UTILIZATION OF *Moringa oleifera* Lam. EXTRACTS AS NATURAL ANTIOXIDANT IN GOAT (*Capra hircus* Linnaeus) MEAT PRODUCTS

By

AL-ZAIDI MOHAMMED AWAD KADHIM

Thesis Submitted to School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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DEDICATIONS

Assalam for all, first and foremost. This work was purely for the sake of Allah, and after relying on Allah, the implementation of the project was initiated. Therefore, praise be to Allah, it was a simple fruit of success that we put for society and humanity as a whole. The most important element and the active role in the success of that experiment was Professor Awis. For that, all thanks to him. Appreciation and respect for his role in helping me and overcoming all the obstacles that I faced throughout the study period. Thanks are extended to all the respected members of the discussion committee. Dr. Ismail, Dr. Shokri, and Dr. Muhamad Faris for their distinguished role in addition to the friends of the study who helped me a lot and had a vital role and great help to complete the experiment successfully, special thanks Dr. Muideen, Dr. Pavan, Mohammed, Abu Bakar and all Lab Staff in Itafos in UPM. May Allah reward you richly rewarded.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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July 2022

Chairman : Awis Qurni Sazili, PhD
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The present study was envisaged to evaluate the use of *Moringa oleifera* leaf extract as natural preservative in goat meat. Aqueous extract of *Moringa oleiferia* mature leaves (60 days) exhibited strong antioxidant activity as determined by radical-scavenging activity of 1,1-diphenyl 2 picrylhydrazyl (DPPH) as IC50 value 18.54 µg/mL, high total phenolic content (48.36 mg of gallic acid equivalent per g). Based on preliminary trial, five treatments were formulated for marination on goat meat as follows: Control (C, without the *Moringa oleifera* leaves extracts (MOLE) treatment); positive control (PC, treated with 0.1% butylated hydroxytoluene (BHT)); $T_{0.1}$ (treated with 0.1% MOLE); $T_{0.5}$ (treated with 0.5% MOLE) and $T_{1.0}$ (with 1.0% MOLE). The control and the extracts were treated on the *Longissimus dorsi* (LD), *Infraspinatus* (IF), *Biceps femoris* (BF) and *Semimembranous* (SM). The samples were marinated under refrigeration in high-density polyethylene (HDPE) bags for 7 days and assessed for various quality attributes (pH, water holding capacity, moisture, colour profile, and radical scavenging activity) on 1, 3 and 7 days, with sensory and texture profile analysis were assessed day 3 in addition to the above parameters. During refrigeration storage, all marinated muscles exhibited increased thiobarbituric acid reactive substances (TBARS) value, lowered water holding capacity (WHC) and increasing moisture content on day 3 followed by marginal decrease in moisture content on day 7. Within muscles, TBARS value of the control was recorded highest followed by $T_{0.1}$, $T_{0.5}$ and $T_{1.0}$. A significant ($p<0.05$) reduction in redness and increase in yellowness of goat meat upon marination was due to addition of MOLE than in control. MOLE extract when added to meat was found to retard lipid peroxidation. A marked deterioration on sensory attributes was recorded with the advancement of storage period; with control exhibited lowest flavour scores among treatments. Marination with MOLE had significant effect on the mean shear force value of LD muscle. The moisture content of all treatments of all 4 muscles under studies exhibited significantly ($p<0.05$) lower moisture content on 7^{th} day of storage. The pH showed an increasing trend with the advancement of storage days. The results of sensory evaluation showed that all estimation levels are within the scale 8, which lies between moderate like and like very much. The antioxidant activity of MOLE was found to be comparable to BHT.

