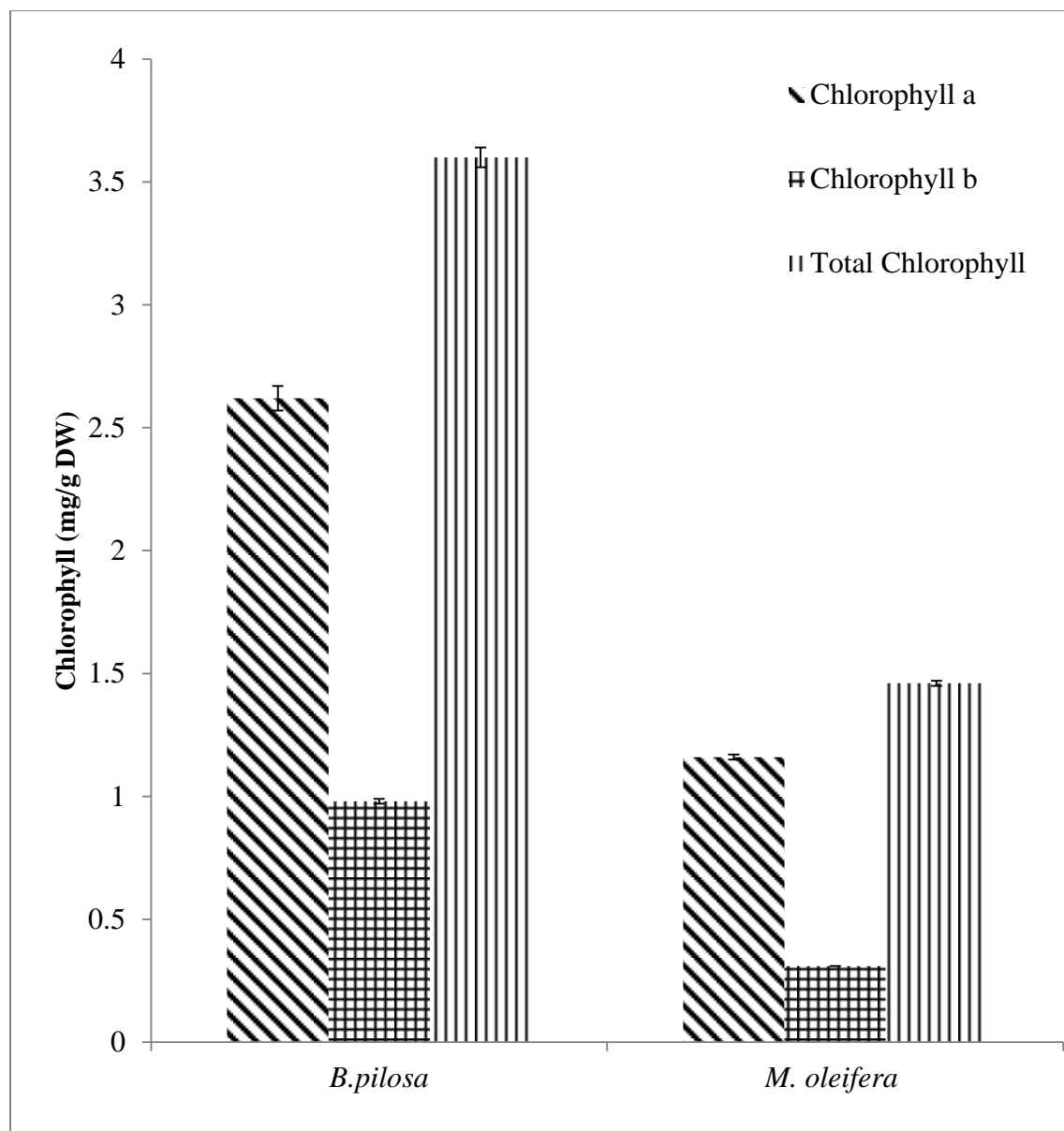


**Table 4. 4 Antioxidant activities of the *Moringa oleifera* and *Biden pilosa* leaf extracts.**

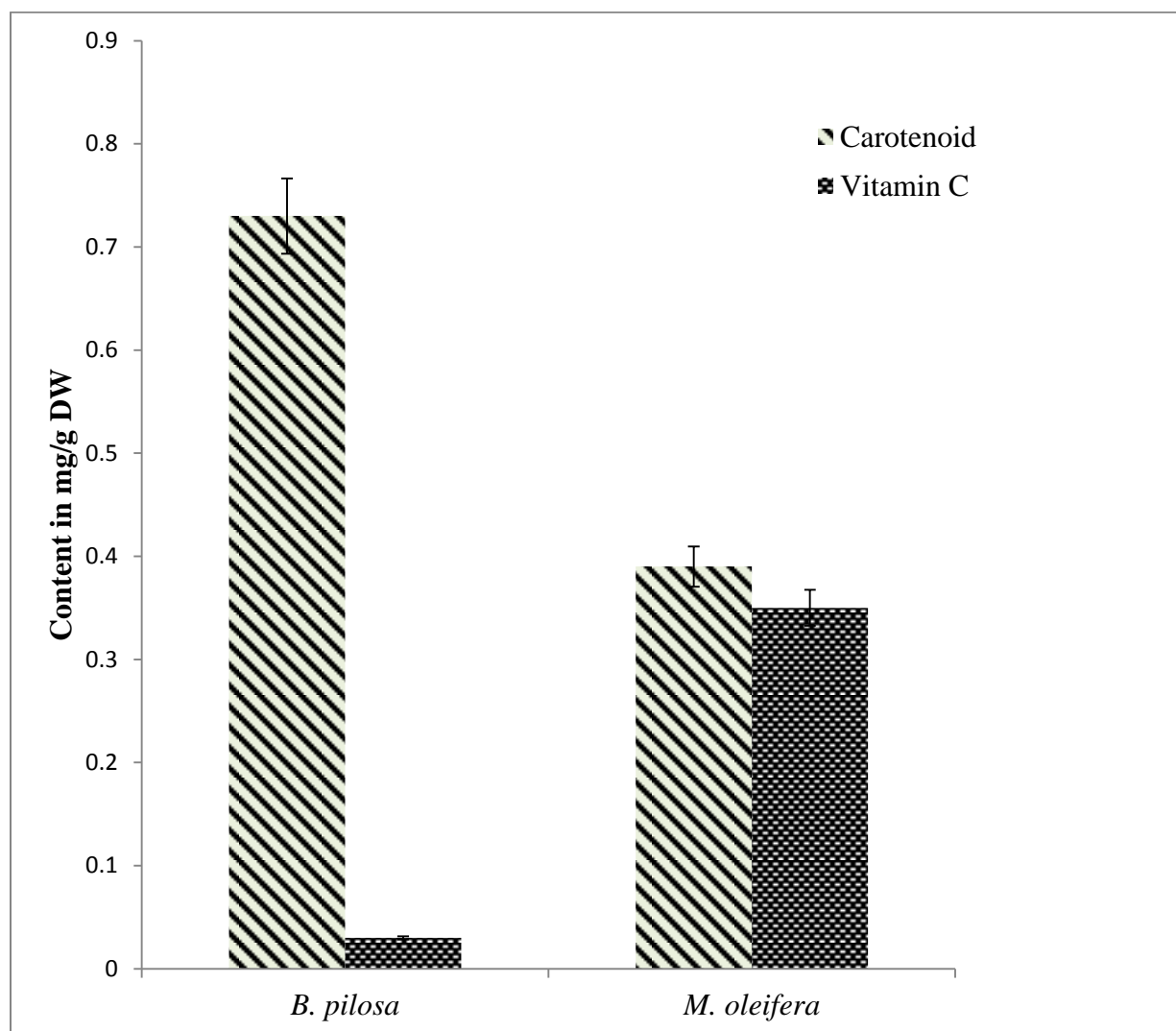
Antioxidant activity	<i>M. oleifera</i>	<i>B. pilosa</i>	Rutin	BHT
DPPH (%)	75.9 <sup>ab</sup> ± 1.12	77.1 <sup>a</sup> ± 0.63	73.8 <sup>bc</sup> ± 0.84	70.6 <sup>c</sup> ± 0.19
ABTS (%)	82.8 ± 1.05	83.24 ± 0.67	79.3 ± 1.34	84.95 ± 0.43

Means within the same row having different superscripts were significantly different (P < 0.05).

DPPH: 2, 2-Diphenyl-2-picrylhydrazyl, ABTS: 2,2-azino- bis-3-ethylbenzothiazoline-6-sulfonic acid, BHT: butylated hydroxytoluene.



**Figure 4. 4 Total chlorophyll content of the *Moringa oleifera* and *Biden pilosa* leaf extracts.**



**Figure 4. 5** Carotenoid and Vitamin C content of *Moringa oleifera* and *Biden pilosa* leaf extracts.

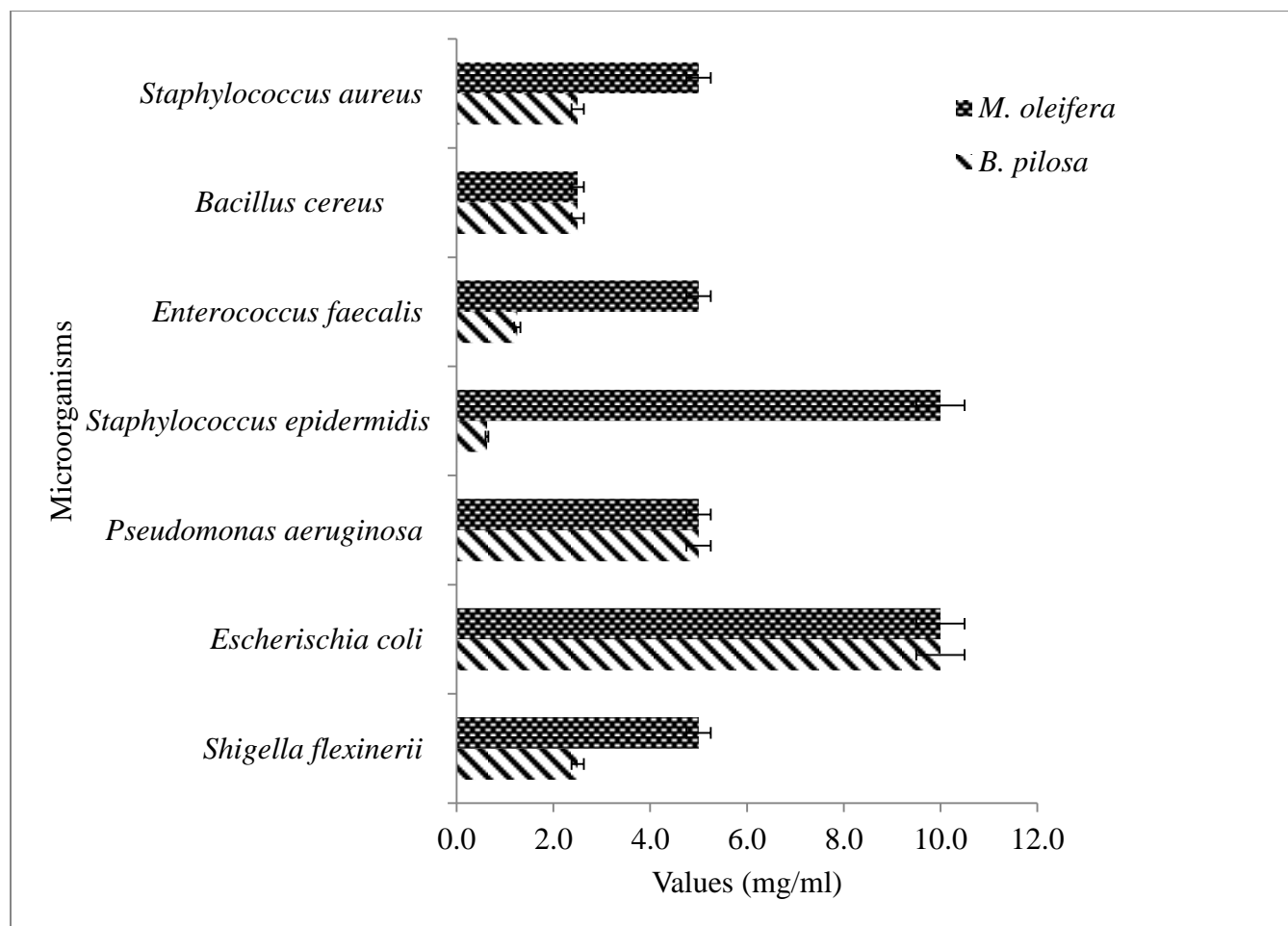
### 4.3.3 *In vitro* antimicrobial activity of plant extracts

The results of *in vitro* antimicrobial activity of the extracts obtained from *M. oleifera* and *B. pilosa*, against the tested organisms are shown in Table 4.5. All the plant extracts tested showed antibacterial activity, but each differs in their activities against some selected food borne micro-organisms. Both extracts showed antimicrobial activity against *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *E. coli*, *S. flexinerii* and *E. faecalis* while *B. cereus* and *P. aeruginosa* were resistant to the extract of *M. oleifera* and *B. pilosa*, respectively. The inhibitory activity of *M. oleifera* and *B. pilosa* extract against the test organism ranged from 9-19mm and 12-17 mm, respectively. The highest inhibitory effect of the extract was observed against *E. coli* and *S. flexinerii* (19 mm) and the lowest against *S. aureus*. The MIC of extracts against test organisms ranged from 0.6 to 10 mg/ml with *B. pilosa* showing higher activity against *S. epidermidis* at 0.6 mg/ml (Figure 4.6). The MBC of the extract varied between 5.0 and 20.0 mg/ml (Figure 4.7).

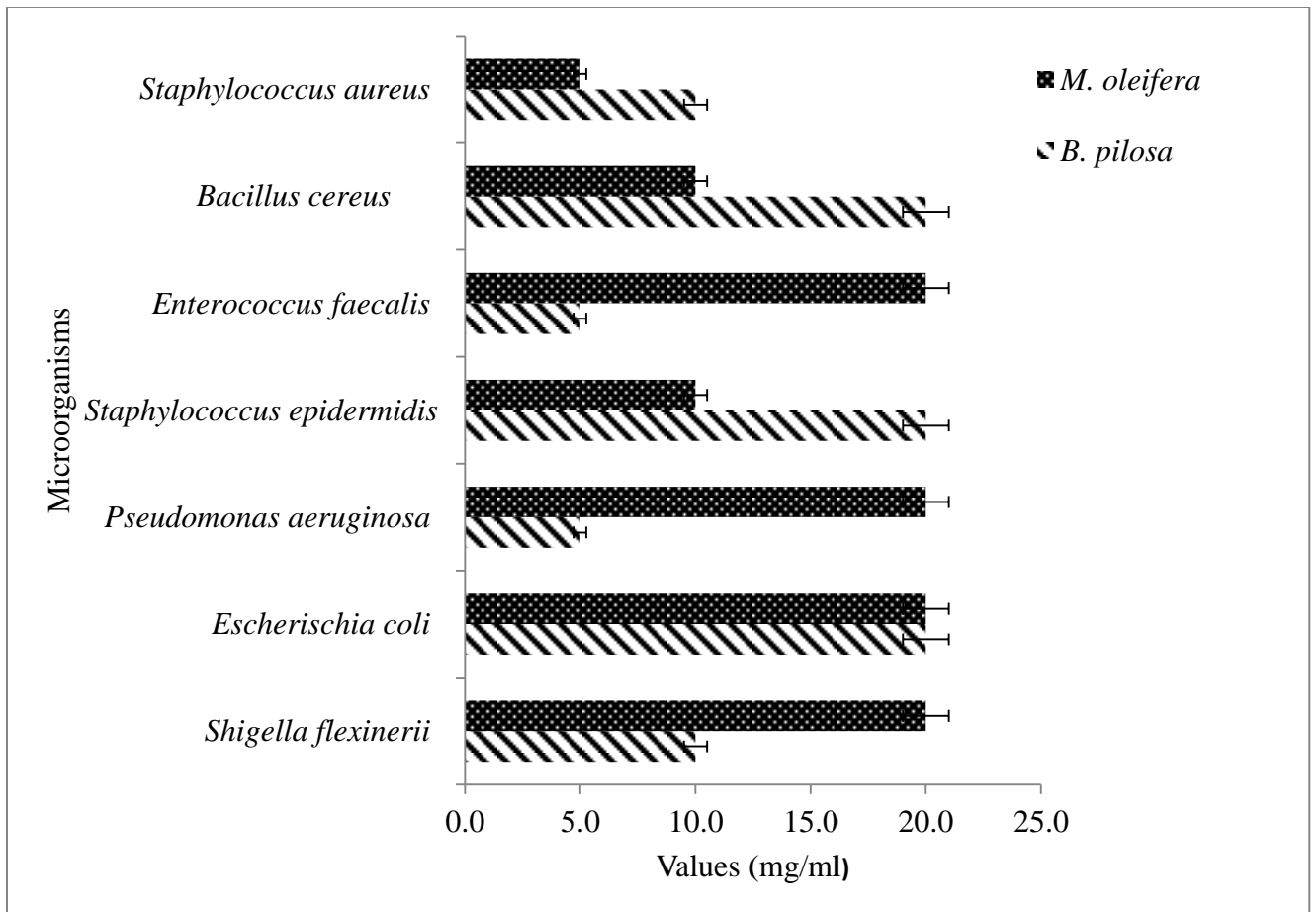
**Table 4. 5 Antimicrobial activities of *Moringa oleifera* and *Biden pilosa* leaf extracts (20mg/ml).**

Microorganism	Gram reaction	Diameter of zone of inhibition (mm)	
		<i>M. oleifera</i>	<i>B. pilosa</i>
<i>Staphylococcus aureus</i> ATCC 33591	+	9 ± 2.3	12 ± 0.3
<i>Bacillus cereus</i> ATCC 10876	+	0 ± 0.00	12 ± 0.9
<i>Staphylococcus epidermidis</i> ATCC 13518	+	14 ± 1.0	12 ± 0.0
<i>Enterococcus faecalis</i> ATCC 49532	+	14 ± 1.0	15 ± 0.0
<i>Pseudomonas aeruginosa</i> ATCC 19429	-	13 ± 0.3	0 ± 0.00
<i>Escherichia coli</i> ATCC 35218	-	19 ± 1.2	16 ± 1.5
<i>Shigella flexinerii</i> ATCC 12022	-	18 ± 1.16	17 ± 0.7
<i>Serratia marcescens</i> ATCC 14041	-	0 ± 0.00	0 ± 0.00

“±” Standard error of mean, “ATCC” American Type Culture Collection



**Figure 4. 6** Minimum inhibitory concentrations (MICs) of *Biden pilosa* and *Moringa oleifera* extracts on the test organisms.



**Figure 4. 7 Minimum bactericidal concentrations (MBCs) of *Biden pilosa* and *Moringa oleifera* extracts on the test organisms.**

#### 4.4 Discussion

Antioxidant compounds are the major constituents of medicinal plants and they possess redox properties that can absorb and neutralize free radicals, quench singlet and triplet oxygen, or decompose peroxide (Adedapo et al., 2008; Moyo et al., 2012a). The results of GC-MS analysis revealed that majority of the phytoconstituents present in the extract of *M. oleifera* and *B. pilosa* contained antimicrobial and antioxidant properties. Most specifically, the GC-MS identified n-Hexadecanoic acid, Hexadecanoic acid ethyl ester, DL-alpha-Tocopherol, Phenol, 2, 2'-methylenebis [6- (1, 1-dimethylethyl)]-4-methyl (BHT) and Phytol compounds, which have been reported to possess antioxidant activity (Di Mambro et al., 2003; Rajeswari et al., 2012; Bharathy and Uthayakumari. 2013; de Moraes et al., 2014; Mujeeb et al., 2014). Also, compounds such as Octasiloxane, 1, 1, 3, 3, 5, 5, 7, 7, 9, 9,11, 11, 13, 13, 15, 15-hexadecamethyl, Linoleic acid ethyl ester, 4-Methyl-2-trimethylsilyloxy-acetophenone, 9,12-Octadecadienoic acid (Z, Z) and 9,12,15-Octadecatrienoic acid ethyl ester (Z, Z, Z) have been reported in many studies to possess antimicrobial activity (Huang et al., 2010; Sethi et al., 2013; Rahman et al., 2014; Penduka et al., 2014; Venkatesh et al., 2014; Musa et al., 2015).

Our results further revealed that the extracts of *M. oleifera* had higher total phenolic, flavonoid, and flavonol content than *B. pilosa*. Phenolic, flavonoid and flavonol compounds are well known to initiate different biological activities in medicinal plants including antimicrobial and antioxidant properties through various mechanisms of action (Oyedemi et al., 2012). However, the antioxidant content of the extracts obtained in this study were slightly higher than those reported by Sreelatha and Padma (2009), Sultana et al. (2009), Cortes-Rojas et al. (2012) and Ogbunugafor et al. (2012). The difference could be attributed to variation in the environment



where the plants were collected, the season, the physiological stage of the plants when they were harvested and extraction method (Taylor and van Staden, 2001).

The DPPH and ABTS radical scavenging activities of *B. pilosa* and *M. oleifera* leaf extracts demonstrated significant strong antioxidant activity, and compared favorably with the standard rutin and BHT which are derivatives of phenolic compounds. In general, the ABTS radical of both leaf extracts showed greater antioxidant activity than DPPH radical. This could be attributed to differences in mechanism of action and reaction of DPPH and ABST radical. The 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) has been reported to be soluble in aqueous and organic solvents, and can therefore determine both hydrophilic and lipophilic antioxidant capacities (Abegg et al., 2012). However, our observations agreed with the findings of Moyo et al. (2012a) who found that scavenging ability of *M. oleifera* extract against ABTS was greater than DPPH radicals. The radical scavenging activities of *M. oleifera* extracts observed in this study were comparable with those reported by Sultana et al. (2009) while that of *B. pilosa* extract were slightly lower than those reported by Adedapo et al. (2012) and Luqman et al. (2012). The total chlorophyll, carotenoid and vitamin C content of the *B. pilosa* plant sample were found to be higher than the *M. oleifera* plant samples. The presence of total chlorophyll, Vitamin C, carotenoid together with synergistic effect of phenolic, flavonoid and flavonol contents could be responsible for stronger free radical scavenging activities displayed by *B. pilosa* extract in this study. The presence of chlorophyll, carotenoid, and vitamin C has been reported to contribute significantly to antioxidant activity of plant species through their ability to scavenge reactive oxygen species, singlet molecular oxygen and peroxy radicals (Ivanova et al., 2005; Lanfer-Marquez et al., 2005; Bunea et al., 2012).

The results of the plant extracts showed broad-spectrum antibacterial activity against gram-positive and gram-negative pathogenic bacteria. The extracts showed significant inhibitory effect against Gram-negative bacteria than Gram-positive bacteria strains. A similar result was also reported by Moyo et al. (2012b) in regard to sensitivity of Gram-negative bacteria to plant extracts. The best antibacterial activity was observed against *E. faecalis* and *S. epidermidis* and the lowest activity was against *E. coli*. However, the extract of *M. oleifera* and *B. pilosa* was found inactive against *B. cereus*, *P. aeruginosa* and *S. marcescens*. The resistant capability of these bacteria against the extract could be explained by disturbance of permeability barrier created by the cell wall or outward membrane of the bacteria (Hayek and Ibrahim, 2012; Nisa et al., 2013). Generally, the extract of *B. pilosa* demonstrated greater antibacterial activity (MIC) against the tested bacteria than *M. oleifera*. This may be attributed to the presence of soluble phenolic and polyphenolic compounds (Igbinosa et al., 2008) as well as huge proportion of the inherent phytoconstituents as revealed by the GC-MS analysis. The highest MBC was observed at 5mg/ml for both extracts. The inhibitory effect, MIC and MBC of the extract against pathogenic bacteria strains indicated that these plants are potential candidates of antimicrobial sources.

#### **4.5 Conclusions**

The plant extracts from *B. pilosa* and *M. oleifera* had the ability to quench free radicals and inhibit the growth of bacteria. The results of the antioxidant activity of this study also confirmed the antiradical activity of *B. pilosa* and *M. oleifera* extracts against DPPH and ABST radical which is comparable with the synthetic antioxidant (BHT). Eradication of these radicals revealed the oxidizing potential of plant extracts against the generation of free radicals in biological

systems. The antimicrobial assay also showed that the extracts possessed potential antibacterial activity against the tested organisms, particularly against gram negative bacteria. Moreover, the presence of these phytochemicals makes *B. pilosa* and *M. oleifera* a potential source of bioactive compounds. In addition, the antioxidant and antimicrobial content of the extracts revealed that these plants are promising candidates as natural preservatives.

#### 4.6 References

- Abegg, M. A., Alabarse, P. V. G., Schuller, A. K. and Benfato, M. S.** 2012. Glutathione levels in and total antioxidant capacity of *Candida* sp. cells exposed to oxidative stress caused by hydrogen peroxide. *Revista da Sociedade Brasileira de Medicina Tropical* **45**:5.
- Adedapo, A., Jimoh, F. and Afolayan, A.** 2011. Comparison of the nutritive value and biological activities of the acetone, methanol and water extracts of the leaves of *Bidens pilosa* and *Chenopodium album*. *Acta Poloniae Pharmaceutica Drug Research* **68 (1)**: 83-92.
- AOAC**, 1995. Official methods for the analysis (16th ed.). Washington, DC: Association of Official Analytical Chemists.
- Bartolome, A. P., Villaseñor, I. M. and Yang, W.** 2013. *Bidens pilosa* L. (Asteraceae): Botanical Properties, Traditional Uses, Phytochemistry, and Pharmacology. *Evidence-Based Complementary and Alternative Medicine* **2013**: 1-51.
- Bharathy, V. and Uthayakumari, F.** 2013. Bioactive Components in leaves of *Jatropha tanjorensis* J.L. Ellis & Saroja by GC-MS Analysis. *International Journal of PharmTech Research* **5**: 1839-1843.
- Bunea, C. I., Pop, N., Babeş, A. C., Matea, C. Dulf, F. V. and Bunea, A.** 2012. Carotenoids, total polyphenols and antioxidant activity of grapes (*Vitis vinifera*) cultivated in organic and conventional systems. *Chemistry Central Journal* **6**:1-66.

**Cortes-Rojas, D. F., Chagas-Paula, D. A., Da Costa, F. B., Souza, C. R. F. and Oliveira, W.**

**P.** 2013. Bioactive compounds in *Bidens pilosa* L. populations: a key step in the standardization of phytopharmaceutical preparations. *Brazilian Journal of Pharmacognosy* **23 (1)**: 28-35.

**Daniells, S.** 2006. Plant extracts beat synthetics in meat preservatives, say study. [www.foodnavigator.com](http://www.foodnavigator.com) accessed on 25/08/2015.

**de Moraes, J., de Oliveira, R. N., Costa, J. P., Junior, A. L.G., de Sousa, D.P., Freitas, R.**

**M., Allegrett, S. M. and Pinto, P. L. S.** 2014. Phytol, a diterpene alcohol from chlorophyll, as a drug against neglected tropical disease schistosomiasis mansoni. *PLOS Neglected Tropical Diseases* **8 (1)**: 2617.

**Di Mambro, V. M., Azzolinib, A. E.C.S., Valimb, Y. M. L. and Fonseca, M. J. V.** 2003.

Comparison of antioxidant activities of tocopherols alone and in pharmaceutical formulations. *International Journal of Pharmaceutics* **262**: 93–99.

**Doolaee, E. H. A., Vossen, E., Raes, K., Meulenaer, B. D., Verhé, R., Paelinck, H. and**

**Smet, S. D.** 2012. Effect of rosemary extract dose on lipid oxidation, colour stability and antioxidant concentrations, in reduced nitrite liver pâtés. *Meat Science* **90**:925-931.

**Gutierrez, J., Barry-Ryan, C. and Bourke, P.** 2009. Antimicrobial activity of plant. Essential

oils using food model media: Efficacy, synergistic potential and interactions with food components. *Food Microbiology* **26**: 142–150.

- Hayek, S. A. and Ibrahim, S. A.** 2012. Antimicrobial activity of Xoconostle pears (Opuntiamatuda) against Escherichia coli O157:H7 in laboratory medium. *International Journal of Microbiology* **2012**: 1-6.
- Huang, C. B., George, B. and Ebersole, J. L.** 2010. Antimicrobial activity of n-6, n-7 and n-9 fatty acids and their esters for oral microorganisms. *Archives of Oral Biology* **55 (8)**: 555–560.
- Igbinosa, O. O., Igbinosa, E. O. and Aiyegoro, O. A.** 2009. Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). *African Journal of Pharmacy and Pharmacology* **3 (2)**: 058-062.
- Ivanova, D., Gerova, D., Chervenkov, T. and Yankova, T.** 2005. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *Journal of Ethnopharmacology* **95**: 145-150.
- Jayawardana, B. C., Liyanage, R., Lalantha, N., Iddamalgoda, S. and Weththasinghe, P.** 2015. Antioxidant and antimicrobial activity of drumstick (*Moringa oleifera*) leaves in herbal chicken sausages. *LWT - Food Science and Technology*, **64**: 1204-1208.
- Jaberian, H., Piri, K. and Nazari, J.** 2013. Phytochemical composition and in vitro antimicrobial and antioxidant activities of some medicinal plants. *Food Chemistry* **136**: 237–244.
- Jorgensen, J H. and Turnidge, J. D.** 2007. Susceptibility test methods: dilution and disk diffusion methods. In Murray P R, Baron E J, Jorgensen J H, Landry M L, Pfaller M A. (ed.), *Manual of clinical microbiology*. ASM Press, Washington, D.C. pp 1152–1172.

- Karre, L., Lopez, K. and Getty, K. J.** 2013. Natural antioxidants in meat and poultry products. *Meat Science* **94** (2): 220-227.
- Khan, M. R., Kihara, M. and Omoloso, A. D.** 2001. Antimicrobial activity of *Bidens pilosa*, *Bischofia javanica*, *Elmerillia papuana* and *Sigesbekia orientalis*. *Fitoterapia* **1** (72): 662-665.
- Kim, S. J, Min, S. A., Shin, H. J., Lee, Y. J., Cho, A. R., Kim, S.Y. and Han, J.** 2013. Evaluation of the antioxidant activities and nutritional properties of ten edible plant extracts and their application to fresh ground beef. *Meat Science* **93**: 715–722.
- Kobus-Cisowska, J., Flaczyk, E., Rudzinska, M., and Kmiecik, D.** 2014. Antioxidant properties of extracts from *Ginkgo biloba* leaves in meatballs. *Meat Science* **97**: 174–180.
- Koolen, H. H. F., da Silva, F. M.A., Gozzo, F. C., de Souza, A. Q. L. and de Souza, A. D.L.** 2013. Antioxidant, antimicrobial activities and characterization of phenolic compounds from buriti (*Mauritia flexuosa* L. f.) by UPLC–ESI-MS/MS. *Food Research International* **51**: 467–473.
- Kumar, Y., Yadav, D. N., Ahmad, T. and Narsaiah, K.** 2015. Recent trends in the use of natural antioxidants for meat and meat products. *Comprehensive Reviews in Food Science and Food Safety* **2015** (00): 1-14 .
- Lanfer-Marquez, U. M., Barros, R. M. C. and Sinnecker, P.** 2005. Antioxidant activity of chlorophylls and their derivatives. *Food Research International* **38**: 885–891.

- Lichtenthaler, H. K.** 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology* **148**: 350–382.
- Liyana-Pathiranan, C. M., Shahidi, F., and Alasalvar, C.** 2006. Antioxidant activity of Cherry laurel fruit (*Laurocerasus officinalis* Roemi.) and its concentrated juice. *Food Chemistry* **99**: 121–128.
- Lobo, V., Patil, A., Phatak, A. and Chandra, N.** 2010. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Review* **4(8)**: 118–126.
- Luqman, S., Srivastava, S., Kumar, R., Maurya, A. K. and Chanda, D.** 2012. Experimental Assessment of Moringa oleifera Leaf and Fruit for Its Antistress, Antioxidant, and Scavenging Potential Using In Vitro and In Vivo Assays. *Evidence-Based Complementary and Alternative Medicine* **2012**: 1-12.
- Mohamed, H. M. H., Mansour, H. A. and Farag, M. D.** 2011. The use of natural herbal extracts for improving the lipid stability and sensory characteristics of irradiated ground beef. *Meat Science* **87**: 33–39.
- Moyo, B., Masika, P. J., Hugo, A. and Muchenje, V.** 2011. Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. *African Journal of Biotechnology* **10 (60)**: 12925-12933.
- Moyo, B., Oyedemi, S., Masika, P. J. and Muchenje, V.** 2012a. Polyphenolic content and antioxidant properties of *Moringa oleifera* leaf extracts and enzymatic activity of liver from goats supplemented with *Moringa oleifera* leaves/sunflower seed cake. *Meat Science* **91**: 441–447.



- Moyo, B., Masika, P. J. and Muchenje, V.** 2012b. Antimicrobial activities of *Moringa oleifera* Lam leaf extracts. *African Journal of Biotechnology* **11 (11)**: 2797-2802.
- Mujeeb, F., Bajpai, P. and Pathak, N.** 2014. Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of *Aegle marmelos*. *BioMed Research International* **2014**: 1-11.
- Musa, A. M., Ibrahim, M. A., Aliyu, A. B., Abdullahi, M. S., Tajuddeen., Ibrahim, H and Oyewale, A. O.** 2015. Chemical composition and antimicrobial activity of hexane leaf extract of *Anisopus mannii* (Asclepiadaceae). *Journal of Intercultural Ethnopharmacology* **4 (2)**: 129-133.
- Nisa, H., Kamili, A. N., Bandh, S. A., Amin, S., Lone, B. A. and Parray, J. A.** 2013. Phytochemical screening, antimicrobial and antioxidant efficacy of different extracts of *Rumex entatus* L. - A locally used medicinal herb of Kashmir Himalaya. *Asian Pacific Journal of Tropical Disease* **3 (6)**: 434-440.
- National Toxicology Program, Department of Health and Human Services (NTP).** 2011. "Butylated Hydroxyanisole, Report on Carcinogens, Twelfth Edition. <http://www.ntp.niehs.nih.gov/go/roc> Accessed on 20/07/2014.
- Ogbunugafor, H., Igwo-Ezikpe, M., Igwilo, I., Ozumba, N., Adenekan, S., Ugochukwu, C., Onyekwelu, O. and Ekechi, A.** 2010. *In vitro* and *In vivo* evaluation of antioxidant properties of *Moringa oleifera* ethanolic leaves extract and effect on serum lipid indices in rat. *Macedonian Journal of Medical Sciences* **5 (4)**:397-403.

- Ordenez, A. A .L., Gomez, J. G., Vattuone, M. A. and Isla, M. I.** 2007 Antioxidant activities of *Sechium edule* (Jacq.) Swart extracts. *Food Chemistry* **97**:452-458.
- Oyedemi, S. O, Oyedemi, B. O., Arowosegbe, S. and Afolayan1, A. J.** 2012. Phytochemicals analysis and medicinal potentials of hydroalcoholic extract from *Curtisia dentata* (burm.f) c.a. Sm stem bark. *International Journal of Molecular Science* **13 (5)**: 6189–6203.
- Penduka, D., Buwa, L., Mayekiso, B., Basson, A. K. and. Okoh, A. K.** 2014. Identification of the antilisterial constituents in partially purified column chromatography fractions of garcinia kola seeds and their interactions with standard antibiotics. *Evidence-Based Complementary and Alternative Medicine* **2014**: 1-8.
- Rahman, M. M., Rahman, M. M., Akhter, S., Jamal, M. A., Pandeya, D. R., Haque, M. A., Alam, M. F. and Rahman, A.** 2010. Control of coliform bacteria detected from diarrhea associated patients by extracts of *Moringa oleifera*. *Nepal Medical College Journal* **12 (1)**: 12-19.
- Rahman, M. M., Ahmad, S. H., Mohamed, M. T. M. and Ab Rahman, M. Z.** 2014 Antimicrobial compounds from leaf extracts of *Jatropha curcas*, *Psidium guajava*, and *Andrographis paniculata*. *Scientific World Journal* **2014**: 1-8.
- Rajeswari, G., Murugan, M. and Mohan, V. R.** 2012. GC-MS analysis of bioactive components of *Hugonia mystax* L. (Linaceae). *Research Journal of Pharmaceutical, Biological and Chemical Sciences* **3**: 301.

- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. A.** 1999. Antioxidant activity applying an improved ABTS radical cation decolourising assay. *Free Radical Biology and Medicine* **26**: 1231–1237.
- Sanchez-Ortega, I., Garcia-Almendarez, B. E., Santos-Lopez, E. M., Amaro-Reyes, A., Barboza-Corona, J. E. and Regalado, C.** 2014. Antimicrobial edible films and coatings for meat and meat products preservation. *Scientific World Journal* **2014**: 1-18.
- Sethi, A., Amandeep, R. P., Shukla, D., Bhatia, A. and Singh, R. P.** 2013. Identification of phytochemical constituents from biologically active pet ether and chloroform extracts of the flowers of *Allamanda violacea* A.DC (Apocynaceae). *Asian Journal of Plant Science and Research* **3 (4)**: 95-108
- Sreelatha, S. and Padma, P. R.** 2009. Antioxidant activity and total phenolic content of moringa oleifera leaves in two stages of maturity. *Plant Foods for Human Nutrition* **64**:303-311.
- Sultana, B., Anwar, F. and Ashraf, M.** 2009. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules* **14**: 2167-2180.
- Tajkarimi, M. M., Ibrahim, S. A. and Cliver, D. O.** 2010. Antimicrobial herb and spice compounds in food. *Food Control* **21**:1199–1218.
- Taylor, J. L. S. and van Staden, J.** 2001. The effect of age, season and growth conditions on anti-inflammatory activity in *Eucomis autumnalis* (Mill.) Chitt. *Plant extracts. Plant Growth Regulation* **34 (1)**: 39-47.

- Venkatesh, R., Vidya, R. and Kalaivani, K.** 2014. Gas chromatography and mass spectrometry analysis of solanum villosum (mill) (solanaceae). *International Journal of Pharma Sciences and Research* **5 (12)**: 5283-5287.
- Williams, G. M., Iatropoulos, M. J. and Whysner, V.** 1999. Safety assessment of butylated hydroxyanisole and butylated hydroxytoluene as antioxidant food additives. *Food and Chemical Toxicology* **37**: 1027–1038.
- Wolfe, K., Wu, X. and Liu, R. H.** 2003. Antioxidant activity of apple peels. *Journal of Food Chemistry* **51**: 609–614.
- Yadav, A. S. and Singh, R. P.** 2004. Natural preservatives in poultry meat products. *Natural product Radiance* **2**:3.
- Yang, W.** 2014. Botanical, pharmacological, phytochemical, and toxicological aspects of the antidiabetic plant *Bidens pilosa* L. *Evidence-Based Complementary and Alternative Medicine* **2014**: 1-14.
- Yassaa, H. D. and Tohamy, A. F.** 2014. Extract of *Moringa oleifera* leaves ameliorates streptozotocin-induced Diabetes mellitus in adult rats. *Acta Histochemica* **116**: 844–854.

## **Chapter 5: Antioxidant and antimicrobial activities of *Moringa oleifera* and *Biden pilosa* leaf extracts in fresh ground beef**

*(Submitted to Food Research International)*

### **Abstract**

The objective of this study was to compare the antimicrobial and antioxidant activities of *Moringa oleifera* and *Biden pilosa* leaf extracts on ground beef quality from Bonsmara and non-descript cattle during 6 days refrigeration storage. Fresh ground beef from each breed were treated BHT (positive control, 0.02%w/w), *M. oleifera* (ML, 0.05 and 0.1% w/w) and *B. pilosa* (BP, 0.05 and 0.1% w/w) leaf extracts and compared with beef samples without any additive (negative control). The pH, instrumental color (CIE L\*, a\*, b\*), oxidative stability, total viable counts (TVC) and lactic acid bacteria (LAB) counts were determined after the storage period. The results revealed higher pH values in control and BHT treatment than ground beef samples treated with extracts ( $p > 0.05$ ). Addition of ML and BP leaf extracts significantly ( $p < 0.05$ ) improved the hunter (L\*, a\* and b\*) values and sensory quality of the ground beef compared to control. Similarly, the formation of TBARS in ground beef samples treated with extracts were significantly ( $p < 0.05$ ) lower compared to control and BHT treatment. Breed had little effect on most parameters except redness (a\*) and TBARS formation in which ground beef samples from Bonsmara cattle demonstrated higher oxidative stability than non-descript ( $p < 0.05$ ). The bacterial counts of beef samples containing ML and BP leaf extract samples were relatively lower than control and BHT treated samples. In conclusion, the addition of *M. oleifera* and *B. pilosa* leaf extracts in ground beef show that they are very effective against lipid oxidation and have potential as natural antioxidants.

**Key words:** Ground beef, microbes, natural antioxidant, lipid oxidation

## 5.1 Introduction

Application of plant extracts in processed meat products as natural antioxidants and preservative agents has continued to receive a considerable attention in recent times due to their ability to prolong shelf life and enhance consumer health. As natural antioxidants, plant extracts are very rich in bioactive compounds (Chapter 4) and they can donate hydrogen ions to inhibit free radical formation and/or interrupt propagation of autoxidation in muscle food (Brewer, 2011). As potential preservative agents, plant extracts possess huge bioactive compounds which are capable of disrupting and degrading the cytoplasmic membrane and cell wall of spoilage microorganisms (Kim et al., 2013; Krishnan et al., 2014) and also improve the physicochemical qualities of processed meat products (Velasco and Williams, 2011; Shah et al., 2015).

Presently, processed meats represent a large percentage of muscle foods consumed in the Western world (Mccurdy, 2009; Soladoye et al., 2015) because they are easily accessible and relatively inexpensive compared with traditional fresh meat cuts (de Oliveira et al., 2012). However, due to production process, almost all processed meats including ground or minced beef are easily susceptible to lipid and pigment oxidation (Nam et al., 2010). Recent studies have shown that the grinding of meat usually disrupt the muscle cell membranes and expose the lipid membranes to metal ions which in turn act as pro-oxidants to initiate oxidation (Banerjee et al., 2012; Kim et al., 2013). Beside this, Mccurdy (2009) also reported that the grinding of meat may facilitate the distribution and multiplication of any pathogens present in the meat before processing. Both the initiation of oxidation process in ground meat reduces their shelf-life and compromises the physical and nutritional quality of meat by generating rancid flavor and

oxidized compounds (aldehydes, ketones and organic acids) which are detrimental to consumer health (Saad et al., 2007; Lara et al., 2011; Hygreeva et al., 2014).

To deal with the undesirable changes referred to above and reduce the use of synthetic preservatives, extracts from plant sources are added to meat and meat products as natural additives (Shah et al., 2015). Interestingly, extracts from Moringaceae (*Moringa oleifera* Lam.) and Asteraceae (*Biden pilosa* Linn.) plant families are known to contain rich antioxidant compounds (Adedapo et al., 2011; Moyo et al., 2012a). The leaves of these plants have been used for centuries as dietary ingredients or supplements (Hazra et al., 2012; Bartolome et al., 2013). Recent studies on their application have showed that they possess great biological activities such as antidiabetes, antitumor, anti-inflammation, anticancer and antibacterial (Dai and Mumper, 2010; Alikwe et al., 2014). Reports on their nutritional contents have also showed that they are rich in proteins (including essential amino acids), vitamins, beta-carotene, minerals and low in fat and carbohydrates (Adedapo et al., 2011; Moyo et al., 2012b; Bartolome et al., 2013). The antioxidant and biological activities of these plants have been attributed to the presence of phytochemicals including flavonoids and other phenolics in their leaf extracts (Al-Owaisi et al., 2014).

Despite the above-mentioned qualities, limited studies are available on the efficacy of *M. oleifera* extracts (Hazra et al., 2012; Muthukumar et al., 2012; Shah et al., 2015) and to our knowledge, the preservative effect of extracts from the leaves of *B. pilosa* in meat products as potential antioxidants have not been studied. Also, the effect of breed/genotype on pigment and lipid oxidation of meat products treated with natural antioxidants has not been fully known.

Evidence has shown that oxidative stability of pigment and lipid in processed meat varies with breed, feeding diet and rearing systems (Xie et al., 2012; Martino et al., 2014). Therefore, the objective of this study was to investigate and compare the effect of *M. oleifera* and *B. pilosa* leaf extracts on the colour stability and lipid oxidation of *Muscularis longissimus thoracis et lumborum* muscle between Bonsmara and non-descript cattle. Prior to application of the extracts in meat samples, the phytochemical constituents and antioxidant activities of the plant leaves were also determined (Chapter 4).

## **5.2 Materials and Methods**

### **5.2.1 Plant sample and extract preparation**

*Biden pilosa* and *M. oleifera* leaf stalks were obtained from the University of Fort Hare farm (South Africa) and Moringa South Africa Ltd, respectively. The dry plant samples (200 g) were exhaustively macerated with 800 ml of ethanol-water solution (7:3) at room temperature for 2 days with agitation. Each extract was separated from the residue by filtration using Whatmann no.1 filter paper and then concentrated under reduced pressure at 55 °C using a rotary evaporator. The extract solvent was removed by freeze-drying and the dried extracts were used for the determination of the antioxidant activity at concentration of 1mg/ml. Determination of the nutritive values of the plants were carried out on the dry samples. All analyses were done in triplicate. The dried powder of plant extracts were then stored at 20<sup>0</sup> C for further analysis.

### **5.2.2 Meat sample preparation**

Fresh beef samples (*Muscularis longissimus thoracis et lumborum*, LTL) were obtained from Bonsmara (n = 40) and non-descripts (n = 40) cattle at a high throughput commercial abattoir in the Eastern Cape Province, South Africa. All the animals were between the ages of 4-5 years and



final live body weight of 450-500kg. The animals were slaughtered by exsanguination after stunning with a captive bolt stunner at voltage of 300 V and a current of 5A. About 200g of beef sample were collected from LTL of each carcass and processed after 48 hours *post mortem*. The beef samples were cut into small cubes after the removal of visible fat and connective tissues. They were then minced in a sterile meat grinder (CombinMax600, China). A portion (1200g) of the ground beef were randomly assigned to one of the following treatments: (1) NC (negative control, meat without additives); (2) BP (meat with 0.05% and 0.1% (w/w) *B. pilosa* extract); (3) ML (meat with 0.05% and 0.1% (w/w) *M. oleifera* extract), (4) BPML ( meat with 0.05% and 0.1% (w/w) Bide and Moringa extract) and (5) PC (positive control, meat with 0.02% (w/w) BHT). Immediately after adding the extracts and BHT, the samples were aerobically packed in low density polyethylene bags and stored at  $4 \pm 1$  °C for 6 days and then analyzed for pH, instrumental color attribute, thiobarbituric acid reactive substances (TBARS), sensory attributes (odor and general acceptability) and microbial counts. The preservation experiment was carried out in four replicates per treatment.

### **5.2.3 pH determination**

The pH of the fresh ground beef sample was determined as described by Muthukumar et al. (2012) with slight modifications. A 5g portion of the sample was blended in 25ml of deionized distilled water for 60s using homogenizer (Model Polytron® PT 2500 E Stand Dispersion Device, Kinematica AG, Switzerland). The pH values were measured using a standardized electrode attached to a digital pH meter (CRISON Instruments S.A., Alella, Spain).

### **5.2.4 Instrumental colour determination**

Colour changes in fresh ground beef during storage were performed using Hunter Lab Minolta colorimeter (BYK-Gardener GmbH, USA) with 20 mm aperture set for illumination D65 at 10<sup>0</sup>

standard observer angles. The colour coordinates CIE L\* (lightness), a\* (redness) and b\* (yellowness) were measured perpendicular to the ground beef surface at three different points after calibration using the standard green, black and white colour samples. All the color parameters (L\*, a\*, and b\*) were obtained from the mean of readings taken from four samples per treatment.

### **5.2.5 Determination of lipid oxidation**

The lipid oxidation of the fresh ground beef was determined by quantifying the thiobarbituric acid reactive substances (TBARS) in 5g of sample using the aqueous acid extraction method of Raharjo et al. (1992). The values of thiobarbituric acid-reacting substances (TBARS) obtained were multiplied by 10 and expressed as micrograms of malonaldehyde (MDA) per gram of meat. All TBARS analysis was carried out in four replicates per treatment.

### **5.2.6 Microbiological quality of ground beef**

Samples for microbial analysis were taken immediately after addition of extracts and again after 3 and 6 days of refrigerated storage for determination of total viable counts (TVC) and lactic acid bacteria (LAB). Ten grams of meat sample was homogenized with 90 mL of 0.1 % sterile buffered peptone water in a Stomacher bag for 2 min at room temperature. For each sample, appropriate serial ten-fold dilutions were prepared by diluting 1 mL of homogenate in 9 mL of 0.1 % peptone water. The pour-plate method was used for the determination of microbial counts. Total viable counts (TVC) were determined using standard plate count agar (Oxoid CM0463) after incubation for 48 h at 37 °C. Determination of lactic acid bacteria was carried out on deMan, Rogosa, and Sharpe (MRS, Oxoid CM0359) medium after 72 h incubation at 30°C.

Microbiological counts were expressed as the log<sub>10</sub> of colony-forming units per gram of ground beef (log CFU/g). All data presented are the mean values of three replicates.