Addition of MOLE did not affect any of the sensory attributes of marinated goat meat. The MOLE at a level of 1% was sufficient to protect goat meat against oxidative rancidity comparable to BHT added samples.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PENGGUNAAN *Moringa oleifera* Lam. EKSTRAK SEBAGAI ANTIOKSIDAN SEMULAJADI DALAM PRODUK DAGING KAMBING (*Capra hircus* Linnaeus)

Oleh

AL-ZAIDI MOHAMMED AWAD KADHIM

Julai 2022

Pengerusi : Awis Qurni Sazili, PhD
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Kajian ini telah dijalankan untuk menilai kesan penggunaan ekstrak daun *Moringa oleifera* sebagai pengawet semulajadi dalam daging kambing. Ekstrak akueus daun Moringa oleiferia matang (60 hari) mempamerkan aktiviti antioksidan yang tinggi seperti yang telah dibuktikan melalui aktiviti penghapusan radikal 2,2-diphenyl-1-picrylhydrazyl (DPPH) yang menunjukkan nilai IC50 sebanyak 18.54 µg/mL, kandungan fenolik yang tinggi (48.36 mg asid gallik bagi setiap g). Berdasarkan beberapa ujian awal, lima rawatan telah diformulasikan untuk perapan iaitu kawalan (tanpa MOLE dan BHT), Kawalan positif (PC) dengan 0.1% butylated hydroxytoluene (BHT), $T_{0.1}$ (dengan 0.1% MOLE) $T_{0.5}$ (dengan 0.5% MOLE) dan T1 (dengan 1.0% MOLE) untuk 4 jenis otot iaitu, *Longissimus dorsi* (LD), Infraspinatus (IF), Biceps femoris (BF) dan semimembranous (SM); membawa kepada jumlah 20 sampel untuk setiap eksperimen. Sampel telah diperap di dalam suhu sejuk di dalam beg HDPE selama 7 hari dan telah dinilai untuk pelbagai ciri-ciri kualiti (pH, kapasiti pegangan air, lembapan, profil warna, bahan tindak balas asid tiobarbiturik TBARS, kiraan plate count (SPC) dan kiraan psikrofilik) pada hari 1, 3 dan 7. Analisis deria (20 panel jurulatih) dan analisis profil tekstur pada hari ke-3 telah dijalankan sebagai tambahan kepada parameter di atas. Semasa penyimpanan penyejukan, semua otot yang diperap menunjukkan peningkatan nilai thiobarbituric acid reactive substances (TBARS), menurunkan kapasiti pegangan air dan meningkatkan kandungan lembapan pada hari ke-3 diikuti dengan penurunan kecil dalam kandungan lembapan pada hari ke-7. Hasil keputusan untuk otot pula menunjukkan, nilai TBARS untuk rawatan kawalan dicatatkan sebagai yang tertinggi diikuti oleh $T_{0.1}$, $T_{0.5}$ dan $T_{1.0}$; manakala nilai TBARS $T_{1.0}$ adalah setanding dengan sampel kawalan positif (PC). Pengurangan ketara ($P <0.05$) untuk nilai kemerahan dan peningkatan nilai kekuningan daging kambing semasa perapan adalah disebabkan oleh penambahan MOLE berbanding dalam rawatan kawalan. Apabila ekstrak MOLE ditambah kepada daging, ianya didapati dapat melambatkan peroksidasi lipid. Kemerosotan yang ketara pada ciri-ciri deria telah direkodkan dengan pertambahan tempoh penyimpanan; dengan rawatan kawalan mempamerkan skor perisa yang paling rendah antara rawatan. Aktiviti antioksidan

ekstrak MOLE didapati setanding dengan BHT. Penambahan ekstrak MOLE tidak menjejaskan sebarang sifat deria daging kambing yang diperap. Ekstrak MOLE pada tahap 1% adalah mencukupi untuk melindungi daging kambing daripada ketengikan oksidatif yang setanding dengan sampel yang telah ditambah BHT.

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This thesis was submitted to the Senate of University Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ABTS	Measures the relative ability of antioxidants to scavenge
APA	Anti-peroxide activity
ANOVA	Analysis of Variance
AQ	Aqueous solvent
AOA	Antioxidant activity
BHA	Butylated hydroxyl anisole
BHT	Butylated hydroxyl toluene
CHL	Chlorophyll
DPPH	2,2-diphenyl-1-picrylhydrazyl
GAE\G.D.W	Gallic acid equilibrium curve equivalent
MOLE	Moringa oliefera leaves extract
MDA	Malondialdehyde salt
PWFC	Protein water fat coefficient
PWC	protein water coefficient
PH	Potential of hydrogen
PCA	Principal component analysis
FRAP	Ferric reducing/antioxidant potential
TBA	2-thiobarbituric acid
TCA	Trichloroacetic acid
TPC	Total Plate Count
TBARS	Thiobarbituric Acid Reactive Substance
TFC	Total Flavonoid Content
TAC	Total Antioxidant capacity
TPA	Texture profile analysis