### **5.2.7 Sensory Evaluation of the ground beef**

A panel of 9 judges was used for the sensory analysis. All the panelists were postgraduate students in Animal and Meat Science program of the University of Fort Hare. The Panelists were asked to evaluate odour intensity and overall acceptability of raw ground beef samples on 9-point descriptive scale: 1, dislike extremely; 2, dislike very much; 3, dislike moderately; 4, dislike slightly; 5, neither like nor dislike; 6, like slightly; 7, like moderately; 8, like very much; 9, like extremely (Maqsood et al., 2015). All raw meat samples were coded with 3-digit random codes and offered to the panelist in the random order. Samples were presented to panelist just after opening the covering material (plastic bag) to score odour first followed by overall acceptability. Samples were evaluated at day 0 and 3.

### **5.2.8 Statistical analysis**

Data on pH, color and lipid oxidation of ground beef was analyzed using Generalized Linear Models procedures of SAS (version 9.1.3 of 2007) with plant extracts and breed as source of variations. Microbial data were transformed into logarithms of the number of colony forming units (cfu/g) before analysis. Differences in mean values were computed using Tukey's Studentised Range (HSD) procedures for multiple comparisons.

## 5.3 Results

### 5.3.1 Effect of *Moringa oleifera* and *Biden pilosa* leaf extracts on pH of raw ground beef during storage period (4 °C)

The pH values on fresh ground beef during storage at 4°C are shown in Table 5.1. Ground beef samples from Bonsmara breed showed higher ( $P < 0.05$ ) pH values, ranging from  $5.23 \pm 0.04$  to  $6.88 \pm 0.28$  than the non-descript meat samples, ranging from  $5.09 \pm 0.03$  to  $6.12 \pm 0.05$ . Although, the overall pH values of the raw ground beef across the treatment did not differ significantly ( $P > 0.05$ ). However, all the beef samples treated with plant extracts had lower pH values compared to control and BHT treatments during the storage period.

### 5.3.2 Effect of *Moringa oleifera* and *Biden pilosa* leaf extracts on Hunter Lab color of raw ground beef during storage period (4 °C)

Results on the Hunter Lab color ( $L^*$ ,  $a^*$  and  $b^*$ ) of ground beef from Bonsmara and non-descript cattle is presented on Tables 5.2, 5.3 and 5.4. All the colors of fresh ground beef samples were slightly affected across treatments during the storage period. At day 0 and 6 of storage, there was no significant difference in overall L-values across the treatments. However, at day 3 of storage, beef samples treated with extracts and BHT exhibited higher L-values ( $p < 0.05$ ) compared to control. The redness ( $a^*$ ) values of the beef samples decreased gradually among the treatments and also with the storage period, although no significant difference was observed at day 0 and 6 of storage. However, at day 3 ( $p < 0.05$ ), beef samples treated with extracts showed higher  $a^*$  value than control ( $10.08 \pm 1.11$ ) and BHT ( $12.22 \pm 2.47$ ) treatments. The yellowness,  $b^*$  values varied significantly among the treatments and also with the storage period. At day 0 and 3, BHT and ML treated samples reflected the lowest  $b^*$  values while BP treated samples showed the

highest value. In all, lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ) of ground beef were however similar among the cattle breeds.

**Table 5. 1 Effect of *Moringa oleifera* (ML) and *Biden pilosa* (BP) leaf extracts on pH of ground beef during storage at 4 °C.**

Storage day	Breed	n	Treatment						
			Control	0.05ML	0.05BP	0.1ML	0.1BP	0.1MLBP	BHT
0	Non-descript	4	5.13 <sup>bcB</sup> ±0.05	5.18 <sup>abcB</sup> ±0.07	5.14 <sup>bcB</sup> ±0.05	5.32 <sup>aA</sup> ±0.13	5.17 <sup>abcA</sup> ±0.08	5.11 <sup>cB</sup> ±0.02	5.29 <sup>abB</sup> ±0.01
	Bonsmara	4	5.23 <sup>cA</sup> ±0.04	5.39 <sup>abA</sup> ±0.05	5.43 <sup>abA</sup> ±0.02	5.31 <sup>bcA</sup> ±0.06	5.42 <sup>abB</sup> ±0.06	5.43 <sup>abA</sup> ±0.10	5.46 <sup>aA</sup> ±0.09
3	Non-descript	4	5.42 <sup>A</sup> ±0.40	5.05 <sup>B</sup> ±0.06	5.11 <sup>A</sup> ±0.12	5.49 <sup>A</sup> ±0.30	5.15 <sup>A</sup> ±0.09	5.27 <sup>A</sup> ±0.42	5.56 <sup>A</sup> ±0.29
	Bonsmara	4	5.66 <sup>A</sup> ±0.60	5.42 <sup>A</sup> ±0.09	5.29 <sup>A</sup> ±1.11	5.52 <sup>A</sup> ±0.27	5.20 <sup>A</sup> ±0.12	5.37 <sup>A</sup> ±0.07	5.15 <sup>B</sup> ±0.08
6	Non-descript	4	6.12 <sup>B</sup> ±0.05	6.11 <sup>A</sup> ±0.47	6.05 <sup>A</sup> ±0.76	6.04 <sup>A</sup> ±0.41	5.64 <sup>A</sup> ±0.46	6.05 <sup>A</sup> ±0.23	5.99 <sup>B</sup> ±0.09
	Bonsmara	4	6.88 <sup>aA</sup> ±0.13	6.51 <sup>abA</sup> ±0.02	6.24 <sup>bcA</sup> ±0.17	6.44 <sup>abA</sup> ±0.18	6.18 <sup>bcA</sup> ±0.42	5.87 <sup>cA</sup> ±0.35	6.64 <sup>abA</sup> ±1.00
0	Overall mean	8	5.18±0.06	5.28±0.12	5.28±0.16	5.31±0.10	5.30±0.15	5.27±0.18	5.37±0.11
3		8	5.54±0.49	5.24±0.21	5.20±0.14	5.51±0.26	5.18±0.11	5.32±0.28	5.36±0.30
6		8	6.50±0.55	6.31±0.40	6.15±0.52	6.24±0.37	5.91±0.50	5.95±0.29	6.31±0.36

Mean with <sup>a-d</sup> superscripts within a row indicates significant differences ( $P \leq 0.05$ ).

Mean with <sup>A-B</sup> superscripts within a column indicates significant differences ( $P \leq 0.05$ ).

Control: no antioxidant, BHT: Butylated hydroxyl toluene, 0.05ML and 0.1ML: *Moringa oleifera* leaf extract, 0.05ML and 0.1ML: *Biden pilosa* leaf extracts.

**Table 5. 2 Effect of *Moringa oleifera* (ML) and *Biden pilosa* (BP) leaf extracts L\* in raw ground beef during storage at 4 °C.**

Storage day	Breed	n	Treatment						
			Control	0.05ML	0.05BP	0.1ML	0.1BP	0.1MLBP	BHT
0	Non-descript	4	29.79 <sup>A</sup> ±2.22	28.23 <sup>A</sup> ±1.17	27.79 <sup>A</sup> ±1.8	28.80 <sup>A</sup> ±5.45	29.37 <sup>A</sup> ±2.93	27.64 <sup>A</sup> ±1.34	29.34 <sup>A</sup> ±0.55
	Bonsmara	4	27.14 <sup>A</sup> ±1.81	27.37 <sup>A</sup> ±2.20	28.12 <sup>A</sup> ±2.38	27.90 <sup>A</sup> ±1.87	28.90 <sup>A</sup> ±0.60	28.04 <sup>A</sup> ±1.11	29.03 <sup>A</sup> ±1.41
3	Non-descript	4	30.85 <sup>abA</sup> ±1.04	28.83 <sup>abB</sup> ±1.55	31.59 <sup>abA</sup> ±3.59	27.72 <sup>ba</sup> ±1.37	31.89 <sup>abA</sup> ±2.26	32.74 <sup>aA</sup> ±0.87	30.81 <sup>abA</sup> ±2.05
	Bonsmara	4	29.86 <sup>A</sup> ±1.02	29.35 <sup>A</sup> ±0.97	29.78 <sup>B</sup> ±1.57	28.76 <sup>A</sup> ±1.24	28.80 <sup>A</sup> ±1.53	30.42 <sup>A</sup> ±2.64	30.38 <sup>A</sup> ±0.98
6	Non-descript	4	29.23 <sup>bcA</sup> ±2.07	28.27 <sup>cb</sup> ±1.03	29.36 <sup>bcA</sup> ±2.61	29.92 <sup>bcB</sup> ±0.70	33.97 <sup>abA</sup> ±1.42	30.19 <sup>abcA</sup> ±1.84	32.22 <sup>abA</sup> ±1.84
	Bonsmara	4	29.48 <sup>A</sup> ±1.02	30.39 <sup>A</sup> ±1.13	30.05 <sup>A</sup> ±2.20	29.38 <sup>A</sup> ±1.72	29.64 <sup>B</sup> ±0.98	30.54 <sup>A</sup> ±1.54	30.43 <sup>A</sup> ±0.76
0	Overall mean	8	28.46±2.35	27.80±1.95	27.95±2.31	28.35±2.10	29.14±1.98	27.84±1.15	29.19±1.00
3		8	30.36 <sup>c</sup> ±1.85	29.09 <sup>ab</sup> ±1.23	30.68 <sup>ab</sup> ±2.75	28.24 <sup>b</sup> ±1.33	30.35 <sup>ab</sup> ±2.42	31.58 <sup>a</sup> ±2.20	31.09 <sup>ab</sup> ±1.52
6		8	29.76±1.54	29.33±1.52	29.71±2.27	29.64±1.25	31.32±1.43	30.37±1.69	31.32±3.18

Mean with <sup>a-d</sup> superscripts within a row indicates significant differences ( $P \leq 0.05$ )

Mean with <sup>A-B</sup> superscripts within a column indicates significant differences ( $P \leq 0.05$ )

Control: no antioxidant, BHT: Butylated hydroxyl toluene, 0.05ML and 0.1ML: *Moringa oleifera* leaf extract, 0.05ML and 0.1ML: *Biden pilosa* leaf extracts.

**Table 5.3 Effect of *Moringa oleifera* (ML) and *Biden pilosa* (BP) extracts on a\* of raw ground meat during storage at 4 °C.**

Storage day	Breed	n	Treatment						
			Control	0.05ML	0.05BP	0.1ML	0.1BP	0.1MLBP	BHT
0	Non-descript	4	14.46 <sup>B</sup> ±1.13	16.92 <sup>A</sup> ±1.96	16.00 <sup>B</sup> ±0.91	16.59 <sup>B</sup> ±1.91	15.31 <sup>B</sup> ±0.95	15.97 <sup>B</sup> ±0.49	14.83 <sup>B</sup> ±0.92
	Bonsmara	4	18.16 <sup>A</sup> ±1.13	17.48 <sup>A</sup> ±1.11	17.70 <sup>A</sup> ±1.02	19.64 <sup>A</sup> ±0.98	19.05 <sup>A</sup> ±1.83	19.68 <sup>A</sup> ±1.01	19.85 <sup>A</sup> ±0.35
3	Non-descript	4	9.69 <sup>ba</sup> ±1.54	14.34 <sup>aA</sup> ±1.69	11.96 <sup>abB</sup> ±1.61	15.13 <sup>aA</sup> ±2.24	13.05 <sup>abA</sup> ±2.37	12.38 <sup>abA</sup> ±0.96	10.24 <sup>bb</sup> ±1.45
	Bonsmara	4	10.47 <sup>ca</sup> ±1.25	12.43 <sup>bcB</sup> ±1.82	16.78 <sup>aA</sup> ±1.57	11.54 <sup>bcB</sup> ±1.57	11.82 <sup>bcA</sup> ±1.27	10.99 <sup>bcA</sup> ±1.66	14.21 <sup>abA</sup> ±1.24
6	Non-descript	4	9.17 <sup>A</sup> ±0.81	11.24 <sup>B</sup> ±1.42	10.12 <sup>B</sup> ±1.90	9.71 <sup>A</sup> ±1.83	10.43 <sup>A</sup> ±1.32	9.64 <sup>A</sup> ±3.20	10.09 <sup>B</sup> ±2.30
	Bonsmara	4	11.64 <sup>A</sup> ±1.87	13.71 <sup>A</sup> ±1.10	14.99 <sup>A</sup> ±2.58	11.43 <sup>A</sup> ±1.69	11.57 <sup>A</sup> ±3.84	10.89 <sup>A</sup> ±1.22	13.66 <sup>A</sup> ±0.39
0	Overall mean	8	16.31±2.24	17.20±1.51	16.85±1.27	18.11±2.15	17.18±2.40	17.82±2.11	17.34±2.76
3		8	10.08 <sup>b</sup> ±1.11	13.39 <sup>a</sup> ±1.92	14.38 <sup>a</sup> ±2.97	13.33 <sup>ab</sup> ±2.63	12.45 <sup>ab</sup> ±1.88	11.68 <sup>ab</sup> ±1.46	12.22 <sup>ab</sup> ±2.47
6		8	10.40±1.88	12.48±1.77	12.55±3.34	10.57±1.87	10.99±2.73	10.27±2.34	11.92±1.96

Mean with <sup>a-d</sup> superscripts within a row indicates significant differences ( $P \leq 0.05$ ).

Mean with <sup>A-B</sup> superscripts within a column indicates significant differences ( $P \leq 0.05$ ).

Control: no antioxidant, BHT: Butylated hydroxyl toluene, 0.05ML and 0.1ML: *Moringa oleifera* leaf extract, 0.05ML and 0.1ML: *Biden pilosa* leaf extracts.



**Table 5. 4 Effect of *Moringa oleifera* (ML) and *Biden pilosa* (BP) extracts on b\* in raw ground beef during storage at 4 °C.**

Storage time	Breed	n	Treatment						
			Control	0.05ML	0.05BP	0.1ML	0.1BP	0.1MLBP	BHT
0	Non-descript	4	10.42 <sup>A</sup> ±1.38	11.22 <sup>A</sup> ±1.56	10.59 <sup>A</sup> ±2.23	10.91 <sup>B</sup> ±1.29	10.89 <sup>B</sup> ±2.08	11.76 <sup>A</sup> ±3.38	10.59 <sup>A</sup> ±3.17
	Bonsmara	4	12.19 <sup>bA</sup> ±1.33	12.42 <sup>bA</sup> ±0.90	12.57 <sup>bA</sup> ±1.83	13.76 <sup>abA</sup> ±0.58	15.76 <sup>aA</sup> ±0.90	13.60 <sup>abA</sup> ±1.21	14.27 <sup>abA</sup> ±0.85
3	Non-descript	4	10.84 <sup>A</sup> ±0.42	12.80 <sup>A</sup> ±1.20	12.00 <sup>A</sup> ±0.88	12.43 <sup>A</sup> ±1.19	12.53 <sup>A</sup> ±0.78	12.60 <sup>A</sup> ±1.27	11.24 <sup>A</sup> ±0.95
	Bonsmara	4	11.64 <sup>abA</sup> ±0.86	9.70 <sup>cB</sup> ±1.01	12.39 <sup>aA</sup> ±0.76	10.84 <sup>abcA</sup> ±0.70	11.92 <sup>abA</sup> ±0.61	11.31 <sup>abcA</sup> ±0.22	10.43 <sup>bcA</sup> ±0.64
6	Non-descript	4	10.13 <sup>A</sup> ±0.58	10.18 <sup>A</sup> ±1.35	12.40 <sup>A</sup> ±1.47	11.49 <sup>A</sup> ±0.47	12.56 <sup>A</sup> ±0.67	12.26 <sup>A</sup> ±1.25	11.60 <sup>A</sup> ±0.79
	Bonsmara	4	8.85 <sup>bA</sup> ±1.51	9.40 <sup>abA</sup> ±0.71	11.76 <sup>aA</sup> ±2.21	9.79 <sup>abB</sup> ±0.67	11.65 <sup>abA</sup> ±1.07	10.63 <sup>abA</sup> ±1.06	10.36 <sup>abA</sup> ±0.80
0	Overall mean	8	11.30±1.57	11.82±1.34	11.58±2.20	12.33±1.79	13.32±2.99	12.68±2.54	12.43±2.92
3		8	11.24±0.75	11.25±1.95	12.20±0.79	11.63±0.72	12.23±0.72	11.95±1.09	10.83±0.86
6		8	9.49 <sup>ab</sup> ±1.91	9.79 <sup>b</sup> ±1.08	12.08 <sup>a</sup> ±1.78	10.64 <sup>ab</sup> ±1.06	12.10 <sup>a</sup> ±0.96	11.45 <sup>a</sup> ±1.35	10.98 <sup>ab</sup> ±1.00

Mean with <sup>a-d</sup> superscripts within a row indicates significant differences (P ≤ 0.05).

Mean with <sup>A-B</sup> superscripts within a column indicates significant differences (P ≤ 0.05).

Control: no antioxidant, BHT: Butylated hydroxyl toluene, 0.05ML and 0.1ML: *Moringa oleifera* leaf extract, 0.05ML and 0.1ML: *Biden pilosa* leaf extracts.

### **5.3.2 Effect of *Moringa oleifera* and *Biden pilosa* leaf extracts on lipid oxidation of ground beef during storage at 4 °C**

Chemical deterioration, particularly lipid oxidation, is a major factor limiting the shelf-life of processed meat products. The results of TBAS analysis show that application of *M. oleifera* and *B. pilosa* extracts can protect ground beef against lipid oxidation during the storage period (Table 5.5.). Addition of the *M. oleifera* and *B. pilosa* extracts at different concentrations significantly lowered ( $p < 0.05$ ) the formation of TBAS in the beef samples from day 0 to 6 compared to control. At day 6, the overall mean TBAS values of the beef samples containing extracts ranged from  $0.85 \pm 0.17$  ugMDA/g to  $0.94 \pm 0.20$  ugMDA/gg compared to the control and BHT treatments at  $1.14 \pm 0.21$  ugMDA/g and  $0.98 \pm 0.10$  ugMDA/g, respectively. However, the addition of extracts from *B. pilosa* exhibited higher antioxidant activity against TBAS formation at 0.05 and 0.1% than the *M. oleifera* extracts. In overall, the TBAS values of the Bonsmara beef were slightly lower than non-descript beef samples across treatment and storage periods.

**Table 5. 5 Effect of *Moringa oleifera* (ML) and *Biden pilosa* (BP) plant extracts on lipid oxidation (ug mal/g) of ground beef during storage at 4 °C.**

Day of storage	Breed	n	Treatments						
			Control	0.05ML	0.05BP	0.1ML	0.1BP	0.1MLBP	BHT
0	Non-descript	4	0.76 <sup>abA</sup> ±0.12	0.76 <sup>abA</sup> ±0.01	0.84 <sup>aA</sup> ±0.07	0.67 <sup>abcA</sup> ±0.06	0.52 <sup>cA</sup> ±0.01	0.59 <sup>bcB</sup> ±0.03	0.54 <sup>cA</sup> ±0.15
	Bonsmara	4	0.52 <sup>bB</sup> ±0.05	0.69 <sup>abB</sup> ±0.07	0.65 <sup>abA</sup> ±0.15	0.57 <sup>abB</sup> ±0.03	0.63 <sup>abA</sup> ±0.09	0.64 <sup>abA</sup> ±0.01	0.98 <sup>aA</sup> ±0.85
3	Non-descript	4	0.85 <sup>aA</sup> ±0.21	0.86 <sup>aA</sup> ±0.03	0.72 <sup>abA</sup> ±0.05	0.91 <sup>aA</sup> ±0.11	0.75 <sup>abA</sup> ±0.08	0.57 <sup>bB</sup> ±0.00	0.76 <sup>abA</sup> ±0.06
	Bonsmara	4	0.68 <sup>bA</sup> ±0.09	0.62 <sup>bB</sup> ±0.03	0.55 <sup>bB</sup> ±0.06	0.59 <sup>bB</sup> ±0.02	0.60 <sup>bA</sup> ±0.17	0.92 <sup>aA</sup> ±0.11	0.68 <sup>bA</sup> ±0.14
6	Non-descript	4	1.13 <sup>aA</sup> ±0.10	1.15 <sup>aA</sup> ±0.17	0.89 <sup>abA</sup> ±0.19	1.04 <sup>abA</sup> ±0.19	0.71 <sup>b</sup> ±0.01	1.13 <sup>aA</sup> ±0.19	0.93 <sup>abA</sup> ±0.19
	Bonsmara	4	1.16 <sup>aA</sup> ±0.09	1.04 <sup>aA</sup> ±0.16	1.00 <sup>aA</sup> ±0.22	0.81 <sup>aA</sup> ±0.05	1.00 <sup>a</sup> ±0.06	1.05 <sup>aA</sup> ±0.06	1.03 <sup>aA</sup> ±0.06
0	Overall mean	8	0.64±0.04	0.72±0.06	0.74±0.15	0.63±0.06	0.57±0.08	0.61±0.04	0.76±0.39
3		8	0.77±0.17	0.74±0.13	0.63±0.09	0.75±0.18	0.67±0.14	0.74±0.19	0.72±0.10
6		8	1.14±0.21 <sup>a</sup>	1.10 <sup>ab</sup> ±0.16	0.94 <sup>ab</sup> ±0.20	0.92 <sup>ab</sup> ±0.18	0.85 <sup>b</sup> ±0.17	1.09 <sup>ab</sup> ±0.13	0.98 <sup>ab</sup> ±0.10

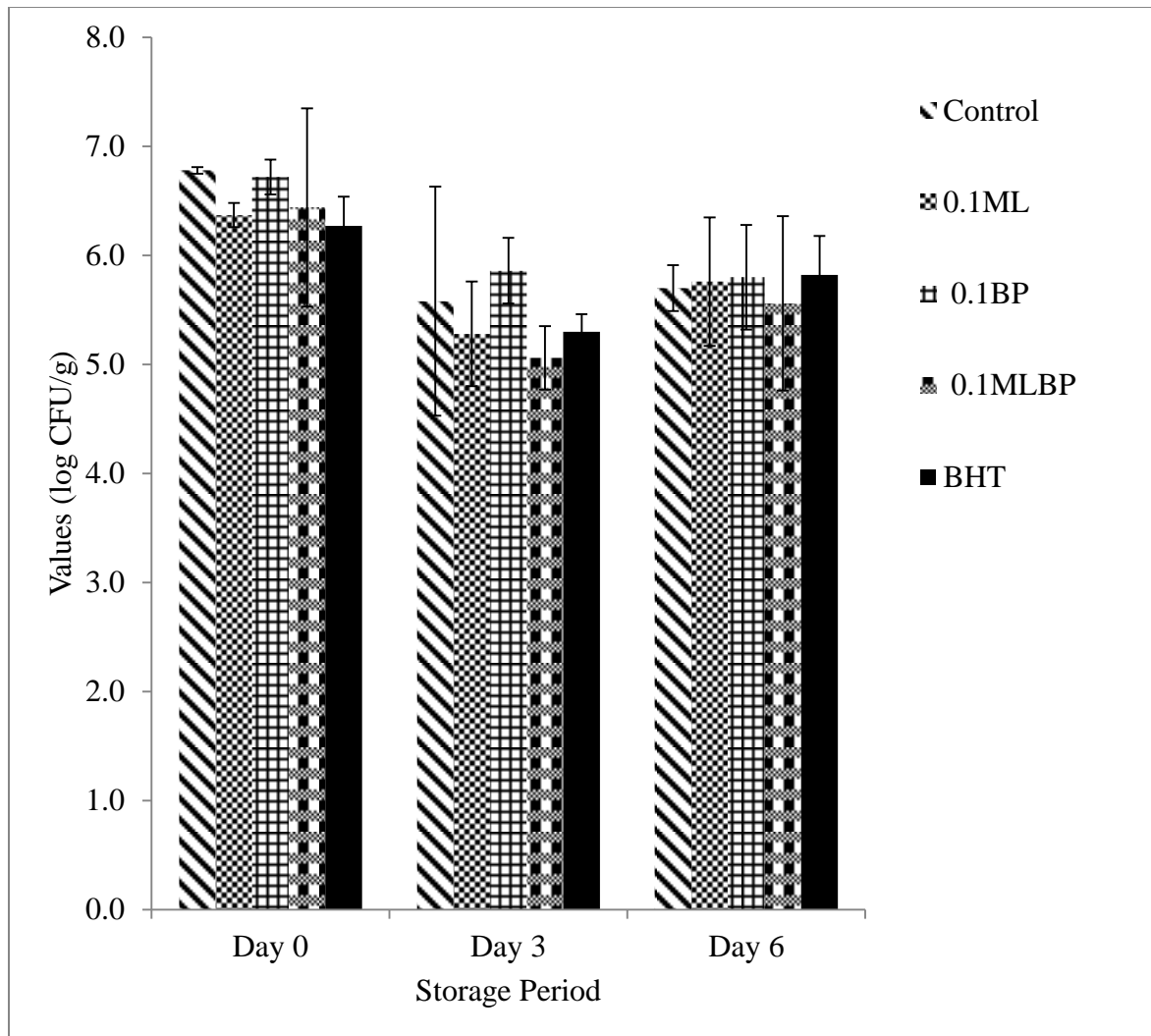
Mean with <sup>a-d</sup> superscripts within a row indicates significant differences (P ≤ 0.05).

Mean with <sup>A-B</sup> superscripts within a column indicates significant differences (P ≤ 0.05).

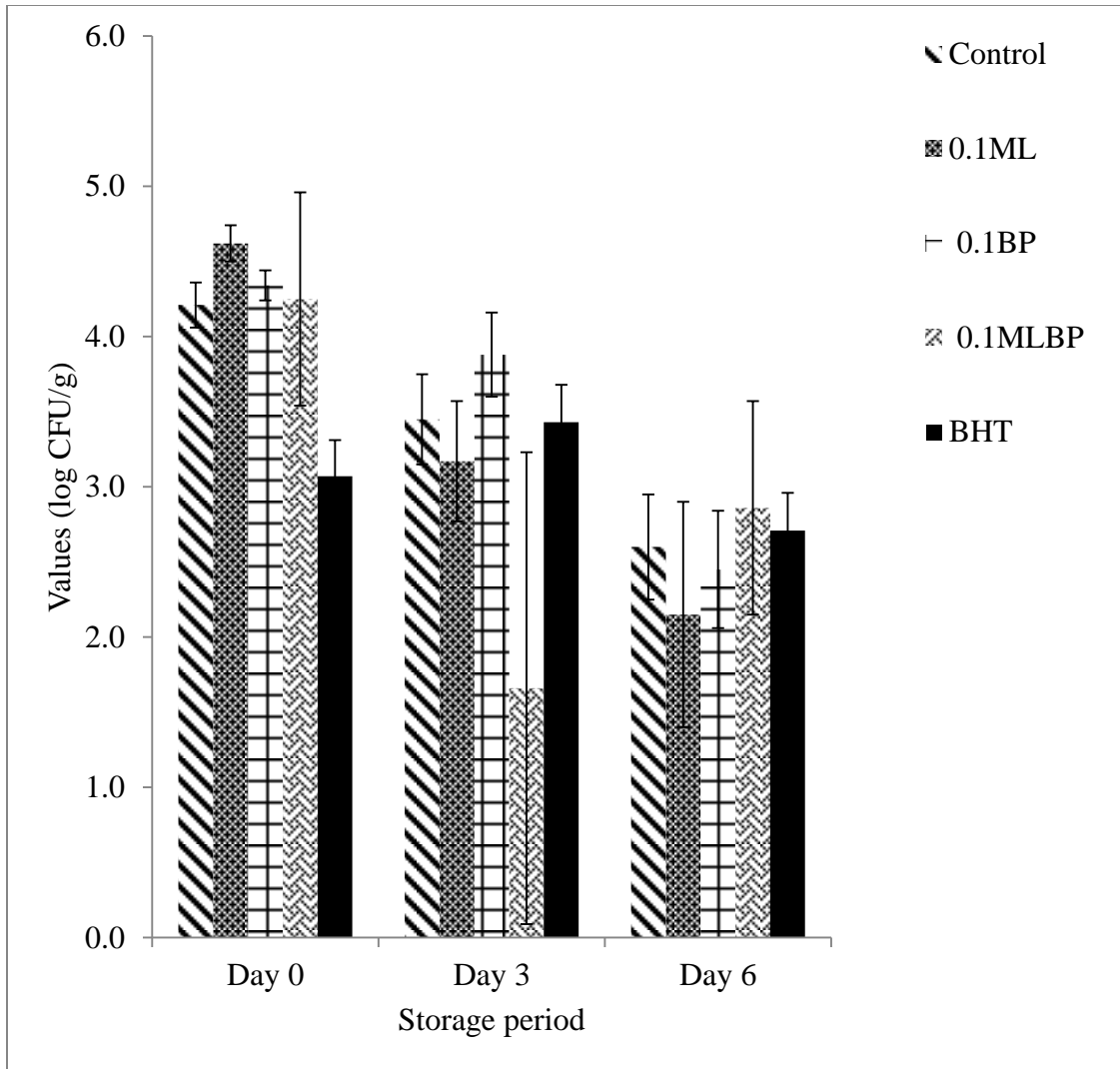
Control: no antioxidant, BHT: Butylated hydroxyl toluene, 0.05ML and 0.1ML: *Moringa oleifera* leaf extract, 0.05ML and 0.1ML: *Biden pilosa* leaf extracts. Each value was multiplied by 10.

### **5. 3. 3 Effect of *Moringa oleifera* and *Biden pilosa* leaf extracts on microbial quality of raw ground beef during storage at 4 °C**

Figure 5.1 and 5.2 shows the effect of plant extracts and storage time on microbial quality of raw ground beef. Total microbial counts decreased significantly ( $P < 0.05$ ) from day 0 to 3 in both control and treated meat samples. The initial total viable counts across the treatment ranges from  $6.27 \pm 0.37$  to  $6.78 \pm 0.32$  log CFU/g with the control having the highest value. However at day 3, the control group had the highest total bacterial counts of  $5.80 \pm 1.05$  log CFU/g, followed by BHT treatment ( $5.30 \pm 5.58$  log CFU/g) and least in beef containing MLBP extracts ( $5.06$  log CFU/g). Similar trend was also observed for lactic acid bacteria counts across the treatments. At day 0, the lactic acid bacteria count ranged from  $3.07 \pm 0.24$  to  $4.62 \pm 0.53$  log CFU/g. While at day 3, beef samples containing ML ( $3.17 \pm 0.43$  log CFU/g) and MLBP ( $1.66 \pm 0.24$  log CFU/g) extracts demonstrated lowest lactic acid bacteria counts compared to control. However, at day 6, both the total microbial and lactic acid bacterial counts were not significantly affected by the extracts compared to control and BHT treatments.



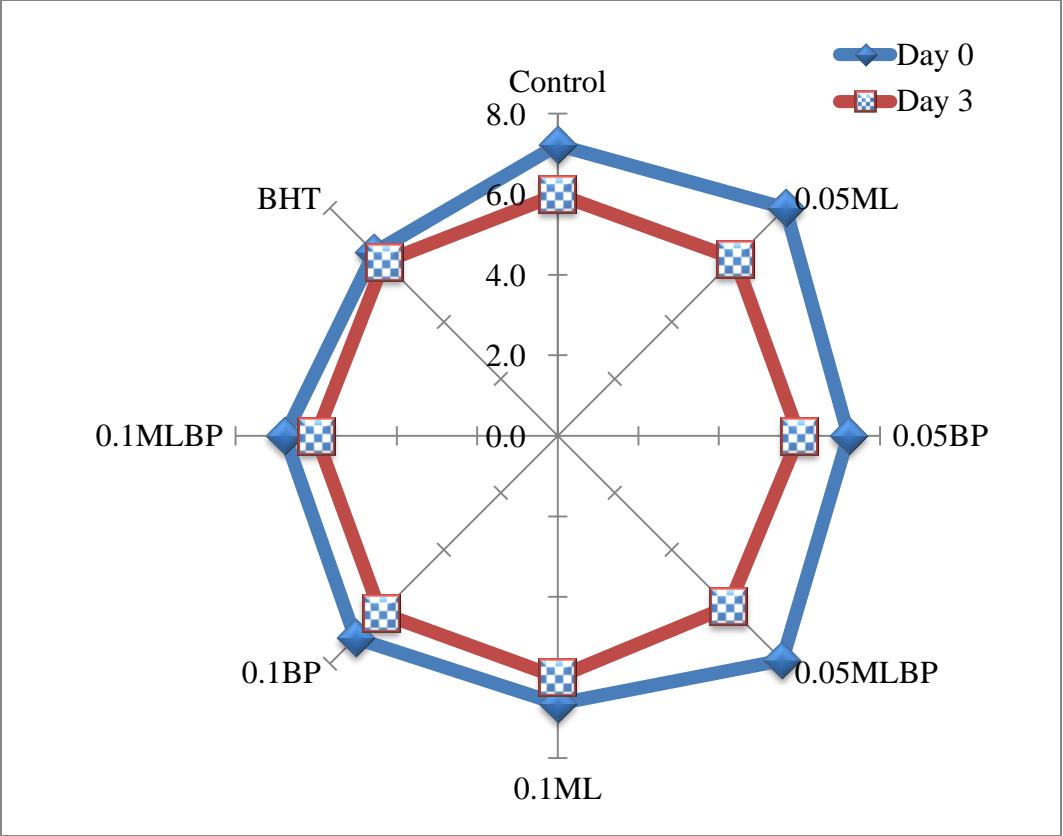
**Figure 5. 1 Effect of *Moringa oleifera* (ML) and *Biden pilosa* (BP) leaf extracts on total viable counts of ground beef during storage at 4 °C.**



**Figure 5. 2 Effect of *Moringa oleifera* (ML) and *Biden pilosa* (BP) leaf extracts on lactic acid bacteria counts of ground beef during storage at 4 °C.**

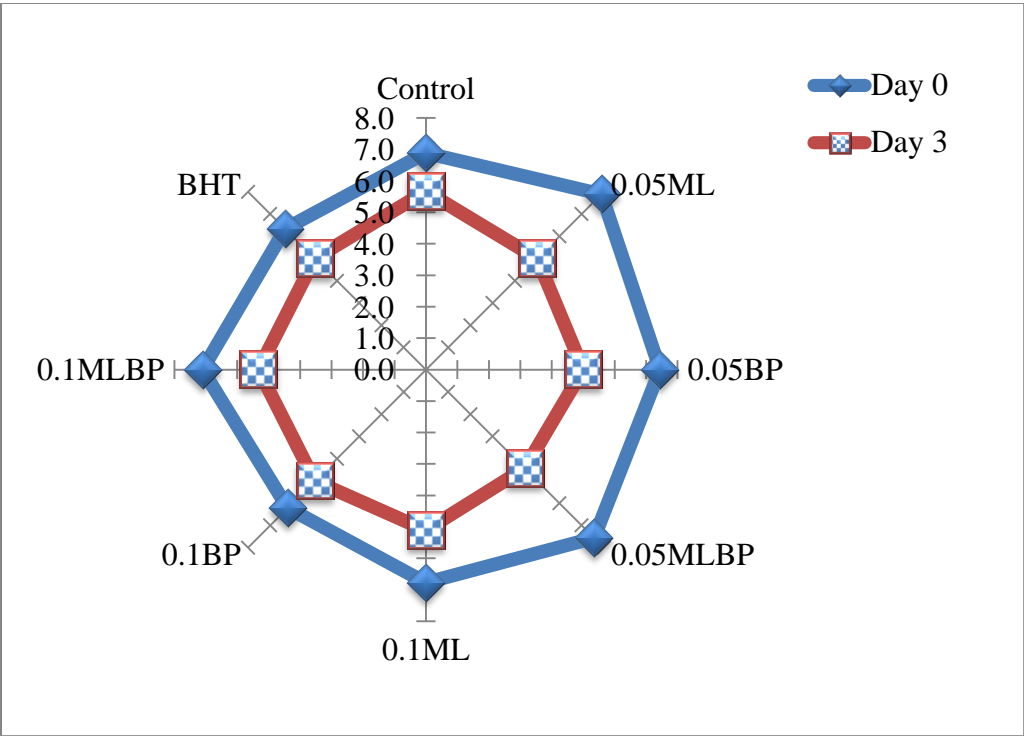
#### **5. 3. 4 Effect of *Moringa oleifera* and *Biden pilosa* leaf extracts on microbial quality and sensory evaluation of raw ground beef during storage at 4 °C**

The sensory scores assessed for odour and overall acceptances of ground beef treated without and with extracts on day 0 and 3 of storage are presented in Figure 5.3 and 5.4. At day 0 of storage, the results of the odour and overall acceptability scores were significantly ( $P < 0.05$ ) higher in ground beef treated with extracts at 0.05%w/w and well comparable with control at 0.1%w/w. However, at day 3 of storage, the odour and overall acceptability scores were not significantly different ( $P > 0.05$ ) across treatments.



**Figure 5. 3 Effect of *Moringa oleifera* (ML) and *Biden pilosa* (BP) leaf extracts on odour of ground beef during storage at 4 °C.**





**Figure 5. 4 Effect of *Moringa oleifera* (ML) and *Biden pilosa* (BP) leaf extracts on overall acceptability of ground beef during storage at 4 °C.**

## 5.4 Discussion

The gradual increase in pH values from day 0- 6 could be attributed to the utilization of amino acids by bacteria after the exhaustion of stored glucose during the protein break down. This consequently leads to formation and accumulation of ammonia that increases the pH values over the storage period (Gill, 1983; Krishnan et al., 2014; Shah et al., 2015). Shah et al. (2015) have also reported similar results with a progressive increase of pH values in raw beef treated with *M. oleifera* leaf extracts during the storage period. In general, ground beef samples from Bonsmara breed showed higher pH values than the non-descript beef samples. These differences could be explained by rate of utilization of amino acids by aerobic bacteria and formation of low-weight nitrogen molecules and ammonia compounds which has been reported to vary between breeds (Hernandez et al., 2004; Virgilia et al., 2007; Shah et al., 2015).

The hunter color values of the raw beef samples were slightly affected across treatments during the storage period. L-values increased steadily across treatments, with beef samples treated with extracts and BHT having the highest values at day 3. Similar trend in L\* values have been reported by Muthukumar et al. (2013) and Shah et al. (2015) for beef steaks and pork patties treated with *M. oleifera* leaf extracts.. However, from day 0 to 3, the raw ground beef containing plant extracts showed intense red color (a-value), higher than the control and BHT. Kim et al. (2013) and Liu et al. (2014) in their study also found higher a-values in fresh ground beef treated with natural antioxidants compared to control. Reduction in a\* values during storage has been associated with oxidation of myoglobin, formation of metmyoglobin and oxidation of lipid component of meat products (Mancini and Hunt, 2005; Shah et al., 2015). Krishnan et al. (2014) have reported the possibility of pigment oxidation to catalyze lipid oxidation and generate free

radicals which may oxidize iron atom or denature the myoglobin molecules, thereby causing decrease in meat color. Yellowness,  $b^*$  values varied significantly among the treatments and also with the storage period. At day 0 and 3, BHT and ML treated samples reflected the lowest  $b^*$  values while BP treated samples showed the highest value. Similarly, Muthukumar et al. (2012) and Shah et al. (2015) had reported a decrease in  $b^*$  values for raw pork and beef patties treated with MLE during storage. Moreso, Rojas and Brewer (2008) also found a decrease in  $b$ -values of beef patties containing natural antioxidants. However, fresh ground meat from Bonsmara breed exhibited higher colour stability, particularly redness, than the non-descript breed. This could be due to differences in their genotypic characteristic. Lynch et al. (2002) had earlier reported a significant difference in colour of beef from three breeds during storage period.

The results of the present study on TBARS show that adding *M. oleifera* and *B. pilosa* extracts can protect ground beef against lipid oxidation. The extracts were able to reduce the formation of TBARS in the beef samples when compared to control and synthetic antioxidants (BHT). The inhibitory effects of the extracts against the TBARS formation could be attributed to the inherent phenolic content, phytoconstituents and antioxidant activity (Chapter Four). Several studies have reported a positive correlation between phytochemical content or antioxidant activity of plant extracts and reduction in lipid oxidation in meat products (Jayathilakan et al., 2007; Devatkal et al., 2010). The antioxidant activity of phytochemical compounds in extracts have been associated with the hydroxyl group linked to the aromatic ring, which is capable of donating hydrogen atoms with electrons and neutralizing free radicals (Krishnan et al., 2014). However, the addition of *B. pilosa* extracts exhibited higher antioxidant activity at 0.05 and 0.1% than the *M. oleifera* extracts. Possible reasons for higher antioxidant activity of *B. pilosa* extracts on TBAS values

could be linked to inherent phytochemicals and the presence of antioxidant compound such as Phenol, 2, 2'-methylenebis [6- (1, 1-dimethylethyl)]-4-methyl (BHT) which is absent in *M. oleifera* extracts. Overall, the TBARS values of the Bonsmara beef were slightly lower than Non-descript beef samples across treatments and storage periods. Since both animals were raised on natural pasture, this difference in their TBARS values could be attributed to factors such as inherent endogenous antioxidants, and composition and distribution of unsaturated fatty acids in triacylglycerol molecule, which has been reported to influence the rate of lipid oxidation in muscle food (Wsowicz et al., 2004). This result is in agreement with the report of Xie et al. (2012) who found that Limousin beef samples had significant lower TBARS values than Qinchuan cattle breed.

The results further showed that the microbial population decreased significantly with addition of the natural extracts in raw ground meat during the storage days. This is in accordance with findings of Kim et al. (2013) who reported great reduction in microbial counts of raw beef patties treated with natural antioxidants. The antimicrobial activities of plant extracts have been linked to the presence of active compounds, although their mode of mechanism has not been fully understood (Kim et al., 2013; Krishnan et al., 2014). However, the observed gradual decrease in the bacteria counts with the increasing storage day in this study could be due to intrinsic factors such as high proteins and fat content coupled with some extrinsic factors (temperature and oxygen) which have been reported to affect the behavior of bacteria in food ecosystems and also act synergistically with preservatives such as antimicrobial agents (El Abed et al., 2014). Evidence has shown that food protein and fat contents can bind and/or solubilized phenolic compounds to reduce their availability for antimicrobial activity during storage (El Abed et al.,

2014). However, our results are in contrast with studies of Muthukumar et al. (2012) Kim et al. (2013) and Krishnan et al. (2014) who found a progressive increase in microbial counts of raw meat samples containing plant extracts as storage time (4<sup>0</sup>C) increased. Finally, the results of sensory score on odour and overall acceptability showed that inclusion of plant extracts can improve the sensory characteristics of meat (Velasco and William, 2011).

## **5.5 Conclusions**

This study has revealed that both *M. oleifera* and *B. pilosa* leaf extracts have substantial amounts of phytochemical compounds as well as phenolic acids with significant free radical scavenging activity. The application of the *M. oleifera* and *B. pilosa* leaf extracts at 0.05 and 0.1% concentration can delay the formation of lipid and pigment oxidation in meat products during refrigerated storage. It also showed that the antioxidant potential of *B. pilosa* is much greater than *M. oleifera*. Moreso, both *M. oleifera* and *B. pilosa* leaf extracts could be used as a potential source of antioxidants to replace synthetic antioxidants without causing any adverse effects on sensory attributes of meat products.

## 5.6 References

- Adedapo, A., Jimoh, F. and Afolayan, A.** 2011. Comparison of the nutritive value and biological activities of the acetone, methanol and water extracts of the leaves of *Bidens pilosa* and *Chenopodium album*. *Acta Poloniae Pharmaceutica Drug Research* **68 (1):** 83-92.
- Alikwe, P. C. N., Ohimain, E. I. and Omotosho, S. M.** 2014 Evaluation of the proximate, mineral, phytochemical and amino acid composition of *Bidens pilosa* as potential feed/feed additive for non-ruminant livestock. *Animal and Veterinary Sciences* **2 (2):** 18-21.
- Al-Owaisi, M., Al-Hadiwi, N. and Khan, S, A.** 2014. GC-MS analysis, determination of total phenolics, flavonoid content and free radical scavenging activities of various crude extracts of *Moringa peregrina* (Forssk.) Fiori leaves. *Asian Pacific Journal of Tropical Biomedicine* **4 (12):** 964-970.
- Bartolome, A. P., Villasenor, I. M. and Yang, W.** 2013. *Bidens pilosa* L. (Asteraceae): Botanical Properties, Traditional Uses, Phytochemistry, and Pharmacology. *Evidence-Based Complementary and Alternative Medicine* **2013:** 1-51.
- Banerjee, R., Verma, A. K., Das, A. K., Rajkumar, V., Shewalkar, A. A. and Narkhede, H. P.** 2012. Antioxidant effects of broccoli powder extract in goat meat nuggets. *Meat Science* **91:** 179–184.
- Brewer, M. S.** 2011. Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. *Comprehensive Reviews in Food Science and Food Safety* **10 (4):** 221–247.

- Dai, J. and Mumper, R. J.** 2010. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules* **15**: 7313–7352.
- de Oliveira, T. L. C., de Carvalhob, S. M., Soares, R. de., Andradeb, M. A., Cardosob, M. das., Ramos, E. M. and Piccolia, R. H.** 2012. Antioxidant effects of *Satureja montana* L. essential oil on TBARS and color of mortadella-type sausages formulated with different levels of sodium nitrite. *LWT - Food Science and Technology* **45**: 204-212.
- Devatkal, S. K. and Naveena, B. M.** 2010. Effect of salt and pomegranate fruit byproduct powders on colour and oxidative stability of raw ground goat meat during refrigerated storage. *Meat Science* **85**: 306–311.
- El Abed, E., Kaabi, B., Smaali, M. I., Chabbouh, M., Habibi, K., Mejri, M., Marzouki, M. N and Ahmed, S. B. H.** 2014. Chemical composition, antioxidant and antimicrobial activities of *Thymus capitata* essential oil with its preservative effect against *Listeria monocytogenes* inoculated in minced beef meat. *Evidence-Based Complementary and Alternative Medicine* **2014**: 1-11.
- Gill, C. O.** 1983. Meat spoilage and evaluation of the potential storage life of fresh meat. *Journal of Food Protection* **46**: 444– 452.
- Jayathilakan , K., Sharma, G.K., Radhakrishna, K. and Bawa, A.S.** 2007. Antioxidant potential of synthetic and natural antioxidants and its effect on warmed-over-flavour in different species of meat. *Food Chemistry* **105**: 908–916.
- Hazra, S., Biswas, S., Bhattacharyya, D., Das, S. K. and Khan, A.** 2012. Quality of cooked ground buffalo meat treated with the crude extracts of *Moringa oleifera* (Lam.) leaves. *Journal of Food Science and Technology* **49 (2)**: 240–245.

- Hernandez, P., Zomeno, L., Arinob, B. and Blasco, A.** 2004. Antioxidant, lipolytic and proteolytic enzyme activities in pork meat from different genotypes. *Meat Science* **66**: 525–529.
- Hygreeva, D., Pandey, M. C. and Radhakrishna, K.** 2014. Potential applications of plant based derivatives as fat replacers, antioxidants and antimicrobials in fresh and processed meat products. *Meat Science* **98**: 47–57.
- Karre, L., Lopez, K. and Getty, K. J.** 2013. Natural antioxidants in meat and poultry products. *Meat Science* **94** (2): 220-227.
- Kim, S., Cho, A.R. and Han, J.** 2013. Antioxidant and antimicrobial activities of leafy green vegetable extracts and their applications to meat product preservation. *Food Control*, **29**: 112–120.
- Krishnan, K. R., Babuskina, S., AzhaguSaravanaBabu, P., Sasikala, M., Sabina, K., Archana, G. Sivarajan, M. and Sukumar, M.** 2014. Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. *International Journal of Food Microbiology* **171**: 32–40.
- Lara, M. S., Gutierrez, J. I., Timon, M. and Andres, A. I.** 2011. Evaluation of two natural extracts (*Rosmarinus officinalis* L. and *Melissa officinalis* L.) as antioxidants in cooked pork patties packed in MAP. *Meat Science* **88**: 481–488.
- Liu, F., Xu, Q., Dai, R. and Ni, Y.** 2015. Effects of natural antioxidants on colour stability, lipid oxidation and metmyoglobin reducing activity in raw beef patties. *ACTA Scientiarum Polonorum Technologia Alimentaria* **14** (1): 37–44.



- Lynch, A., Buckley, D. J., Galvin, K., Mullen, A. M., Troy, D. J. and Kerry, J. P.** 2002. Evaluation of rib steak color from Friesian, Hereford and Charolais heifers pastured or overwintered prior to slaughter. *Meat Science* **61** (3): 227–232.
- Mancini, R. A. and Hunt, M. C.** 2005. Current research in meat colour. *Meat Science* **71**: 100–121.
- Martino, G., Mugnai, C., Compagnone, D., Grotta, L., Del Carlo, M. and Sarti, F.** 2014. Comparison of Performance, Meat Lipids and Oxidative Status of Pigs from Commercial Breed and Organic Crossbreed. *Animals* **4**: 348-360.
- Maqsood, S., Abushelaibi, A., Manheem, K., Al Rashedi, A. and Kadim, I T.** 2015. Lipid oxidation, protein degradation, microbial and sensorial quality of camel meat as influenced by phenolic compounds. *LWT - Food Science and Technology* **63**: 953-959
- Mccurdy, S.** 2009. Ground Beef: Safe Handling and Cooking. [www.foodsafetynews.com](http://www.foodsafetynews.com)  
Accessed on 18/05/2015.
- Moyo, B., Oyedemi, S., Masika, P. J. and Muchenje, V.** 2012a. Polyphenolic content and antioxidant properties of *Moringa oleifera* leaf extracts and enzymatic activity of liver from goats supplemented with *Moringa oleifera* leaves/sunflower seed cake. *Meat Science* **91**: 441–447.
- Moyo, B., Masika, P. J., Hugo, A. and Muchenje, V.** 2012b. Nutritional characterization of *Moringa (Moringa oleifera Lam.)* leaves. *African Journal of Biotechnology* **10** (60): 12925-12933.
- Muthukumar, M., Naveena, B. M., Vaithyanathan, S., Sen, A. R., and Sureshkumar, K.** 2012. Effect of incorporation of *Moringa oleifera* leaves extract on quality of ground pork patties. *Journal of Food Science and Technology* **51** (11): 3172-3180.