CHAPTER 1

INTRODUCTION

1.1 Background

Red meat has a vital role in providing a healthy and nutritionally balanced diet, mainly contributed by the sources of animal proteins in the human diet (Ekmekcioglu et al., 2018). Meanwhile, the increase in the global population will require more meat and meat products, mostly for the developing country (FAO, 2011). The growing meat market provides a vast opportunity for livestock rearing and the meat industry in developed nations. Nevertheless, increasing livestock production and the safe processing and marketing of hygienic meat and meat products represent a big challenge (FAO, 2019). Therefore, despite increasing meat production, the reduction of meat wastage especially within processing, distribution, and storage, should also be considered in order to meet the growing demand and ensure food security.

World population is projected to increase to reach 9 billion by 2050, most of which is forecasted in developing countries. Meanwhile, the consumption of meat is continuously increasing worldwide because of the increase in the human population. Although the consumption of meat is increasing worldwide significant portion of meat and meat products are spoiled every year (Addis, 2015). However, there is significant portion of meat and meat products are spoiled especially from microbial spoilage and lipid oxidation (Addis, 2015), which leads to the application of antioxidants in the meat and meat products (Islam et al., 2018). Meat preservation is a vital process for production and transporting of meat for a long duration without spoiling texture, colour, and nutritional value after the development and rapid growth of supermarkets (Nychas et al., 2008). The aims of preservation methods are to inhibit microbial spoilage and to minimize oxidation and enzymatic spoilage.

Antioxidants have a major role when added to meat products to prevent lipid oxidation, retard the development of off-flavours, and improve colour stability. In the food industry, they can be divided into natural and synthetic antioxidants. BHA (butylated hydroxyanisole), BHT, PG (propyl gallate), and TBHQ (tert-butylhydroquinone) are examples of synthetic antioxidants; while ingredients obtained from natural sources which exhibit anti-oxidative potential in a food model system are considered as natural antioxidants. These antioxidants play a very important role in the food industry. However, synthetic antioxidants have been identified as toxicological and carcinogenic agents in some studies (Abraham et al., 1986; Ahmad et al., 1995; Sarafian et al. 2002; Faine et al., 2006). Thus, the food industry selected natural products over artificial ones. Consequently, the food market is demanding natural antioxidants, free of synthetic additives and still orientated to diminish the oxidation processes in high-fat meat and meat products. The majority of natural antioxidants are phenolic compounds, and the most important are the tocopherols, flavonoids, and phenolic acids, which may be found widely in plant part, such as grains, fruits, nuts, seeds, leaves, roots, arils, and barks (Santos-Sánchez et al., 2019). *Moringa oleifera* is a natural antioxidant being focus of research by food technologist recently. *M. oleifera* contains myriad of phytochemicals possess antioxidant potential. Phenolics and flavonoids are the authentic antioxidants found in *Moringa* leaves that have been reported to be safe and bioactive, in which kaempferol and quercetin are recognized as the most effective antioxidants in *Moringa* leaves (Fahey, 2005; Sreelatha and Padma, 2009).

Moringa oleifera is an ornamental plant native to tropical and subtropical. All parts of the *Moringa* plant are edible, with leaves and pods used most frequently. Abundant studies have been done to evaluate the antioxidative capacity of the *M. oleifera* (Sreelatha and Padma, 2009; Ogbunugafor et al., 2011; Fitriana et al., 2016; Feihrmann et al., 2017). From the abundant research works which showed the antioxidative capacity of the *M. oleifera*, subsequent several studies have been done to evaluate the application of *M. oleifera* as a food additive. The antioxidant potential of *M. oleifera* leaf extract has been shown for the lipid stabilization of sunflower oil (Anwar et al., 2006), butter (Nadeem et al., 2013), and cream cheese (Mohamed et al., 2018). Meanwhile, Feihrmann et al. (2017) showed that *M. oleifera* leaf extract was effective to inhibit lipid oxidation in beef. This finding was also supported by Islam et al. (2018), who concluded that the effects of *M. oleifera* leaf extract as a natural antioxidant can be used in beef meatballs preparation instead of synthetic antioxidants (BHA) by dose-dependent effects. Thus, this showed that *M. oleifera* leaf extract is a good candidate as a food additive in meat. However, to the best of our knowledge, there is no evidence of *M. oleifera* as preservation on goat meat products (Hocquette et al., 2010).