- Nam, K. C., Seo, K. S., Jo, C. and Ahn, D. U.** 2014. Electrostatic spraying of antioxidants on the oxidative quality of ground beef. *Journal of Animal Science* **89 (3)**:826-832.
- Raharjo, S., Sofos, J. N. S. and Schmidt, G. R.** 1992. Improved speed specificity, and limit of determination of an aqueous acid extraction thiobarbituric acid-C<sub>18</sub> method for measuring lipid peroxidation in beef. *Journal of Agriculture and Food Chemistry* **40**: 2182-2185.
- Rojas, M. C. and Brewer, M. S.** 2008. Effect of natural antioxidants on oxidative stability of frozen, vacuum-packaged beef and pork. *Journal of Food Quality* **31 (2)**: 173–188.
- Saad, B., Yong, Y.S., Mohd, A.N., Noorhasani, H., Abdussalam, S.M.A., Muhammad, I.S., Saida, F.S., Khariuddin, M.T. and Kamarudzaman, A.** 2007. Determination of synthetic phenolic antioxidants in food items using reversed-phase HPLC. *Food Chemistry* **105**:389–394.
- Shah, M. A., Don Bosco, S. J and Mir, S. A.** 2015. Effect of *Moringa oleifera* leaf extract on the physicochemical properties of modified atmosphere packaged raw beef. *Food packaging and shelf life* **3**: 31 – 38.
- Soladoye, O. P., Juárez, M. L., Aalhus, J. L., Shand, P. and Estévez, M.** 2015. Protein Oxidation in Processed Meat: Mechanisms and Potential Implications on Human Health. *Comprehensive Reviews in Food Science and Food Safety* **14 (2)**: 106–122
- Statistical Analysis System (SAS), 2007. In: SAS/STAT Guide to Personal Computers, Version 9.1.3, Statistical Analysis System Institute Inc. Cary, NC”.**
- Velasco, V. and Williams, P.** 2011. Improving meat quality through natural antioxidants. *Chilean Journal of Agricultural Research* **71**: 2.

- Virgilia, R., Saccania, R., Gabbaa, L., Tanzia, E., and Soresi Bordini, C.** 2007. Changes of free amino acids and biogenic amines during extended ageing of Italian dry-cured ham. *LWT-Food Science and Technology* **40**: 871–878.
- Wslowicz, E., Gramza, A., Heoe, M., Jeleñ, H. H., Korczak, J., Maecka, M., Mildner-Szkudlarz, S., Rudzińska, M., Samotyja, U. and Zawirska-Wojtasiak, R.** 2004. Oxidation of lipids in food. *Polish journal of food and nutrition sciences* **13/54 (1)**: 87–100.
- Xie, X., Meng, Q., Cui, Z. and Ren, L.** 2012. Effect of Cattle Breed on Meat Quality, Muscle Fiber Characteristics, Lipid Oxidation and Fatty Acids in China. *Asian-Australas Journal Animal Science* **25 (6)**: 824–831.

## **Chapter 6: Effect of thermal treatment on fatty acids, minerals and tenderness of beef and liver from Bonsmara and non-descript cattle**

*(Submitted to Journal of Food Composition and Analysis)*

### **Abstract**

The objective of this study was to compare the effect of thermal treatment on fatty acids, minerals and tenderness of beef (*Muscularis longissimus thoracis et lumborum*) and liver muscle from Bonsmara (n=40) and non-descript (n=40) cattle raised on natural pasture. Beef and liver samples were thermal-processed at 65<sup>0</sup>C and 85<sup>0</sup>C for 120 and 60 minutes, respectively, using sous vide cooking techniques. The results did not reveal any significant breed effect (P > 0.05) on fatty acid composition of raw beef and liver muscles. However, higher concentrations of monounsaturated fatty acids (MUFA) and lower concentrations of polyunsaturated fatty acids (PUFA) were observed in raw beef than liver sample. The liver samples from Bonsmara and non-descript cattle had a higher percentage of intramuscular fat content of 4.67 ± 0.53% and 4.44 ± 0.53% respectively, and fat free dry matter of 27.51 ± 1.05% and 25.73 ± 1.05%, respectively, than the beef samples (P < 0.05). The application sous vide thermal method significantly increased (P < 0.05) the total intramuscular fat and FFD contents of cooked beef and liver compared to raw meat. Beef and liver from Bonsmara showed higher (P < 0.05) Warner-Bratzler Shear Force (WBSF) values at 65<sup>0</sup>C and 85<sup>0</sup>C cooking temperatures than those from non-descript samples. The total percentage of saturated fatty acid (SFA), MUFA, PUFA, n-6, n-3 retained after cooking was higher in liver than beef samples (P > 0.05). The results further revealed higher contents of Na, Mg, Zn and Se in raw and cooked beef than liver samples. In general all beef samples from Bonsmara cattle exhibited higher mineral contents than those from the non-descript cattle (P < 0.05).

It was concluded that application of sous vide thermal method could be used to minimize the amount of nutrient loss in beef and liver samples during cooking.

**Key words:** Beef, fatty acids, liver, minerals, sous vide technique, tenderness

## 6.1 Introduction

In the meat industry, the oxidation stability of lipids, protein and minerals plays an important role in ensuring the nutritional, technological and sensory qualities of meat during thermal treatments. Generally, oxidation of lipid is believed to occur during cold storage (Chapter 5) and also thermal treatment. Oxidation of lipid fatty acids and minerals during thermal treatment has a profound impact on organoleptic attributes of meat products (Legako et al., 2015). Studies have shown that thermal treatment can induce free radical production and loss of essential fatty acids through lipid oxidation and other nutritive values in meat products (Rodriguez-Estrada et al., 1997; Alfaia et al., 2010). It has also been reported that thermal oxidation can cause the dimerization of lipid degradation products and decomposition of saturated fatty acids (SFA) to produce different volatile compounds, such as saturated and unsaturated aldehydes and ketones in muscle foods (Mottram, 1998; Legako et al., 2015). Particularly, polyunsaturated fatty acids (PUFA) have been found to be more susceptible to oxidation than monounsaturated (MUFA) and saturated fatty acids due to their low melting point during thermal treatments (Alfaia et al., 2010; Legako et al., 2015).

Any loss of MUFA and PUFA (omega-3 and omega-6) through lipid oxidation during thermal treatment will reduce the nutritional quality and biological benefit of meat products (Alfaia et al., 2010). Studies have shown that the dietary intake of MUFA and PUFA (omega-3 and omega-6) in right proportions help to reduce the risk of atherosclerosis, diabetes, obesity, hypertension, cancer and improve immunomodulation (Chin and Dart 1995; Stephen et al., 2010; Nantapo et al., 2015). Equally, the mineral content of meat has been considered important for both health and regulation of several enzymes in the human body (Hosseini et al., 2014). The loss of macro

and micro minerals during thermal processing has been reported to cause changes in muscle structure, which decreases the water holding capacity, sensory and nutritional quality of meat (Gerber et al., 2009; Czerwonka and Szterk, 2015; Lopes et al., 2015). Thus, the nutritional value of meat lies in its content and bioavailability of essential macro and micro elements, especially after cooking (Lopes et al., 2015).

In order to minimize the nutrient loss while optimizing palatability and shelf life of meat during thermal treatment, many food processors now use sous-vide cooking technique to replace the conventional methods such as frying, microwaving and grilling (Sanchez del Pulgar et al., 2012; Roldan et al., 2014). Most of these conventional cooking methods have been demonstrated to significantly decrease the concentration of PUFA, omega-3 and omega-6 and minerals in cooked meat (Alfaia et al., 2010; Lopes et al., 2015). Sous-vide technique refers to process of cooking meat in vacuum-sealed pouches inside temperature-controlled water environment for long periods of time (Roldan et al., 2014). This method allows heat to be efficiently transferred from the water (or steam) to the meat sample. It also allows greater control over degree of doneness compared to other cooking methods (Baldwin, 2012). However, there is paucity of information on the effect of sous vide cooking method on the nutritional composition of beef and liver in terms of the fatty acid and mineral composition. Beef is a potential source of high quality proteins, lipid (fatty acids), vitamins, minerals and other bioactive compounds which are absent in vegetables and other sources (Sanchez-Ortega et al., 2014). Also, liver meat has been reported to contain higher micro nutrients than other corresponding muscles (Jayathilakan et al., 2012; Li et al., 2014). Studies have shown that liver is traditionally consumed in a number of countries around the world especially in South East Asia, Australia and Africa (Fatma and Mahdey, 2010).

In some cookery, liver meat is considered as primal food that has health benefits and is consumed by people of all ages (Fayemi and Muchenje, 2014). It has been established that factors such as muscle type, breed, age, diet, production region and species of animal can greatly influence the fatty acid and mineral composition of raw meat including liver (Lopes et al., 2015). Therefore, the aim of this study was to evaluate the effect of sous vide cooking on fatty acid composition of beef and liver from Bonsmara and non-descript cattle.

## **6.2 Materials and Methods**

### **6.2.1 Experimental animals and sample preparation**

A total of 80 cows from two breeds [(Bonsmara (n = 40) and non-descripts (n = 40)] with final live body weight of 450 to 500kg and an average age of 4-5 years were used in this study. All the animals were reared on natural pasture and slaughtered at a high throughput commercial abattoir in the Eastern Cape Province of South Africa. Animals were slaughtered by exsanguination after stunning with a captive bolt stunner at voltage of 300 V and a current of 5A. A representative sample (100-250g) of *Muscularis longissimus thoracis et lumborum* was excised from each carcass and processed after 48 hours *post mortem*. Liver samples from each animal were also collected to determine the cooking loss, Warner Brazlter Shear Force (WBSF), fatty acid profile and mineral composition. Meat samples were thermal processed at temperatures of 65<sup>0</sup>C (medium well) and 85<sup>0</sup>C (well done) for 120 and 60 minutes, respectively, using sous vide method (Vaudagna et al., 2002; Garcia-Segovia et al., 2007; Alfaia et al., 2010). Prior to cooking, about 40g of meat samples were weighed into a vacuum plastic bag and sealed using vacuum sealer (Genesis, 80-GVS, South Africa). Thereafter, the meat samples were submerged in thermostated water baths that had been preheated to 65<sup>0</sup>C and 85<sup>0</sup>C and maintained within the water bath for 120 and 60 minutes, respectively. After cooking, samples were removed from



the water bath, submerged in cold water for 10 minutes (4<sup>0</sup>C) and cooled at room temperature. The meat samples (beef and liver) for fatty acid and mineral determination were vacuum packed immediately after cooling and stored at -23<sup>0</sup>C until analysis.

### **6.2.2 Determination of cooking loss and Warner–Bratzler (WB) shear force on beef and liver samples**

Immediately after cooking, the beef samples (n=40) and liver samples (n=40) from each breed were removed from the vacuum bags and allowed to cool for an average of 10 minutes at room temperature to determine the cooking loss and tenderness. Cooking loss was evaluated as differences in the weight of raw and cooked meat samples. Warner–Bratzler Shear Force (WBSF) values were determined on meat samples as indicators of tenderness using a Universal Instron apparatus. Three specimen measuring 10mm core diameter were cored from each cooked meat sample after cooling and sheared perpendicularly to the muscle fibre orientation at a constant speed of 400mm/ minutes (one shear in the center of each core). The maximum force (N) required to shear for each specimen was measured and the mean was recorded for tenderness.

### **6.2.3 Determination of fatty acid profile of raw and cooked beef and liver samples**

Total lipid of the fresh and cooked beef (n=4) and liver (n=4) samples from each breed was quantitatively extracted as described by Folch et al. (1957), using chloroform and methanol in a ratio of 2:1. An antioxidant (butylated hydroxytoluene, BHT) was added at a concentration of 0.001 % to the chloroform: methanol mixture. The fat extracts were dried in a rotary evaporator under vacuum and the extracts were dried overnight in a vacuum oven at 50<sup>0</sup>C, using phosphorus pentoxide as moisture absorbent. Total extractable fat was determined gravimetrically from the extracted fat and expressed as percent fat (w/w) per 100g tissue.

Thereafter, the extracted fat muscle was stored in a polytop (glass vial, with push-in top) under a blanket of nitrogen and frozen at  $-20^{\circ}\text{C}$  for analysis of fatty acids. An aliquot (30mg) of muscle lipid were converted to methyl esters by base-catalysed transesterification, in order to avoid CLA isomerisation, with sodium methoxide (0.5 M solution in anhydrous methanol) during 2 h at  $30^{\circ}\text{C}$ , as described by Alfaia et al. (2007). Fatty acid methyl esters (FAMES) from muscle were quantified using a Varian 430 flame ionization GC, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2  $\mu\text{m}$  film thicknesses). The analysis was performed using an initial isothermic period ( $40^{\circ}\text{C}$  for 2 minutes) and gradually increased at a rate of  $4^{\circ}\text{C}/\text{minute}$  until a final temperature of  $230^{\circ}\text{C}$  was reached. Finally the isothermic period of  $230^{\circ}\text{C}$  was maintained for 10 minutes. FAMES n-hexane (1 $\mu\text{l}$ ) was injected into the column using a Varian CP 8400 Autosampler. The injection port and detector were both maintained at  $250^{\circ}\text{C}$ . Hydrogen, at 45 psi, was used as the carrier gas, while nitrogen was employed as the makeup gas. Galaxy Chromatography Data System Software recorded the chromatograms.

Fatty acid methyl ester samples were identified (Table 6.1) by comparing the retention times of FAME peaks from samples with those of standards obtained from Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich Aston Manor, Pretoria, South Africa). Conjugated linoleic acid (CLA) standards were obtained from Matreya Inc. (Pleasant Gap, Unites States). These standards included: cis-9, trans-11 and trans-10, cis-12-18:2 isomers. Nonadecanoic acid (C19:0) (SIGMA N553377 – 1G) was used as the internal standard to improve quantitative FAME estimation. Fatty acids were expressed as the proportion of each individual fatty acid to the total of all fatty acids present in the sample. Fatty acid data were used to calculate the following ratios of FAs: total SFAs; total MUFAs; total PUFAs; PUFA/SFA;  $\Delta^9$

desaturase index (C18:1c9/C18:0); total omega-6; total omega-3; the ratio of omega-6 to omega-3 ( $n-6/n-3$ ). Atherogenicity index (AI) was calculated as  $(C12:0 + 4 \times C14:0 + C16:0)/(MUFA + PUFA)$  (Chilliard et al., 2003).

#### **6.2.4 Determination of mineral constituents of raw and cooked beef and liver samples**

The elemental constituents of potassium (K), sodium (Na), magnesium (Mg), calcium (Ca), Zinc (Zn) and selenium (Se) in beef (n=4) and liver (n=4) samples were determined after dry ashing mineralisation, as described by Gorsuch (1970) and Tomovic et al. (2015). Five gram of meat sample was weighed into a porcelain crucible and dried in a laboratory oven at 105<sup>0</sup>C for 3h. After drying, the samples were incinerated in a muffle furnace at 550<sup>0</sup>C overnight until the white ash formed. The ash was solubilized with 20 ml of HNO<sub>3</sub> (0.5 N) in an Erlenmeyer flask and heated to reduce the volume to 5ml. The solution was then filtered through ash-free filter paper into a 50-mL volumetric flask. Each sample solution was made up with dilute HNO<sub>3</sub> (0.5 N) to a final volume of 50 mL and analyzed by flame atomic absorption spectrometry.

#### **6.2.5 Statistical analysis**

The Statistical Analysis System (SAS version 9.1.3 of 2007) was used for all the analyses. PROC GLM procedure of SAS was used to consider the effect of breed and sous vide cooking temperature on the fatty acid profiles, mineral content, cooking loss and Warner Bratzler Shear Force (WBSF) values of beef and liver. Significant differences between the least square means were performed using the Fishers' least significance difference (LSD) method of SAS, with significance level of  $P < 0.05$ .

**Table 6. 1 Fatty acid names and Abbreviations**

Common name	Abbreviation	
Saturated fatty acid (SFA)		
Lauric	C12:0	C12:0
Myristic	C14:0	C14:0
Pentadecylic	C15:0	C15:0
Palmitic	C16:0	C16:0
Margaric	C17:0	C17:0
Stearic acid	C18:0	C18:0
Arachidic	C20:0	C20:0
Behenic	C22:0	C22:0
Tricosanoic	C23:0	C23:0
Monounsaturated fatty acids (MUFA)		
Myristoleic	C14:1c9	C14:1
Palmitoleic	C16:1c9	C16:1
Heptadecenoic	C17:1c10	C17:1
Vaccenic	C18:1c7	C18:1 (n-7)
Elaidic	C18:1t9	C18:1 (n-9t)
Oleic	C18:1c9	C18:1 (n-9c)
Polyunsaturated fatty acids (PUFA)		
Conjugated linoleic acid (CLA)	C18:2c9,t11 (n-6) (CLA)	C18:2 CLA
Linoleic	C18:2c9,12 (n-6)	C18:2 (n-6)
$\gamma$ -Linolenic	C18:3c6,9,12 (n-3)	C18:3 (n-6)
$\alpha$ -Linolenic	C18:3c9,12,15 (n-3)	C18:3 (n-3)
Eicosadienoic	C20:2c11,14 (n-6)	C20:2 (n-6)
Eicosatrienoic	C20:3c8,11,14 (n-6)	C20:3 (n-6)
Eicosatrienoic	C20:3c11,14,17 (n-3)	C20:3 (n-3)
Arachidonic	C20:4c5,8,11,14 (n-6)	C20:4 (n-6)
Eicosopentaenoic	C20:5c5,8,11,14,17 (n-3)	C20:5 (n-3)
Docosapentaenoic	C22:5c7,10,13,16,19 (n-3)	C22:5 (n-3)
Docosahexanoic	C22:6c4,7,10,13,16,19 (n-3)	C22:6 (n-3)

## **6.3 Results**

### **6.3.1 Effect of thermal treatment on cooking loss and Warner Bratzler Shear Force**

#### **(WBSF) of beef and liver muscles**

Results of thermal treatment on cooking loss and Warner Bratzler Shear Force (WBSF) of beef and liver from non-descript and Bonsmara cattle are presented in Table 6.2 and 6.3. The results revealed a significant increase ( $P < 0.05$ ) in cooking loss as the temperature increased from 65<sup>0</sup>C to 85<sup>0</sup>C. Beef and liver samples from Bonsmara cattle had higher cooking loss (beef, 42.64% and liver, 34.70%) at 85<sup>0</sup>C than those from non-descript breed. However, at 65<sup>0</sup>C cooking temperature, liver sample from non-descript cattle exhibited lower cooking loss value (21.19%) while the beef samples had higher cooking loss value (31.57%) than those from Bonsmara breed. Furthermore, beef and liver samples from non-descript cattle showed higher WBSF values than those from Bonsmara cattle ( $P < 0.05$ ). At 85<sup>0</sup>C cooking temperature, liver samples exhibited higher WBSF values and beef samples had lower WBSF values.

### **6.3.2 Proximate composition of raw and cooked beef and liver muscles**

Results on proximate analysis of raw beef and liver from cattle are presented on Table 6.4. The results revealed no statistical differences in intramuscular fat, fat free dry matter and moisture contents in liver and beef between the breeds. However, liver samples from Bonsmara and non-descript cattle had a higher percentage of intramuscular fat content of  $4.67 \pm 0.53\%$  and  $4.44 \pm 0.53\%$  respectively, and fat free dry matter of  $27.51 \pm 1.05\%$  and  $25.73 \pm 1.05\%$ , respectively, than the beef samples ( $P < 0.05$ ). Moreover, the moisture contents of the liver samples were much lower than that of beef samples. Similar trend was also observed in the cooked samples with

liver having higher intramuscular fat and fat free dry matter than beef samples (Table 6.5). Application of thermal treatment on beef and liver samples at 85<sup>0</sup>C revealed a higher intramuscular fat and fat free dry matter contents and lower moisture content than those cooked at 65<sup>0</sup>C.

### **6.3.3 Fatty acid composition of raw beef and liver muscles**

Table 6.5 to 6.8 showed the results of the fatty acid composition of raw beef and liver. There was no significant difference in fatty acid composition of raw beef and liver between the breeds ( $P > 0.05$ ). However, among individual saturated fatty acid (SFA), beef samples revealed higher content of C12:0, C15:0 C16:0 and C20:0 and lower contents of C14:0, C17:0 and C18:0 than liver samples ( $P < 0.05$ ) (Table 6.5). Similarly, the individual composition of monounsaturated fatty acids (MUFA) showed that beef had higher contents of C14:1, C17:1, C18:1 (n-7), C18:1 (n-9t) and C18:1 (n-9c) than liver sample (Table 6.6). While the polyunsaturated fatty acid (PUFA) profile of liver revealed higher contents of C18:2 (n-6), C18:3 (n-3), C20:4 (n-6), C20:5 (n-3) and C22:5 (n-3) than beef samples (Table 6.7). In general, the total MUFA values were relatively lower in liver ( $21.08 \pm 1.23\%$ ) than in beef ( $42.53 \pm 1.20\%$ ), whereas PUFA values were higher in liver ( $31.11 \pm 1.19\%$ ) than in beef ( $11.02 \pm 2.74\%$ ). However, the contents of SFA in beef did not differ significantly from the liver samples. Furthermore, it was observed that the values of total n-6, n-3 and PUFA: SFA were higher in liver than in beef muscles. While the values of PUFA/MUFA, n-6/n-3, atherogenicity Index (AI) and desaturase Index (DI) were lower in liver than beef muscles (Table 6.9).

**Table 6. 2 Effect of thermal treatment on cooking loss of beef and liver from Bonsmara and non-descript cattle.**

Meat type	Breed	Temperature		SEM
		65 <sup>0</sup> C	85 <sup>0</sup> C	
<b>Beef</b>	Non-descripts	31.57 <sup>bA</sup>	38.90 <sup>aA</sup>	0.58
	Bonsmara	28.78 <sup>bA</sup>	42.64 <sup>aA</sup>	0.58
<b>Liver</b>	Non-descripts	21.19 <sup>bB</sup>	34.19 <sup>aB</sup>	1.86
	Bonsmara	25.33 <sup>bA</sup>	34.70 <sup>aA</sup>	1.86

**Non-descript (n=40), Bonsmara (n= 40), mean with <sup>a-b</sup> superscripts within a row indicates significant differences between temperature (P ≤ 0.05), Mean with <sup>A-B</sup> superscripts within a column indicates significant differences between breed (P ≤ 0.05).**

**Table 6. 3 Effect of thermal treatment on tenderness (N) of beef and liver from Bonsmara and non-descript cattle.**

Meat type	Breed	Temperature		SEM
		65 <sup>0</sup> C	85 <sup>0</sup> C	
<b>Beef</b>	Non-descripts	49.45 <sup>Aa</sup>	46.75 <sup>aA</sup>	2.22
	Bonsmara	44.29 <sup>bA</sup>	43.26 <sup>bA</sup>	2.22
<b>Liver</b>	Non-descripts	9.59 <sup>b</sup>	13.34 <sup>a</sup>	0.63
	Bonsmara	8.84 <sup>b</sup>	12.98 <sup>a</sup>	0.63

**Non-descript (n=40), Bonsmara (n= 40), mean with <sup>a-b</sup> superscripts within a row indicates significant differences between temperatures (P ≤ 0.05).**



**Table 6. 4 Proximate composition of raw beef and liver muscles.**

Selected nutrient	Breed	Beef	Liver
Intramuscular fat %	Non-descript	2.04 <sup>b</sup> ± 0.47	4.44 <sup>a</sup> ± 0.53
	Bonsmara	2.40 <sup>b</sup> ± 0.47	4.67 <sup>a</sup> ± 0.53
Fat free dry matter %	Non-descript	22.82 <sup>b</sup> ± 0.44	25.73 <sup>a</sup> ± 1.05
	Bonsmara	22.14 <sup>b</sup> ± 0.44	27.51 <sup>a</sup> ± 1.05
Moisture %	Non-descript	75.14 <sup>a</sup> ± 0.75	69.83 <sup>b</sup> ± 1.19
	Bonsmara	75.45 <sup>a</sup> ± 0.75	67.82 <sup>b</sup> ± 1.19

**Non-descript (n=4), Bonsmara (n= 4), mean with <sup>a-b</sup> superscripts within a row indicate significant differences (P ≤ 0.05).**

**Table 6. 5 Proximate composition of cooked beef and liver samples.**

Meat type	Selected nutrient	Breed	65 <sup>0</sup> C	85 <sup>0</sup> C
Beef	Intramuscular fat %	Non-descript	3.63 <sup>a</sup> ± 0.57	2.87 <sup>a</sup> ± 0.69
		Bonsmara	3.63 <sup>a</sup> ± 0.57	4.41 <sup>a</sup> ± 0.69
	Fat free dry matter %	Non-descript	30.11 <sup>b</sup> ± 1.43	36.25 <sup>a</sup> ± 0.79
		Bonsmara	26.97 <sup>b</sup> ± 1.43	37.12 <sup>a</sup> ± 0.79
	Moisture %	Non-descript	66.25 <sup>a</sup> ± 1.23	60.89 <sup>b</sup> ± 0.64
		Bonsmara	69.39 <sup>a</sup> ± 1.23	58.48 <sup>b</sup> ± 0.64
Liver	Intramuscular fat %	Non-descript	5.09 <sup>a</sup> ± 0.23	2.87 <sup>a</sup> ± 0.69
		Bonsmara	5.17 <sup>a</sup> ± 0.23	4.41 <sup>a</sup> ± 0.69
	Fat free dry matter %	Non-descript	26.76 <sup>b</sup> ± 0.99	36.25 <sup>a</sup> ± 0.79
		Bonsmara	25.96 <sup>b</sup> ± 0.99	37.12 <sup>a</sup> ± 0.79
	Moisture %	Non-descript	68.14 <sup>a</sup> ± 1.09	62.43 <sup>b</sup> ± 0.84
		Bonsmara	68.88 <sup>a</sup> ± 1.09	60.41 <sup>b</sup> ± 0.73

**Non-descript (n=4), Bonsmara (n= 4), mean with <sup>a-b</sup> superscripts within a row indicates significant differences (P ≤ 0.05).**

**Table 6. 6 Total saturated fatty acid (SFA) composition of raw beef and liver muscles.**

Parameter	Breed	Beef	Liver
C12:0	Non-descript	0.02 <sup>b</sup> ± 0.01	0.00 <sup>a</sup> ± 0.00
	Bonsmara	0.02 <sup>b</sup> ± 0.01	0.00 <sup>a</sup> ± 0.00
C14:0	Non-descript	2.48 <sup>a</sup> ± 0.52	0.32 <sup>b</sup> ± 0.09
	Bonsmara	2.49 <sup>a</sup> ± 0.52	0.53 <sup>b</sup> ± 0.09
C15:0	Non-descript	0.37 <sup>a</sup> ± 0.02	0.26 <sup>b</sup> ± 0.03
	Bonsmara	0.29 <sup>a</sup> ± 0.02	0.34 <sup>b</sup> ± 0.03
C16:0	Non-descript	26.50 <sup>a</sup> ± 2.02	15.14 <sup>b</sup> ± 0.99
	Bonsmara	27.75 <sup>a</sup> ± 2.02	16.57 <sup>b</sup> ± 0.99
C17:0	Non-descript	1.05 <sup>a</sup> ± 0.04	1.08 <sup>a</sup> ± 0.05
	Bonsmara	0.80 <sup>b</sup> ± 0.04	1.15 <sup>a</sup> ± 0.05
C18:0	Non-descript	18.29 <sup>b</sup> ± 0.64	31.31 <sup>a</sup> ± 1.16
	Bonsmara	15.92 <sup>b</sup> ± 0.64	28.97 <sup>a</sup> ± 1.16
C20:0	Non-descript	0.09 <sup>a</sup> ± 0.02	0.02 <sup>b</sup> ± 0.02
	Bonsmara	0.08 <sup>a</sup> ± 0.02	0.05 <sup>a</sup> ± 0.02
C22:0	Non-descript	0.00 <sup>a</sup> ± 0.00	0.09 <sup>b</sup> ± 0.02
	Bonsmara	0.00 <sup>a</sup> ± 0.00	0.02 <sup>b</sup> ± 0.02
C23:0	Non-descript	0.00 <sup>a</sup> ± 0.00	0.20 <sup>b</sup> ± 0.02
	Bonsmara	0.00 <sup>a</sup> ± 0.00	0.20 <sup>b</sup> ± 0.02
Total SFA	Non-descript	48.76 ± 2.49	49.17 ± 0.33
	Bonsmara	47.35 ± 2.94	47.82 ± 0.19

**Non-descript (n=4), Bonsmara (n= 4), mean with <sup>a-b</sup> superscripts within a row indicates significant differences (p ≤ 0.05)**

**Table 6.7 Total monounsaturated fatty acid (MUFA) composition of raw beef and liver muscles.**

Parameter	Breed	Beef	Liver
C14:1	Non-descript	0.46 ± 0.10	0.00 <sup>a</sup> ± 0.00
	Bonsmara	0.44 <sup>a</sup> ± 0.10	0.01 <sup>a</sup> ± 0.01
C16:1	Non-descript	2.39 <sup>a</sup> ± 0.65	0.60 <sup>b</sup> ± 0.12
	Bonsmara	4.18 <sup>a</sup> ± 0.65	0.98 <sup>b</sup> ± 0.12
C17:1	Non-descript	0.16 <sup>b</sup> ± 0.05	0.00 <sup>a</sup> ± 0.00
	Bonsmara	0.22 <sup>b</sup> ± 0.05	0.00 <sup>a</sup> ± 0.00
C18:1 (n-7)	Non-descript	1.63 <sup>a</sup> ± 0.87	1.32 <sup>a</sup> ± 0.09
	Bonsmara	1.62 <sup>a</sup> ± 0.87	1.49 <sup>a</sup> ± 0.09
C18:1 (n-9t)	Non-descript	0.14 <sup>b</sup> ± 0.26	2.17 <sup>a</sup> ± 0.23
	Bonsmara	0.74 <sup>b</sup> ± 0.26	2.00 <sup>a</sup> ± 0.23
C18:1 (n-9c)	Non-descript	35.45 <sup>a</sup> ± 1.22	14.65 <sup>b</sup> ± 1.05
	Bonsmara	35.33 <sup>a</sup> ± 1.22	16.58 <sup>b</sup> ± 1.05
Total MUFA	Non-descript	40.22 <sup>a</sup> ± 1.90	20.11 <sup>b</sup> ± 2.38
	Bonsmara	42.53 <sup>a</sup> ± 1.20	21.08 <sup>b</sup> ± 1.23

**Non-descript (n=4), Bonsmara (n= 4), mean with <sup>a-b</sup> superscripts within a row indicates significant differences (P ≤ 0.05).**

**Table 6. 8 Total polyunsaturated fatty acid (PUFA) composition of raw beef and liver muscles.**

Parameter	Breed	Beef	Liver
C18:2 CLA	Non-descript	0.44 <sup>a</sup> ± 0.05	0.51 <sup>a</sup> ± 0.06
	Bonsmara	0.40 <sup>a</sup> ± 0.05	0.51 <sup>a</sup> ± 0.06
C18:2 (n-6)	Non-descript	4.76 <sup>b</sup> ± 1.34	6.60 <sup>a</sup> ± 0.39
	Bonsmara	3.86 <sup>b</sup> ± 1.34	5.00 <sup>a</sup> ± 0.39
C18:3 (n-6)	Non-descript	0.00 <sup>a</sup> ± 0.00	0.10 <sup>b</sup> ± 0.03
	Bonsmara	0.00 <sup>a</sup> ± 0.00	0.13 <sup>b</sup> ± 0.03
C18:3 (n-3)	Non-descript	1.32 <sup>b</sup> ± 0.31	2.51 <sup>a</sup> ± 0.28
	Bonsmara	1.09 <sup>a</sup> ± 0.31	1.94 <sup>a</sup> ± 0.28
C20:2 (n-6)	Non-descript	0.00 <sup>a</sup> ± 0.00	0.02 <sup>b</sup> ± 0.02
	Bonsmara	0.00 <sup>a</sup> ± 0.00	0.02 <sup>b</sup> ± 0.02
C20:3 (n-6)	Non-descript	0.00 <sup>a</sup> ± 0.00	0.06 <sup>b</sup> ± 0.02
	Bonsmara	0.00 ± 0.00	0.00 ± 0.00
C20:3 (n-3)	Non-descript	0.00 <sup>a</sup> ± 0.00	0.06 <sup>b</sup> ± 0.02
	Bonsmara	0.00 ± 0.00	0.00 ± 0.00
C20:4 (n-6)	Non-descript	2.28 <sup>b</sup> ± 0.74	7.14 <sup>a</sup> ± 0.40
	Bonsmara	2.26 <sup>b</sup> ± 0.74	7.13 <sup>a</sup> ± 0.40
C20:5 (n-3)	Non-descript	0.61 <sup>b</sup> ± 0.18	3.54 <sup>a</sup> ± 0.27
	Bonsmara	0.70 <sup>b</sup> ± 0.18	3.54 <sup>a</sup> ± 0.27
C22:5 (n-3)	Non-descript	1.17 <sup>b</sup> ± 0.45	7.45 <sup>a</sup> ± 0.38
	Bonsmara	1.36 <sup>b</sup> ± 0.45	7.43 <sup>a</sup> ± 0.38
C22:6 (n-3)	Non-descript	0.00 <sup>a</sup> ± 0.00	1.99 <sup>b</sup> ± 0.33
	Bonsmara	0.00 <sup>a</sup> ± 0.00	2.55 <sup>b</sup> ± 0.33
Total PUFA	Non-descript	11.02 <sup>b</sup> ± 2.74	30.73 <sup>a</sup> ± 2.60
	Bonsmara	10.13 <sup>b</sup> ± 3.36	31.11 <sup>a</sup> ± 1.19

**Non-descript (n=4), Bonsmara (n= 4), mean with <sup>a-b</sup> superscripts within a row indicates significant differences (P ≤ 0.05).**

**Table 6. 9 Fatty acid composition of raw beef and liver muscles.**

Parameter	Breed	Beef	Liver
n-6	Non-descript	7.91 <sup>b</sup> ± 2.16	17.14 <sup>a</sup> ± 0.69
	Bonsmara	6.98 <sup>b</sup> ± 2.16	15.51 <sup>a</sup> ± 0.69
n-3	Non-descript	3.10 <sup>b</sup> ± 0.92	13.59 <sup>a</sup> ± 1.53
	Bonsmara	3.14 <sup>b</sup> ± 0.92	15.60 <sup>a</sup> ± 1.53
PUFA:SFA	Non-descript	0.24 <sup>b</sup> ± 0.08	0.63 <sup>a</sup> ± 0.04
	Bonsmara	0.24 <sup>b</sup> ± 0.08	0.65 <sup>a</sup> ± 0.04
PUFA/MUFA	Non-descript	2.54 <sup>a</sup> ± 0.12	1.64 <sup>b</sup> ± 0.23
	Bonsmara	2.29 <sup>a</sup> ± 0.12	1.50 <sup>b</sup> ± 0.23
n-6/n-3	Non-descript	2.54 <sup>a</sup> ± 0.12	1.38 <sup>b</sup> ± 0.18
	Bonsmara	2.28 <sup>a</sup> ± 0.12	0.99 <sup>b</sup> ± 0.18
Atherogenicity Index	Non-descript	0.58 <sup>a</sup> ± 0.07	0.00 <sup>b</sup> ± 0.03
	Bonsmara	0.59 <sup>a</sup> ± 0.07	0.33 <sup>b</sup> ± 0.03
Desaturase Index	Non-descript	1.95 <sup>a</sup> ± 0.13	0.54 <sup>b</sup> ± 0.09
	Bonsmara	2.24 <sup>a</sup> ± 0.13	0.58 <sup>b</sup> ± 0.09

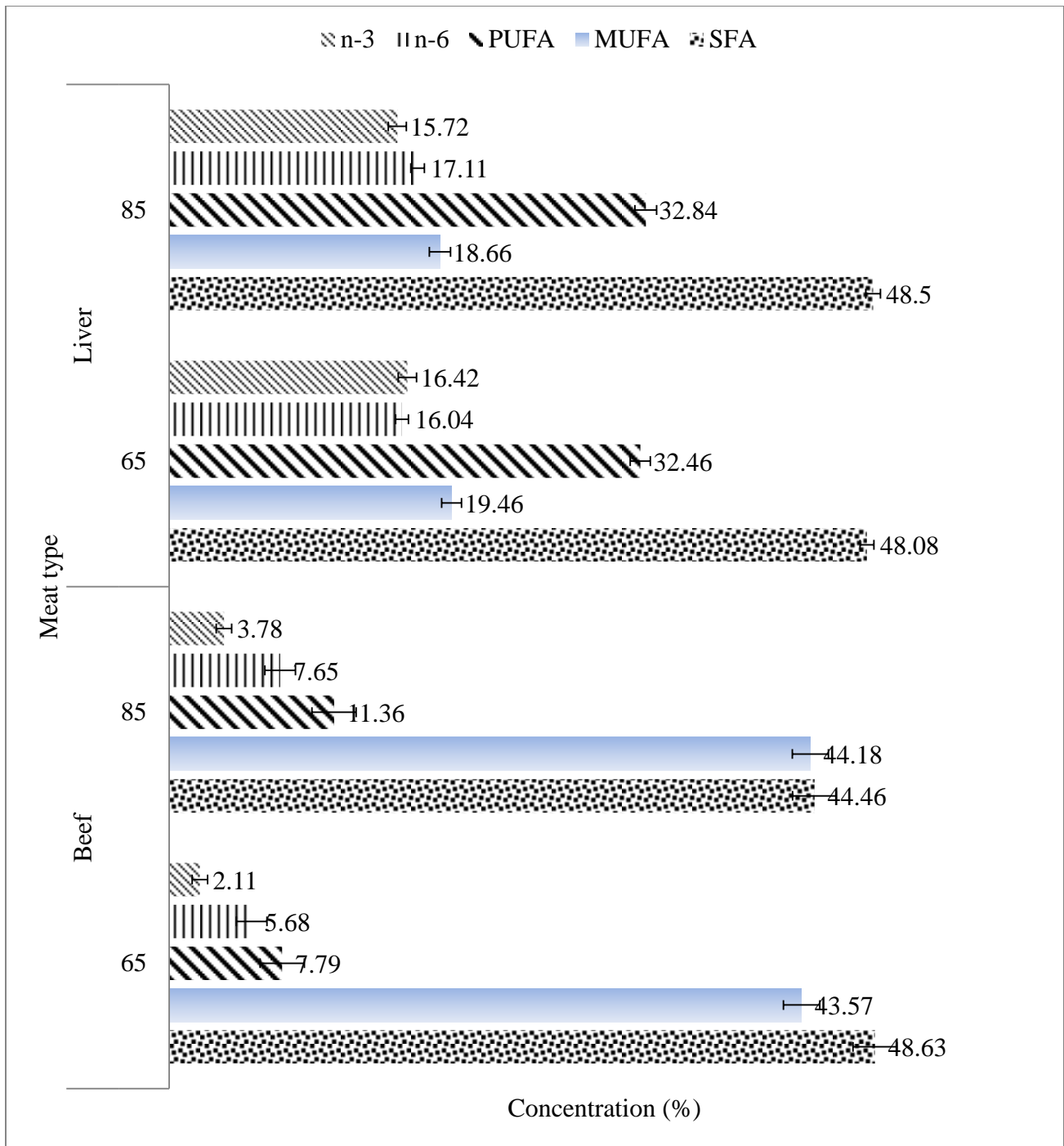
**Non-descript (n=4), Bonsmara (n= 4), mean with <sup>a-b</sup> superscripts within a row indicates significant differences (P ≤ 0.05).**

### **6.3.4 Effect of Sous vide thermal temperature on fatty acid composition of beef and liver muscles**

As shown in Figure 6.1 and 6.2, the total fatty acid composition in beef and liver samples were not significantly ( $P > 0.05$ ) affected by application of sous vide cooking method at 65<sup>0</sup>C and 85<sup>0</sup>C temperatures. However, the content of MUFA, PUFA, n-6, n-3, PUFA/SFA, PUFA/MUFA, n-6/n-3 were higher at 85<sup>0</sup>C than at 65<sup>0</sup>C cooking temperature for both beef and liver samples. The percentage of PUFA, n-6, n-3, PUFA/SFA, PUFA/MUFA, n-6/n-3, atherogenicity index and desaturase index loss in relation to raw samples after cooking at 65<sup>0</sup>C and 85<sup>0</sup>C was higher in beef than liver samples (Figures 6.3 and 6.4).

### **6.3.5. Mineral composition of raw beef and liver muscles**

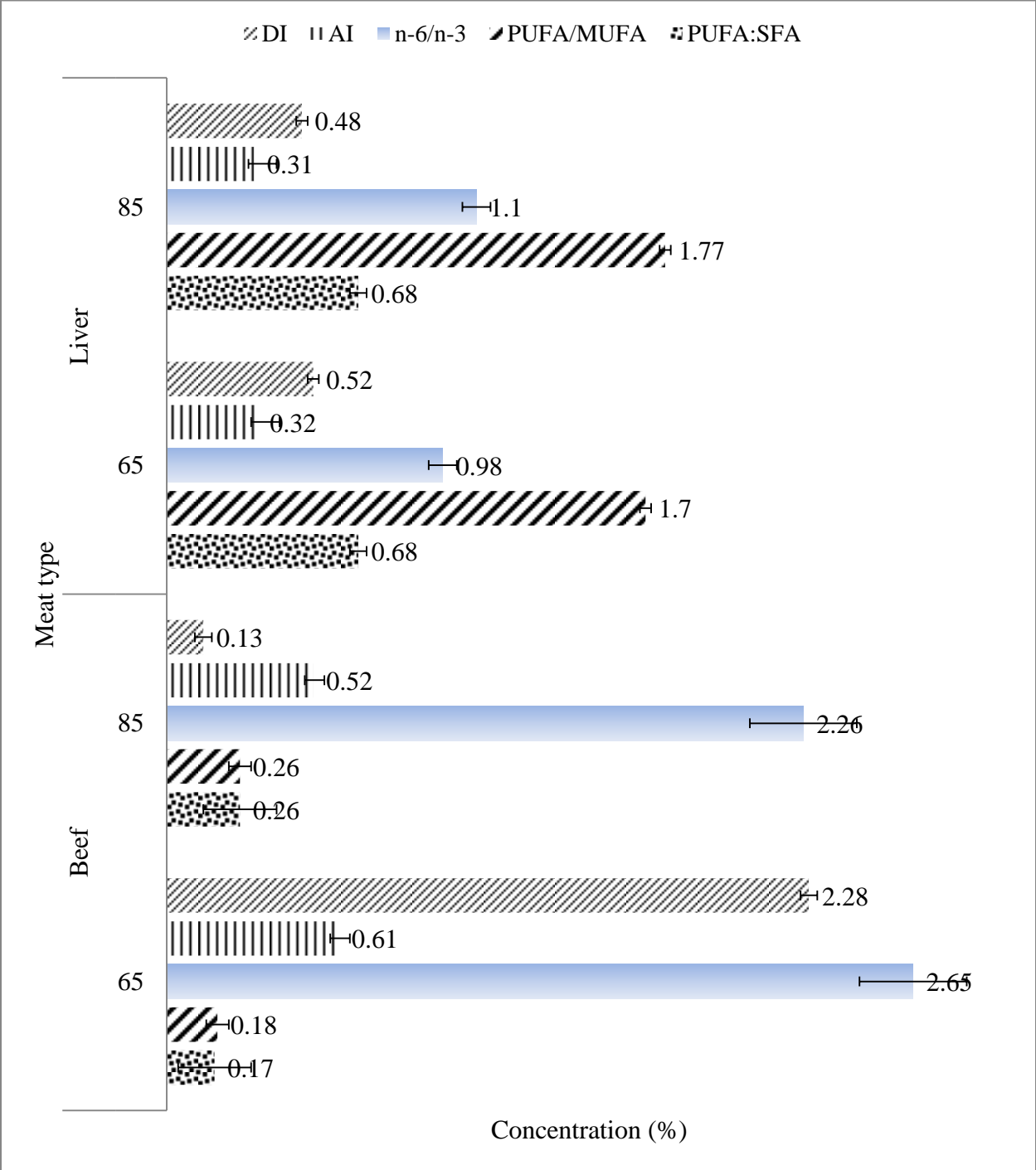
The results of mineral contents in raw beef and liver meat samples are presented in Table 6.9. The major minerals present in raw meat samples were potassium (beef 213.7–221.7mg/100g, liver 171.1-271.8mg/100g), followed by selenium (beef 141.8-350.7mg/100g, liver 88.8-365.0g/100mg), magnesium (beef 52-53.7 mg/100g, liver 41-56.1mg/100g)), sodium (29.3-48.4 mg/100g, liver 32.9-43.9mg/100g), zinc (8.9-19.5mg/100g, liver 8.5-17.3mg/100g) and calcium (12.9-13.9mg/100g, liver 17.0-17.5mg/100g). Among the breeds, the mean content of Na ( $48.40 \pm 0.35$ mg/100g), Mg ( $52.80 \pm 0.22$ mg/100g) and K ( $221.7 \pm 1.41$ mg/100g) were higher in beef than liver muscles from Bonsmara cattle. While on the contrary, liver muscles from non-descript cattle had higher contents of Na ( $43.90 \pm 0.76$ mg/100g), Mg ( $56.10 \pm 0.74$ mg/100g) and K ( $271.1 \pm 3.41$ mg/100g) than the beef muscles. However, the concentration of Ca in liver from Bonsmara ( $17.50 \pm 0.17$ mg/100g) and non-descript ( $17.00 \pm 0.17$ mg/100g) was higher than that of beef muscles.



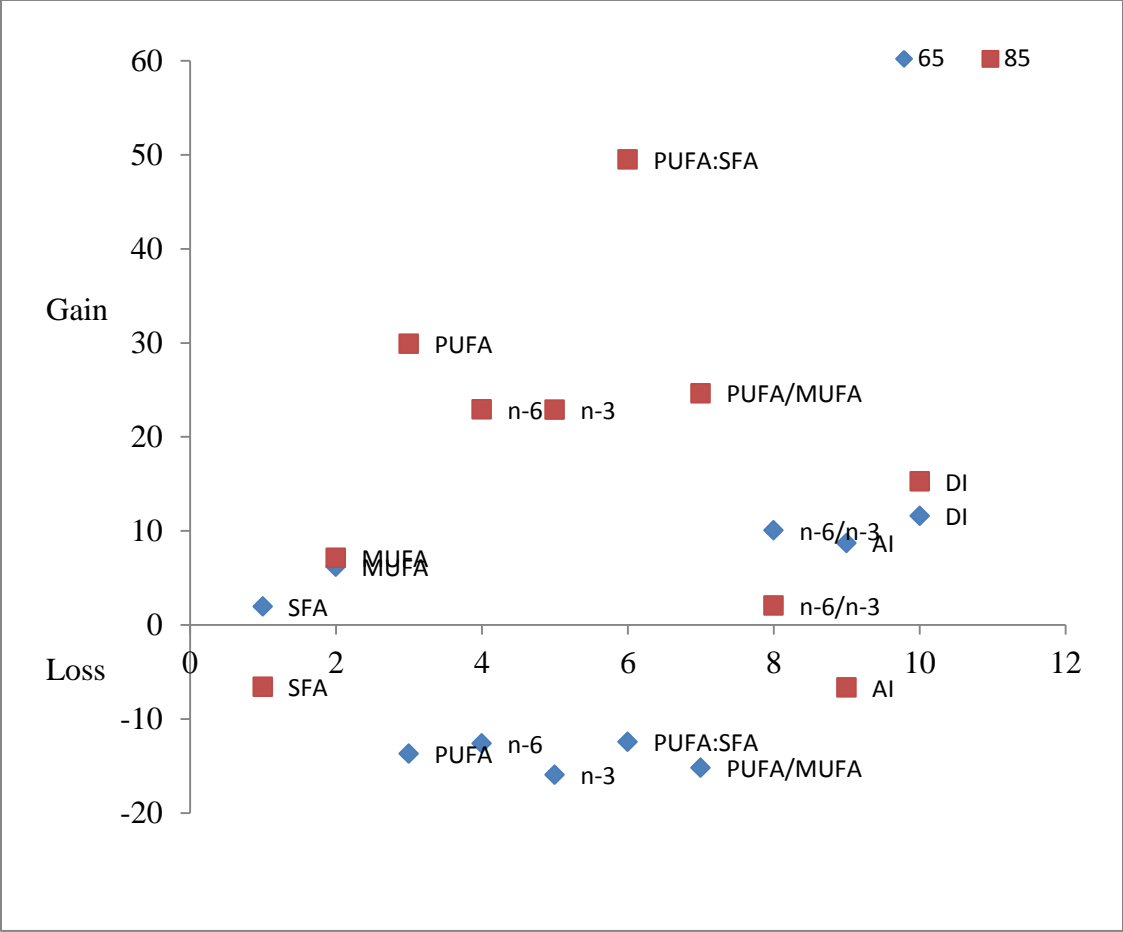
**Figure 6. 1 Fatty acid composition of cooked beef and liver meat samples.**

65 = 65<sup>0</sup>C, 85 = 85<sup>0</sup>C.