1.2 Problem statement

It is clear that the use of industrial/synthetic antioxidants in preserving meat has a negative impact on consumer health such as food allergy, intolerance, nausea, hyperactivity, and even cancer. Preserving meat from natural plant sources and having a positive effect on the health of meat consumers and increases the effectiveness of its preservation period .Different maturity stages of *Moringa oleifera* leaves could result different levels of phenolic compounds. The effect of maturity stages of *Moringa oleifera* leaves on the extraction process is used as a natural antioxidant incorporated to goat meat causes various concentrations of phenolic compounds. However, different type of muscles of the goat meat could be affected differently by the *Moringa oleifera* extracts. The different types of goat muscle after incorporation with the previously selected *Moringa oleifera* leaves extract may improve the physical and chemical properties and extention of goat meat shelf life.

1.3 General objective

The general objective of this study was to determine the effects of *M. oleifera* on the quality of goat meat during storage.

The specific objectives of this study were:

1. To determine the phenolic compound of extracts from different *Moringa oleifera* leaves at different maturity levels (30 days and 60 days) using an extraction method.

2. To determine the effect of *Moringa oleifera* extracts on different type of muscles of goat meat.

1.4 Hypotheses

The total phenolic content may be affected by different age (30-60 days) of *M. oleifera* leaves. The *M. oleifera* leaves extracts in marinade may affect the shelf life and physiochemical properties of different goat muscles.

1.5 Significance of study

The current study focuses on finding natural preservatives from plant sources that are available, affordable, easy to use, and of high biological value. The reason for this is because preservatives contain valuable sources and nutrients such as minerals and vitamins. In addition, they contain highly effective materials such as phenolic compounds, carotenoids, vitamin C, and beta-carotene. These materials are used as antioxidants to prevent food spoilage especially red meat and improve the quality and properties of food and meat processing. Consequently, researchers have been working and developing these sources to be used as food additives. The objective of this study was to use *Moringa* extract as a natural antioxidant as preventive to red meat, especially the various muscles of raw goat meat and to increase their shelf life. In addition, preservatives are good agents to keep the physical and chemical properties intact and, as such, their consumption is healthy and better than the synthetic additives. It is important to mention that the synthetic additives cause several heath problems such as toxicity and cancer. This study is potentially beneficial to consumers' health. This study highlights the severe consequences against food producers to limit the use of the synthetic preservative for the sake of human requirement for health environment.

1.6 Gaps of previous studies

Incorporation of natural antioxidants in red goat meat has been experienced in the industries (Kumar et al., 2015). Antioxidants preserve the quantity and quality of meat by scavenging free radicals that cause lipid oxidation which deteriorates the shelf life of meat and meat products (Manessis et al., 2020). Studies have indicated that the use of *Moringa* extract with red goat meat had a positive significant effect. The extract is biologically characterized as active substances against lipid oxidation and meat spoilage; which increases the shelf life of goat meat. Feihrmann et al. (2017) suggested that *M. oleifera* leaf extract substitutes synthetic antioxidant in beef and, as such, inhibits lipid oxidation of beef. Kenawi and Abd El-Hameed (2018) studied the incorporation of *M. oleifera* leaf extract in buffalo meat product. In the experiment, the pH value of raw and cooked meat patties showed that the pH was not affected by *M. oleifera* and butylated hydroxy toluene (BHT) (Das et al., 2012) and that *M. oleifera* leaf extract is a potential incorporated antioxidant with a privilege of being without adverse effect on the quality. Meanwhile, Rahman et al. (2020) studied the quality and shelf-life of goat meat nuggets incorporated with *M. oleifera* leaf extract at frozen storage. Hence, the gaps in previous studies were in ignoring the effect of maturity age of *Moringa* under a single study. However, these studies did not specify the effect of the *M. oleifera* leaves incorporated with various solvents. The aim of this study was to fill these gaps along with other objectives as listed earlier.

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