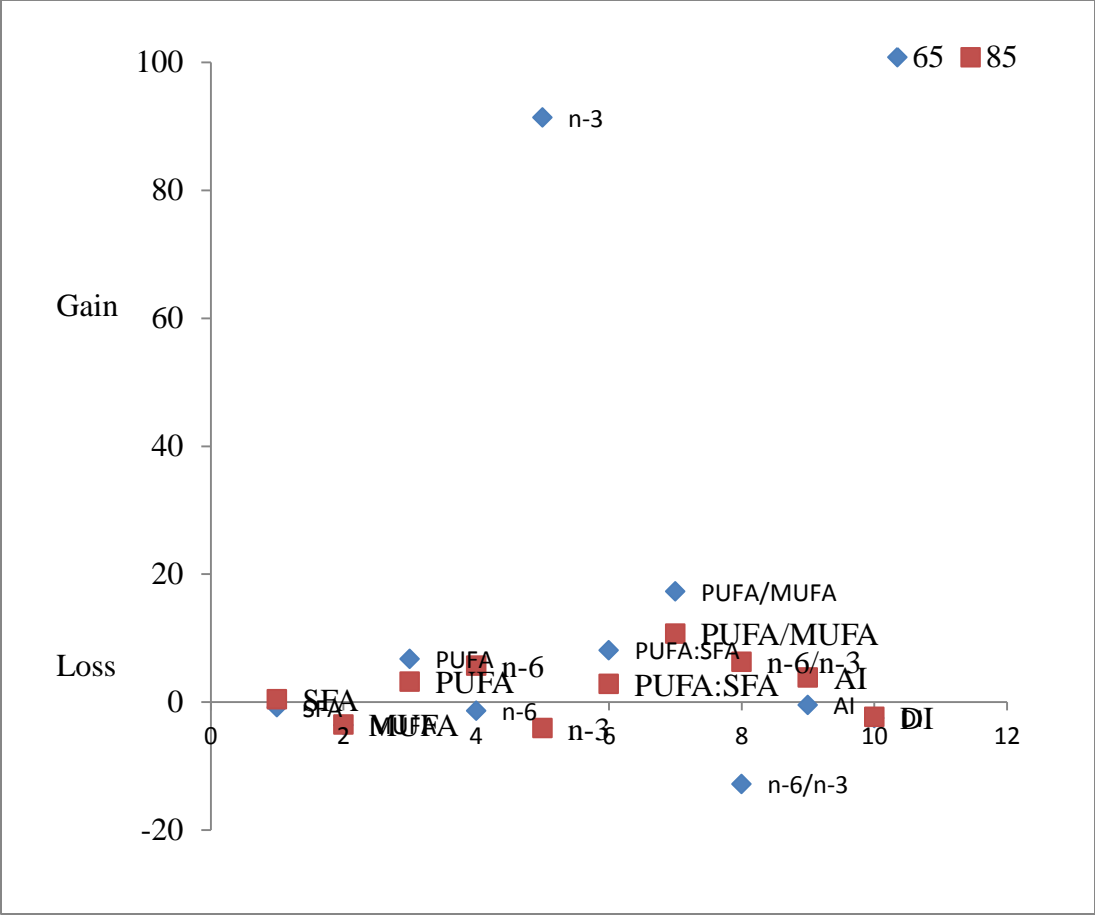


**Figure 6. 2 Health indicator values of fatty acid profile in cooked beef and liver samples. 65 = 65°C, 85 = 85°C.**



**Figure 6. 3 Percentage of fatty acids loss or gain in beef after cooking.**

**n = 8, AI: Atherogenicity Index, DI: Desaturase Index.**



**Figure 6. 4 Percentage of fatty acids loss or gain in liver after cooking.**  
**n = 8, AI: Atherogenicity Index, DI: Desaturase Index.**

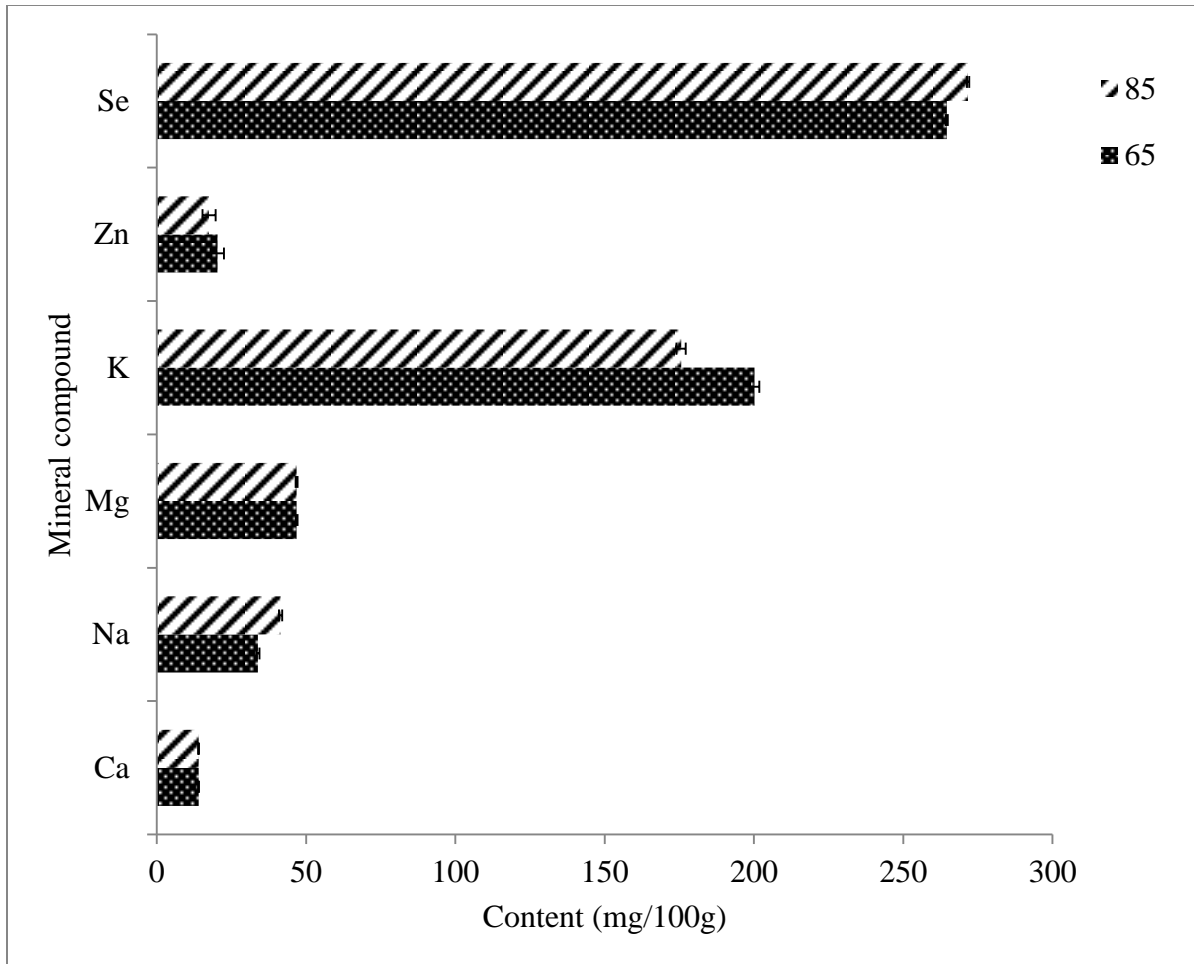
**Table 6. 10 Mineral composition of raw beef and liver samples.**

Minerals	Breed	Beef (mg/100)	Liver (mg/100)
Ca	Non-descript	12.90 <sup>bA</sup> ± 0.08	17.00 <sup>aA</sup> ± 0.17
	Bonsmara	13.90 <sup>bA</sup> ± 0.08	17.50 <sup>aA</sup> ± 0.17
Na	Non-descript	29.30 <sup>aA</sup> ± 0.35	43.90 <sup>aA</sup> ± 0.76
	Bonsmara	48.40 <sup>aA</sup> ± 0.35	32.90 <sup>aA</sup> ± 0.76
Mg	Non-descript	53.70 <sup>aA</sup> ± 0.22	56.10 <sup>aA</sup> ± 0.74
	Bonsmara	52.80 <sup>aA</sup> ± 0.22	41.80 <sup>aA</sup> ± 0.74
K	Non-descript	213.7 <sup>aA</sup> ± 1.41	271.1 <sup>aA</sup> ± 3.41
	Bonsmara	221.7 <sup>aA</sup> ± 1.41	171.8 <sup>aA</sup> ± 3.41
Se	Non-descript	141.8 <sup>aB</sup> ± 2.81	88.80 <sup>aB</sup> ± 2.81
	Bonsmara	350.7 <sup>aA</sup> ± 1.59	365.0 <sup>aA</sup> ± 1.59
Zn	Non-descript	19.60 <sup>aA</sup> ± 0.15	17.30 <sup>aA</sup> ± 0.27
	Bonsmara	8.90 <sup>aB</sup> ± 0.15	8.50 <sup>aB</sup> ± 0.27

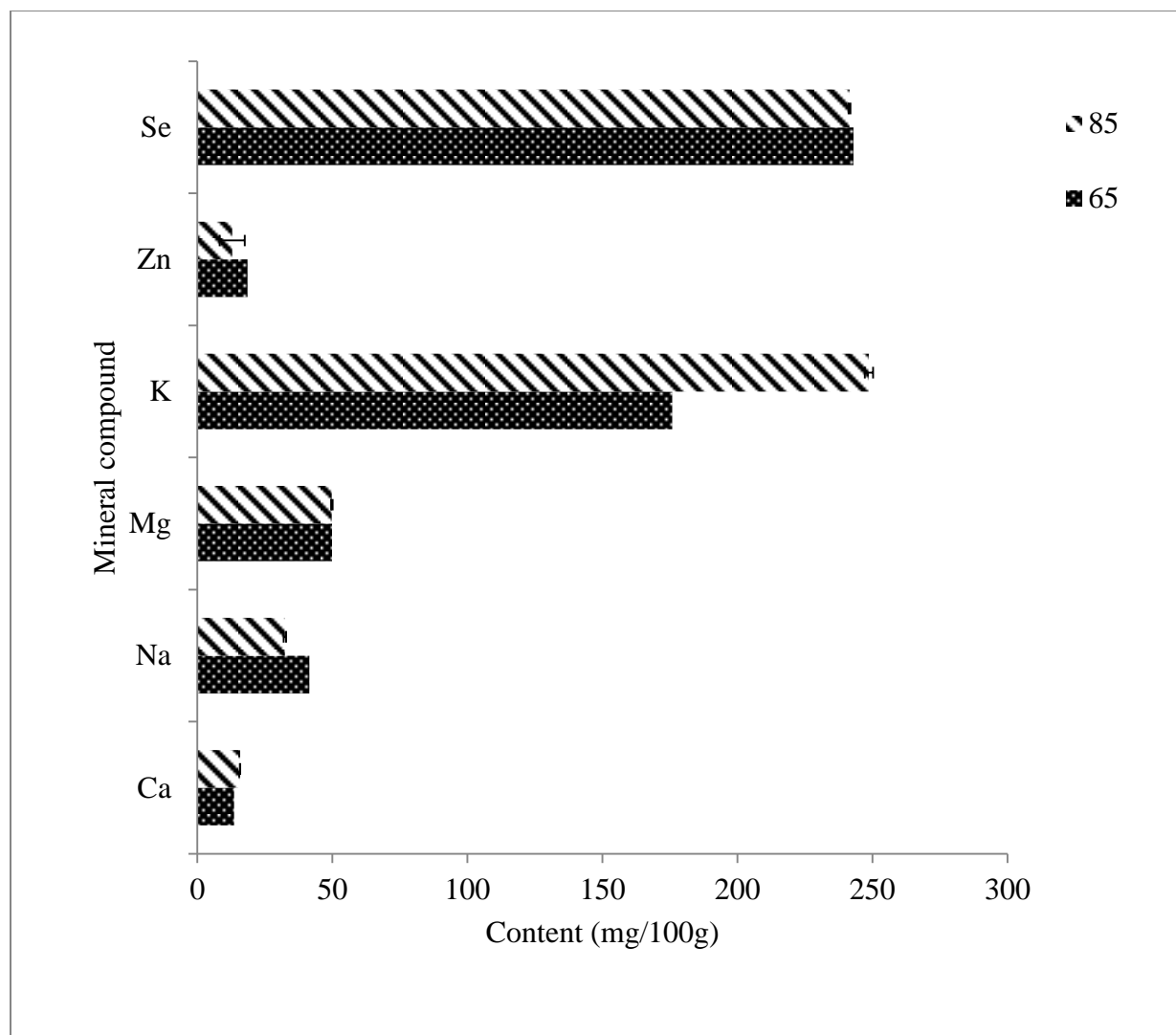
**Non-descript (n=4), Bonsmara (n= 4), mean with <sup>a-b</sup> superscripts within a row indicates significant differences between meat types (P ≤ 0.05). Mean with <sup>A-B</sup> superscripts within a column indicates significant differences between breed (P ≤ 0.05).**

### **6.3.6 Effect of thermal treatment on mineral composition of beef and liver muscles**

As shown in Figures 6.5 and 6.6, application of sous vide cooking method at 65<sup>0</sup>C and 85<sup>0</sup>C temperature had no significant ( $P > 0.05$ ) effect on the concentration of minerals in beef and liver samples. However, among the mineral contents, the mean value of Se and K was numerically higher in beef and liver samples, respectively, after cooking at 85<sup>0</sup>C than at 65<sup>0</sup>C. While on the other hand, the concentration of Zn was lower in both beef and liver samples at the cooking temperature of 85<sup>0</sup>C than at 65<sup>0</sup>C. The concentration of Mg in beef samples after cooking was not different from that of liver samples.



**Figure 6. 5** Effect of of thermal treatments on mineral content of beef from Bonsmara and non-descript cattle; 65 = 65<sup>0</sup>C, 85 = 85<sup>0</sup>C.



**Figure 6. 6 Effect of thermal treatments on mineral content of liver from Bonsmara and non-descript cattle. 65 = 65°C, 85°C.**

## 6.4 Discussion

The gradual increase in cooking loss as the temperature rises from 65<sup>0</sup>C to 85<sup>0</sup>C can be attributed to increase in denaturation of myosin and actin of muscle fibres which has been identified to cause structural changes and expulsion of sarcoplasmic fluid from the muscle fibers (Bertola et al., 1994; Li et al., 2013). Changes in muscle and connective tissue during thermal treatment have been reported to influence muscle texture and cooking loss in processed meat (Murphy and Marks, 2000). The expression of cooking loss has been demonstrated to influence the degree of juiciness and consumer acceptance of meat after thermal treatment (Aaslyng, 2009). However, our results are in agreement with previous studies (Alfaia et al., 2010; Nithyalakshmi and Preetha, 2015) demonstrating that increase in cooking temperature and time increases cooking loss. Moreover, the increase in Warner Bratzler Shear Force (WBSF) values as observed in this study could also be attributed to progressive transformations and solubilization of connective tissue leading to meat tenderization as cooking temperature increased (Garcia-Segovia et al., 2007; Li et al., 2013).

The percentage of intramuscular fat and fat free dry matter of liver sample was relatively higher than those of the beef samples. Increase in intramuscular fat content has been attributed to accumulation of fatty acid in the muscle (Wood et al., 2008). The proportion of intramuscular fat is known generally to enhance palatability and flavor intensity of meat (Hoehne et al., 2012; Legako et al., 2015). Variation in fatty acid composition of raw and cooked beef and liver samples as observed in this study is in line with previous studies where significant differences in the fatty acid profile of liver and beef of cattle raised and finished on pasture was reported (Alfaia et al., 2010; Purchas et al., 2015). Also, the higher proportion of PUFA, n-6 and n-3



observed in raw liver than beef samples could be linked to the ability of liver to synthesize most of n-6 and n-3 long-chain polyunsaturated fatty acids from dietary precursors during lipid metabolism in the body (Araya et al., 2010; Valenzuela et al., 2012). This could also adduce for higher total fat and free fat dry matter found in liver in this study. However, omega-6 PUFA (arachidonic acid) and omega-3 PUFA (eicosapentaenoic and docosahexaenoic acid) have been identified in several physiological functions including regulation of inflammation and prevention of transmissible chronic diseases (Simopoulos, 2008; Stephen et al., 2010; Valenzuela et al., 2012). This result is in line with other studies that have shown that liver meat contained higher levels of polyunsaturated fatty acid than lean tissue (Enser et al., 1998; Liu, 2002; Jayathilakan et al., 2012).

The non-significant difference between raw beef and liver from Bonsmara and non-descript indicated that their genotypes did not have an effect on fatty acid contents since both breeds were reared on natural pasture. Similar results have been reported by Xie et al. (2012) who found no significant difference in fatty acid composition of five breeds of cattle offered the same diet. However, our results are in contrast with the finding of Li et al. (2014) and Orellana et al. (2009) who had reported significant breed effect on fatty acid composition of liver and beef samples, respectively.

The total SFA and MUFA contents that were observed in raw beef and liver were relatively higher than those reported by Li et al. (2014) and Alfaia et al. (2010), respectively. However, the values PUFA in raw liver was higher than those reported by Li et al. (2014) while that of raw

beef was lower than those reported by Alfaia et al. (2010), but comparable with the report of Sarriés et al. (2009) and Legako et al. (2015). Also, the values of PUFA/SFA, PUFA/MUFA, n-6/n-3, AI and DI recorded in raw beef and liver in this study were in contrast with those found in other reports (Sarriés et al., 2009; Alfaia et al., 2010; Li et al., 2014; Purchas et al., 2015). These contrasting differences may be attributed to factors such as slaughter age, diet, meat cut and seasonal variation which have been reported to influence fatty acid composition of ruminant animals (Schmid et al., 2006; Orellana et al., 2009). Application of sous vide cooking technique led to significant loss of moisture and, consequently, a higher intramuscular fat and free fat dry matter content in both liver and beef samples compared to the raw samples. This is similar to the findings of Alfaia et al. (2010) who reported a significant reduction in moisture content and higher increase in intramuscular fat of cooked beef compared to raw beef. Other authors have also reported an increase in nutrient composition of meat (including fat content) as moisture content decreases during cooking (Badiani et al., 2002; Alfaia et al., 2010).

The percentage of fatty acid loss after cooking in this study was relatively lower compared to other studies that used cooking methods such as boiling, grilling and microwaving (Alfaia et al., 2010). Several mechanisms, such as water loss and lipid oxidation, diffusion and exchange that occur during cooking have been suggested to lead to relative changes in FA compositions (Dal Bosco et al., 2001; Alfaia et al., 2010). This indicates that sous vide cooking method was able to minimize nutrient loss and prevent oxidation of fatty acid content by reducing the contact of free oxygen with meat sample (Schellekens, 1996; Oz and Zikirov, 2015). Consequently, sous vide thermal treatment had lower cooking effect on liver samples than beef samples. This indicates that beef is more susceptible to nutrient loss and lipid oxidation than liver meat during cooking.

Considering the mineral composition of liver and beef muscle in this study, the concentration of K and Na in raw beef and liver was slightly lower than the values reported by Czerwonka and Szterk (2015) and Lopes et al., (2015), but higher than that of Reykdal et al. (2014). Equally, the level of Mg observed in raw beef and liver in this study was higher than the values reported by Czerwonka and Szterk (2015) and Duan et al. (2015). The content of Ca, Zn and Se in beef and liver was in contrast with values reported by Reykdal et al. (2014). However, this contrasting difference in the values reported in this study compared to other literature could be attributed to factors such as the type of cuts, age of the animals, sex, diet, genetic factors, physiological state, geographical site of rearing and method of mineral content determination (Tomovic et al., 2011; Mateescu et al., 2013; Czerwonka and Szterk, 2015; Duan et al., 2015). Besides this, many studies have also indicated wide variation in the content of these nutrients in bovine meat, and the limits of these variations have not been fully defined (Greenfield and Southgate, 2003; Tomovic et al., 2011).

The non-significant difference in mineral contents between breed in this study was similar to the report of Duan et al. (2015) who found no significant breed effect in mineral content of cattle. The impact of thermal processing on the mineral content of beef and liver in this study was relatively minimal compared to the findings of Czerwonka and Szterk (2015) who reported great loss of elemental nutrients after cooking different meat cuts through roasting, grilling and frying. This shows that sous vide cooking methods can preserve the elemental constituents of beef and liver during the cooking process than other conventional methods.

## **6.5 Conclusions**

The findings of this study revealed that breed did not have significant effect on fatty acid and mineral composition of raw beef and liver muscles. It also showed that the liver meat had higher lipid, fat dry matter, PUFA, n-6 and n-3 contents than the correspondent beef muscles. It was also observed that the concentration of mineral in liver was similar to that of beef. There was no pronounced effect of sous vide cooking temperature on fatty acid and mineral composition of beef and liver compared to uncooked meat. Moreover, the concentration of PUFA, n-6, and n-3 suffered greater loss in beef than liver after cooking. In comparison to other cooking methods, this study has demonstrated that sous vide cooking method can preserve the nutritional properties of meat products.

## 6.6 References

- Aaslyng, M. D.** 2009. Trend in meat consumption and the need for fresh meat and meat products improved quality. In improving the sensory and nutritional quality of fresh meat, Kerry, J. P. and Ledward, D. (Eds), CRC Press, North American pp 3-19.
- Alfaia, C. M. M, Castro, M. L. F., Martins, S.I.V., Portugal, A. P. V., Alves, S. P. A., Fontes, C. M. G. A., Bessa, R. J. B. and Prates, J. A. M.** 2007. Effect of slaughter season on fatty acid composition, conjugated linoleic acid isomers and nutritional value of intramuscular fat in Barrosã-PDO veal. *Meat Science* **75**: 44–52.
- Alfaia, C. M. M., Alves, S. P., Lopes, A. F., Fernandes, M. J. E., Costa, A S. H., Fontes, C.M.G.A., Castro, M. L.F., Bessa, R. J.B. and Prates, J. A.M.** 2010. Effect of cooking methods on fatty acids, conjugated isomers of linoleic acid and nutritional quality of beef intramuscular fat. *Meat Science* **84**: 769–777.
- Araya, J., Rodrigo, R., Pettinelli, P., Araya, A.V., Poniachik, J. and Videla, L. A.** 2010. Decreased liver fatty acid  $\Delta$ -6 and  $\Delta$ -5 desaturase activity in obese patients. *Obesity* **18**: 1460–1463.
- Badiani, A., Montellato, L., Bochicchio, D., Anfossi, P., Zanardi, E. and Maranesi, M.** 2004. Selected nutrient contents, fatty acid composition, including conjugated linoleic acid, and retention values in separable lean from lamb rib loins as affected by external fat and cooking method. *Journal of Agricultural and Food Chemistry* **52**: 5187–5194.
- Baldwin, D. E.** 2012. Sous vide cooking: A review. *International Journal of Gastronomy and Food Science* **1**: 15–30.

- Bertola, N. C., Bevilacqua, A. E. and Zaritzky, N. E.** 1994. Heat treatment effect on texture changes and thermal denaturation of proteins in beef muscle. *Journal of Food Processing and Preservation* **18**: 31–46.
- Chilliard, Y., Ferlay, A., Rouel, J. and Lamberet, G.** 2003. A review of nutritional and physiological factors affecting goat milk lipid synthesis and lypolysis. *Journal of Dairy Science* **86 (5)**: 1751-1770.
- Chin, J. P. F. and Dart, A. M.** 1995. How do fish oil affect vascular function? *Clinical and Experimental Pharmacology and Physiology* **22**:71–81.
- Czerwonka, M. and Szterk, A.** 2015. The effect of meat cuts and thermal processing on selected mineral concentration in beef from Holstein–Friesian bulls. *Meat Science* **105**: 75–80.
- Dal Bosco, A., Castellini, C. and Bernardini, M.** 2001. Nutritional quality of rabbit meat as affected by cooking procedure and dietary vitamin E. *Journal of Food Science* **66**: 1047–1051.
- Dominguez, R., Gómez, M., Fonseca, S. and Lorenzo, J. M.** 2014. Effect of different cooking methods on lipid oxidation and formation of volatile compounds in foal meat. *Meat Science* **97**: 223–230.
- Duan, Q., Tait Jr. R. G., Schneider, M. J., Beitz, D. C., Wheeler, T. L., Shackelford, S. D., Cundiff, L. V. and Reecy, J. M.** 2015. Sire breed effect on beef longissimus mineral concentrations and their relationships with carcass and palatability traits. *Meat Science* **106**: 25–30.

- Enser, M., Hallett, K. G., Hewett, B., Fursey, G. A. J., Wood, J. D. and Harrington, G.** 1998. The Polyunsaturated Fatty Acid Composition of Beef and Lamb Liver. *Meat Science* **49** (3): 321-327.
- Fatma, H. M. and Mahdey, E. A.** 2010, 'Incidence of *Brucella* species in slaughtered food animals and its edible offal at Beni-suef, Egypt', *Global Veterinaria* **5**: 248–254.
- Fayemi, P. O. and Muchenje, V.** 2014. Farmers' preference for mutton and lamb from natural velds in the Eastern Cape Province of South Africa. *Journal of Food, Agriculture and Environment* **12** (2): 15-19.
- Folch, J., Lees, M., Sloane-Stanley, G. H.** 1957. A simple method for the isolation and purification of total lipids from animal tissue. *Journal of Biological Chemistry* **226**: 497- 509.
- Garcia-Segovia, P., Andres-Bello, A. and Martinez-Monzo, J.** 2007. Effect of cooking method on mechanical properties, color and structure of beef muscle (*M. pectoralis*). *Journal of Food Engineering* **80**: 813–821.
- Gerber, N., Scheeder, M. R. L. and Wenk, C.** 2009. The influence of cooking and fat trimming on the actual nutrient intake from meat. *Meat Science* **81**:148–154.
- Gorsuch, T. T.** 1970. The destruction of organic matter (1st editon). Oxford, New York, Toronto, Sydney, Braunschweig: Pergamon Press Ltd, pp 1-160.
- Greenfield, H. and Southgate, D. A. T.** 2003. Food composition data: Production, management and use. [www.fao.org/fileadmin/templates/food\\_composition](http://www.fao.org/fileadmin/templates/food_composition). Accessed on 11/09/2015
- Hoehne, A., Nuernberg, G., Kuehn, C. and Nuernberg, K.** 2012. Relationships between intramuscular fat content, selected carcass traits, and fatty acid profile in bulls using a F2-population. *Meat Science* **90**: 629–635.

- Hosseini, H., Mahmoudzadeh, M., Rezaei, M., Mahmoudzadeh, L., Khaksar, R., Khosroshahi, K. N. and Babakhani, A.** 2014. Effect of different cooking methods on minerals, vitamins and nutritional quality indices of kutum roach (*Rutilus frisii kutum*). *Food Chemistry* **148**: 86–91.
- Jayathilakan, K., Sultana, K., Radhakrishna, K. and Bawa, A. S.** 2012. Utilization of by products and waste materials from meat, poultry and fish processing industries: a review. *Journal of Food Science and Technology* **49** (3): 278–293.
- Legako, J. F., Dinh, T. T. N., Miller, M. F. and Brooks, J. C.** 2015. Effects of USDA beef quality grade and cooking on fatty acid composition of neutral and polar lipid fractions. *Meat Science* **100**: 246–255.
- Li, C., Wang, D., Xu, W., Gao, F. and Zhou, G.** 2013. Effect of final cooked temperature on tenderness, protein solubility and microstructure of duck breast muscle. *LWT - Food Science and Technology* **51**:266-274.
- Li, R., Yu, Q., Han, L. and Cao, H.** 2014. Nutritional Characteristics and Active Components in Liver from Wagyu×Qinchuan Cattle. *Korean Journal for Food Science of Animal Resources* **34** (2): 214-220.
- Liu, D. C.** 2002. Better utilization of by-products from the meat industry. *EFTC Extension Bulletins* **304**: 2462.
- Lopes, A. F., Alfaia, C. M. M., Partidário, A. M. C. P. C., Lemos, J. P. C. and Prates, J. A. M.** 2015. Influence of household cooking methods on amino acids and minerals of Barrosã-PDO veal. *Meat Science* **99**: 38–43.
- Mateescu, R. G., Garrick, D. J., Tait, R. G., Jr., Garmyn, A. J., Duan, Q., Liu, Q., Mayes, M. S., Van Eenennaam, A. L., VanOverbeke, D. L., Hilton, G. G., Beitz, D. C. and**



- Reecy, J. M.** 2013. Genome-wide association study of concentrations of iron and other minerals in longissimus muscle of Angus cattle. *Journal of Animal Science* **91**: 3593–3600.
- Mottram, D. S.** 1998. The chemistry of meat flavour. In *flavor of meat, meat products and seafood*, shahidi, F. (Ed.), Blackie Academic and Professional: London, UK, 1998; pp 5–26.
- Murphy, R. Y. and Marks, B. P.** 2000. Effect of meat temperature on proteins, texture, and cook loss for ground chicken breast patties. *Poultry Science* **79**: 99-104.
- Nantapo, C. W., Muchenje, V., Nkukwana, T. T., Hugo, A., Descalzo, A., Grigioni, G. and Hoffman, L. C.** 2015. Socio-economic dynamics and innovative technologies affecting health-related lipid content in diets: Implications on global food and nutrition security. *Food Research International* **76 (4)**: 896-905.
- Nithyalakshmi, V. and Preetha, R.** 2015. Effect of cooking conditions on physico-chemical and textural properties of Emu (*Dromaius novaehollandiae*) meat. *International Food Research Journal* **22 (5)**: 1924-1930.
- Orellana, C., Peña, F., García, A., Perea, J., Martos, J., Domenech, V. and Acero, R.** 2009. Carcass characteristics, fatty acid composition, and meat quality of Criollo Argentino and Braford steers raised on forage in a semi-tropical region of Argentina. *Meat Science* **81**: 57–64.
- Oz, F. and Zikirov, E.** 2015. The effects of sous-vide cooking method on the formation of heterocyclic aromatic amines in beef chops. *LWT - Food Science and Technology* **64**: 120-125.

- Purchas, R. W., Wilkinson, B.H.P., Carruthers, F. and Jackson, F.** 2015. A comparison of the trans fatty acid content of uncooked and cooked lean meat, edible offal and adipose tissue from New Zealand beef and lamb. *Journal of Food Composition and Analysis* **41**: 151–156.
- Reykdal, O., Rabieh, S., Steingrimsdottir, L. and Gunnlaugsdottir, H.** 2014. Minerals and trace elements in Icelandic dairy products and meat. *Journal of Food Composition and Analysis* **24**: 980–986.
- Rodriguez-Estrada, M. T., Penazzi, G., Caboni, M. F., Bertacco, G. and Lercker, G.** 1997. Effect of different cooking methods on some lipid and protein components of hamburgers. *Meat Science* **45** (3): 365–375.
- Roldan, M., Antequera, T., Armenteros, M. and Ruiz, J.** 2014. Effect of different temperature–time combinations on lipid and protein oxidation of sous-vide cooked lamb loins. *Food Chemistry* **149**: 129–136.
- Sanchez del Pulgar, J., Gazquez, A. and Ruiz-Carrascal, J.** 2012. Physico-chemical, textural and structural characteristics of sous-vide cooked pork cheeks as affected by vacuum, cooking temperature, and cooking time. *Meat Science* **90**: 828–835.
- Sánchez-Ortega, I., García-Almendárez, B. E., Santos-López, E., Amaro-Reyes, A., Barboza-Corona, J. E. and Regalado, E.** 2014. Antimicrobial edible films and coatings for meat and meat products preservation. *Scientific World Journal* **2014**: 18.
- Sarries M. V., Murray, B. E., Moloney, A. P., Troy, D. and Beriain, M. J.** 2009. The effect of cooking on the fatty acid composition of longissimus muscle from beef heifers fed rations designed to increase the concentration of conjugated linoleic acid in tissue. *Meat Science* **81**: 307–312.

- Schellekens, M.** 1996. New research issues in sous-vide cooking. *Trends in Food Science and Technology* **7 (8)**: 256–262.
- Schmid, A., Collom, M., Sieber, R. and Bee, G.** 2006. Conjugated linoleic acid in meat and meat products: A review. *Meat Science* **73**: 29–41.
- Simopoulos, A. P.** 2008. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Experimental Biology and Medicine* **233**: 674–688.
- Statistical Analysis System (SAS), 2007.** In: *SAS/STAT Guide to Personal Computers, Version 9.1.3*, Statistical Analysis System Institute Inc. Cary, NC”.
- Stephen, N. M., Jeya, S. R., Jeyasekaran, G. and Sukumar, D.** 2010. Effect of different types of heat processing on chemical changes in tuna. *Journal of Food Science and Technology* **47 (2)**:174–181.
- Tomovic, V. M., Petrovic, L. S., Tomovic, M. S., Kevrešan, Z. K. and Dzinic, N. R.** 2011. Determination of mineral contents of semimembranosus muscle and liver from pure and crossbred pigs in Vojvodina (northern Serbia). *Food Chemistry* **124**: 342–348.
- Valenzuela, R., Espinosa, A., González-Mañán, D., D'Espessailles, A., Fernández, V., Videla, L. A. and Tapia, G.** 2012. N-3 Long-Chain Polyunsaturated Fatty Acid Supplementation Significantly Reduces Liver Oxidative Stress in High Fat Induced Steatosis. *PLoS One* **7 (10)**: 46400.
- Vaudagna, S. R., Sánchez1, G., Neira, M. S., Insani, E. M., Picallo, A. B., Gallinger, M. M. and Lasta, J. A.** 2002. Sous vide cooked beef muscles: effects of low temperature–long time (LT–LT) treatments on their quality characteristics and storage stability. *International Journal of Food Science and Technology* **37 (4)**: 425–441.

- Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P.R., Richardson, R. I., Hughes, S. I. and Whittington, F. M.** 2008. Fat deposition, fatty acid composition and meat quality: A review. *Meat Science* **78**: 343–358.
- Xie, X., Meng, Q., Cui, Z. and Ren, L.** 2012. Effect of Cattle Breed on Meat Quality, Muscle Fiber Characteristics, Lipid Oxidation and Fatty Acids in China. *Asian-Australas Journal Animal Science* **25 (6)**: 824–831.

## **Chapter 7: General Discussion, Conclusions and Recommendations**

### **7.1 General Discussion and Conclusions**

Antioxidants play an important role in meat industry, as they provide protection against microbial growth and lipid oxidation in meat and meat products during storage and supply chain. Nowadays, antioxidants from natural sources are generally preferred over synthetic antioxidants for use in meat products because of their numerous bioactive compounds and potential health benefits. Thus, this study was designed to determine the activities of natural antioxidants and thermal treatment on meat quality from Bonsmara and non-descript cattle. In chapter three of this study, a survey was conducted to assess the level of consumer awareness on application of antioxidants in meat and meat products. It was evident from the results that more than half of the respondents had not heard about application of antioxidants as preservatives in meat products. This could be due to the fact that antioxidants are not predominantly advertised or mentioned on meat labels (Venkatesh, 2011) and more so, information regarding its application and benefits are rarely heard on public media. However, among the respondents that were aware of antioxidants and their application, the majority of them clearly indicated they knew about the use of both natural antioxidants and synthetic antioxidants as preservatives in meat products. This group of respondents also expressed their great concerns on application and potential health consequences of synthetic antioxidant on consumer health. Many studies have shown that application of synthetic antioxidants can cause cancers and cardiovascular diseases (Sarafian et al., 2002; Faine et al., 2006; Kumar et al., 2015).

With this knowledge, the efficacy of plant-derived antioxidants as preservatives to inhibit lipid oxidation, microbial growth and improve the physicochemical properties of meat products during

storage is currently being studied. In chapter 4 and 5, the antioxidant and antimicrobial activities of *Biden pilosa* and *Moringa oleifera* leaf extracts were investigated on meat quality from Bonsmara and non-descript cattle. Prior to application in meat, plant extracts (*Biden pilosa* and *Moringa oleifera* leaf) were screened for antioxidant potentials (Chapter 4). It was found that *Biden pilosa* and *Moringa oleifera* leaf extracts contained huge amounts of volatile compounds which has been reported for antioxidant and antimicrobial activities in food products (Bharathy and Uthayakumari. 2013; de Moraes et al., 2015; Mujeeb et al., 2015). The *in vitro* antioxidant activities of the extracts also revealed that the plants possessed strong scavenging capacity (DPPH and ABTS) against free radicals production in muscle food.

Upon application of the extracts in meat samples, it was observed that all the meat samples treated with *Biden pilosa* and *Moringa oleifera* plant extracts had lower pH values and higher a-value (redness) compared to control and BHT treatments during the storage period. This finding is also consistent with other studies who had reported the ability of natural antioxidants to improve and increase the colour (redness) of fresh ground beef when compared to control (Balentine et al., 2006; Kim et al., 2013; Liu et al., 2015). Additionally, the results of TBARS analysis showed that application of *M. oleifera* and *B. pilosa* extracts could protect meat products against lipid oxidation and extend its shelf life during the storage period. The inhibitory effects of these extracts against lipid oxidation has been attributed to the inherent phenolic content, phytoconstituents and antioxidant activity (Moyo et al., 2012; Krishian et al., 2014). It was also observed that breed did not have much effect on most of the parameters except for TBARS values, in which ground meat from Bonsmara exhibited lower lipid oxidation than non-descript cattle. This is indicating that Bonsmara cattle had better antioxidant ability than non-

descript cattle. Similar results had been reported by Xie et al (2012) who found a significant lower TBARS values in beef sample of Limousin than Qinchuan cattle breed.

Furthermore, the efficacy of these extracts was tested on sensory and microbiological qualities of the meat samples (Chapter 5). It was discovered that beef treated with extract had better sensory quality and lower microbial counts compared to control and BHT treatments. Evidence has shown that inclusion of plant extracts can improve their sensory quality and reduce food pathogens (Velasco and William, 2011). In general, the beneficial effect of producing meat products containing natural antioxidants is to prolong shelf life, improve quality and enhance consumer health. In chapter 6, the fatty acids and mineral composition of raw and cooked beef and liver meat were studied. The results revealed higher concentrations of MUFAs and a lower concentration of PUFAs in raw beef than liver meat. This is in agreement with other studies that have shown that liver contained lower levels of monounsaturated fatty acids and higher levels of polyunsaturated fatty acid than lean tissue (Enser et al, 1998; Liu 2002; Jayathilakan et al., 2012). The presence of MUFAs and PUFAs are known to promote consumer health by reducing the risk of developing coronary heart diseases (Stephen et al., 2010, Nantapo et al., 2015). The mineral content of raw beef showed higher content of Na, Mg and Se than liver samples. While on the other hand, the concentration of Ca and K was higher in liver than beef samples. Studies have shown that the amount of fatty acids and minerals contributed greatly to technological, sensory and nutritional quality of meat (Wood et al., 2003; Nieto and Ros, 2012). Application of sous vide method did not significantly affect the concentration of fatty acids and minerals in beef and liver meat samples after cooking. Meanwhile, the percentage of fatty acids and minerals lost during cooking in this study was relatively lower compared to other studies that used cooking

methods such as boiling, grilling and microwaving (Alfaia et al., 2010; Czerwonka and Szterk, 2015). This indicated that sous vide cooking method is more efficient in minimizing nutrient loss in meat products than other conventional methods.

In conclusion, this current study has demonstrated that application of *B. pilosa* and *M. oleifera* leaf extracts and sous vide cooking method can be used to preserve the quality of meat during storage. However, due to consumers' safety concerns on the use of synthetic antioxidants, the application of plant-derived antioxidants, particularly *B. pilosa* and *M. oleifera* leaf extracts, in commercial meat and meat products production should be considered. Evidence from this study has also shown that the use of sous vide thermal technique could be effective in preventing nutrient loss in meat products during cooking.



### 7.3 Recommendations

Based on the findings from this study, the following recommendations are offered for for further study:

- More research should be conducted on consumers' perception on the application of natural antioxidants as preservative in on meat and meat products.
- Research on the antioxidant and antimicrobial activities of essential oil from *Moringa oleifera* and *Biden pilosa* and their effectiveness on meat quality will be needed.
- Research should also be conducted on synthetization, characterization and commercialization of bioactive compounds in plant extracts in order to improve their application in meat products.
- Qualitative study on the effect of natural antioxidants on physicochemical properties of meat from more than two breeds should be considered.
- Studies on the effect of sous vide cooking technique on meat protein, vitamins and amino acid profile should also be considered.

#### 7.4 References

- Alfaia, C.M.M., Alves, S. P., Lopes, A. F., Fernandes, M . J. E., Costa, A S. H., Fontes, C.M.G.A., Castro, M. L.F., Bessa, R. J.B. and Prates, J. A.M.** 2010. Effect of cooking methods on fatty acids, conjugated isomers of linoleic acid and nutritional quality of beef intramuscular fat. *Meat Science* **84**: 769–777.
- Balentine, C.W., Crandall, P.G., O’ Bryan, C.A., Duong, D.Q. and Pohlman, F.W.** 2006. The pre- and post-grinding application of rosemary and its effects on lipid oxidation and color during storage of ground beef. *Meat Science* **73 (3)**: 413-421.
- Bharathy, V. and Uthayakumari, F.** 2013. Bioactive components in leaves of *Jatropha tanjorensis* J. L. Ellis and Saroja by GC-MS Analysis. *International Journal of PharmTech Research* **5**: 1839-1843.
- Czerwonka, M. and Szterk, A.** 2015. The effect of meat cuts and thermal processing on selected mineral concentration in beef from Holstein–Friesian bulls. *Meat Science* **105**: 75–80.
- de Moraes, J., de Oliveira, R. N., Costa, J. P., Junior, A. L.G., de Sousa, D.P., Freitas, R. M., Allegrett, S. M. and Pinto, P. L. S.** 2014. Phytol, a diterpene alcohol from chlorophyll, as a drug against neglected tropical disease *Schistosomiasis mansoni*. *PLOS Neglected Tropical Diseases* **8 (1)**: 2617.
- Enser, M., Hallett, K. G., Hewett, B., Fursey, G. A. J., Wood, J. D. and Harrington, G.** 1998. The polyunsaturated fatty acid composition of beef and lamb liver. *Meat Science* **49(3)**: 321-327.

- Faine, L. A., Rodrigues, H. G., Galhardi, C. M., Ebaid, G. M. X., Diniz, Y. S., Fernandes, A. A. H. and Novelli, E. L. B.** 2006. Butyl hydroxytoluene (BHT)-induced oxidative stress: Effects on serum lipids and cardiac energy metabolism in rats. *Experimental and Toxicologic Pathology* **57**:221–226.
- Jayathilakan, K., Sultana, K., Radhakrishna, K. and Bawa, A. S.** 2012. Utilization of byproducts and waste materials from meat, poultry and fish processing industries: a review. *Journal of Food Science and Technology* **49** (3): 278–293.
- Kim, S., Min, S. C., Shin, H., Lee, Y., Cho, A. R., Kim, S. Y. and Han, J.** 2013. Evaluation of the antioxidant activities and nutritional properties of ten edible plant extracts and their application to fresh ground beef. *Meat Science* **93**: 715–722.
- Krishnan, K. R., Babuskina, S., AzhaguSaravanaBabu, P., Sasikala, M., Sabina, K., Archana, G. Sivarajan, M. and Sukumar, M.** 2014. Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. *International Journal of Food Microbiology* **171**: 32–40.
- Kumar, Y., Yadav, D. N., Ahmad, T. and Narsaiah, K.** 2015. Recent trends in the use of natural antioxidants for meat and meat products. *Comprehensive Reviews in Food Science and Food Safety* **00**: 1-14.
- Liu, D. C.** 2002. Better utilization of by-products from the meat industry. *EFTC Extension Bulletins* **304**:2462.
- Liu, F., Xu, Q., Dai, R. and Ni, Y.** 2015. Effects of natural antioxidants on colour stability, lipid oxidation and metmyoglobin reducing activity in raw beef patties. *ACTA Scientiarum Polonorum Technologia Alimentaria* **14** (1): 37–44.

- Moyo, B., Oyedemi, S., Masika, P. J. and Muchenje, V.** 2012. Polyphenolic content and antioxidant properties of *Moringa oleifera* leaf extracts and enzymatic activity of liver from goats supplemented with *Moringa oleifera* leaves/sunflower seed cake. *Meat Science* **91**: 441–447.
- Mujeeb, F., Bajpai, P. and Pathak, N.** 2014. Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of *Aegle marmelos*. *BioMed Research International* **2014**: 1-11.
- Nantapo, C. W., Muchenje, V., Nkukwana, T. T., Hugo, A., Descalzo, A., Grigioni, G. and Hoffman, L. C.** 2015. Socio-economic dynamics and innovative technologies affecting health-related lipid content in diets: Implications on global food and nutrition security. *Food Research International* **76 (4)**: 896-905.
- Nieto, G and Ros, G.** 2012. Modification of fatty acid composition in meat through diet: effect on lipid peroxidation and relationship to nutritional quality – A Review. In: *A. Catala, Editor, Biochemistry, Genetics and Molecular Biology "Lipid Peroxidation"*, Chapter 12. <http://www.intechopen.com/books/lipid-peroxidation> Accessed on 18/08/2015.
- Sarafian, T. A., Kouyoumjian, S., Tashkin, D. and Roth, M. D.** 2002. Synergistic cytotoxicity of 9-tetrahydrocannabinol and butylated hydroxyanisole. *Toxicology Letters* **133**:171–179.
- Stephen, N. M., Jeya, S. R., Jeyasekaran, G. and Sukumar, D.** 2010. Effect of different types of heat processing on chemical changes in tuna. *Journal of Food Science and Technology* **47 (2)**:174–181.

**Velasco, V. and Williams, P.** 2011. Improving meat quality through natural antioxidants.

*Chilean Journal of Agricultural Research* **71**: 2.

**Venkatesh, R.** 2011. A Review of the Physiological Implications of Antioxidants in Food.

<https://www.wpi.edu> Accessed on 20/10/2015.

**Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Whittington, F. M. and Richardson, R. I.**

2003. Effects of diets on fatty acids and meat quality. *Options Méditerranéennes Series A* **67**: 133-141.

**Xie, X., Meng, Q., Cui, Z. and Ren, L.** 2012. Effect of Cattle Breed on Meat Quality, Muscle

Fiber Characteristics, Lipid Oxidation and Fatty Acids in China. *Asian-Australas Journal Animal Science* **25 (6)**: 824–831.

## 8: Appendices

### Appendix 8.1: Consumer's awareness on the use of antioxidants as preservatives in meat and meat products

#### A. Demographic characteristics

Please tick:

1. Gender: Male  Female
2. Age group: < 20  20-25  26-30  31-40  41-50  >50
3. Education level: Primary  Secondary (Matric)  Tertiary  Others

#### B. Application of antioxidants in meat products

1. Do you eat meat? Yes  No
2. In your own view, what are the causes of spoilage/deterioration in meat products?  
Oxidation  Microorganism  Both  Not sure
3. Have you heard about the use of antioxidants as preservatives in processed meat?  
Yes  No

If yes; please tick the type of antioxidant you know?

Natural (plant-derived) antioxidants  Both   
Synthetic (artificial) antioxidant  Others (specify)  .....

4. How did you get to know about the use of antioxidants in meat products?  
School  Internet source  Friends  Radio programs  TV programs
5. In which forms do you think natural antioxidants are used to preserve meat products?  
As supplement in animal feed   
As substance (essential oil/extract) to be applied directly on meat   
All of the above   
Not sure

6. Are you aware that some synthetic (artificial) antioxidants are reported to have toxic effect on human health?

Yes  No

7. How concerned are you about the effects of synthetic antioxidants on your Health?

Very much  Slightly  Somewhat  Not at all

**Appendix 8.2: Natural antioxidants against lipid–protein oxidative deterioration in meat and meat products: A review**





## Review

## Natural antioxidants against lipid–protein oxidative deterioration in meat and meat products: A review



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## ABSTRACT

Oxidation is a well-known non-microbial cause of quality loss in meat. Oxidative stress occurs due to uneven generation of free radicals reactive oxygen species (ROS) and reactive nitrogen species (RNS) which triggers oxidative and/or nitrosative stress and damage of macromolecules including the lipid and protein fractions. Failure of synthetic antioxidants to combat multiple health risks associated with this stress and maintenance of functional integrity of oxidised meat hitherto remains a challenge to the meat industry. A search for a viable alternative amidst the unexploited novel sources of natural antioxidants stands as a sustainable option for preserving the meat quality. In this paper, the potential use of bioactive compounds in medicinal plants is reviewed as phytoremedy against lipid–protein oxidation. Synergistic antimicrobial potentials of these natural antioxidants are also revealed against oxidative deterioration in meat and meat products and, for enhancing their functional properties.

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### 1. Introduction

Oxidation is one of the major causes of quality deterioration in meat. Meat becomes susceptible to oxidative deterioration due to high concentrations of unsaturated lipids, heme pigments, metal catalysts and a range of oxidizing agents in the muscle tissue. Oxidative deterioration in any type of meat manifests in form of discoloration, development of off flavour, formation of toxic compounds, poor shelf life, nutrient and

drip losses, respectively (Contini et al., 2014; Palmieri & Sblendorio, 2007). Under normal physiologic conditions, the molecular oxygen undergoes a series of reactions that leads to the generation of free radicals. A small portion (about 2–5%) of the oxygen consumed during the metabolic reaction is converted to free radicals in the form of reactive oxygen species (ROS). These free radicals, particularly, the reactive oxygen species (ROS) and reactive nitrogen species (RNS), play key regulatory roles in several homeostatic processes by interacting with proteins, fatty acids and nucleic acids. They act as intermediate agents in essential oxidation–reduction reactions (Moylean et al., 2014; Wiseman and Halliwell, 1996).

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