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EFFECT OF MORINGA OLEIFERA ON THE SAFETY AND QUALITY OF SEMI-DRY FERMENTED SAUSAGE

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INTRODUCTION

In many countries all over the world, *Moringa oleifera* (*M. oleifera*) is used as a rich source of food due to its nutritional, antioxidant and antimicrobial benefits; as well as its ability to survive in diverse climatic conditions. Generally, the plant is known to be a fast growing and multi-functional plant with varying applications in agriculture, medicine, animal and human nutrition areas (**Ndubuaku *et al*., 2015**). Different parts of *M. oleifera*, including the leaf, root, bark, seed, flower and pod are edible and contain compounds that are important for human and animal health (**Kadhim and AL-Shammaa, 2014**).

Consumption of this plant has been reported to provide significantly amounts of essential nutrients and health promoting phytochemicals for humans (**Bamishaiye *et al*., 2011**). *M. oleifera* leaves contain higher proportion of vitamins C and A, calcium, potassium, iron and proteins than those found in other food such as orange, carrots, milk, bananas, yoghurt and spinach, respectively (**Gopalakrishnan *et al*., 2016**). Therefore, *M. oleifera* leaves have been widely used to compensate the malnutrition among infants, pregnant women and nursing mothers as well as increase milk production in lactating mothers.

Dietary supplementation with *M. oleifera* leaves has been observed to protect humans against iron deficiency and oxidative stress (**Saini *et al*., 2014**). Furthermore, the application of *M. oleifera* leaves in animals feed as a source of protein, antibiotic and antioxidant compounds has been reported to improve the growth performance, meat oxidative stability, organoleptic quality as well as reducing the rate of microbial growth in meat products after processing and storage (**Nkukwana *et al*., 2014**). The seeds of *M. oleifera* have also been used as an effective coagulant and antimicrobial agent (**Saini *et al*., 2016**), while the bark has been used a rich source of fiber (**Duke, 2001**). Moreover, the other parts of *M. oleifera* such as roots, stems, flowers and fruits have been reported to have high levels of protein, fatty acids, mineral and vitamins (**Shih *et al*., 2011**).

Based on the aforementioned quality of *M. oleifera*, several researchers directed to use this plant for improving the nutritional quality, storage stability and safety of meat and meat products either through its inclusion in animal diets or by direct application into meat products.

Meat and meat products are highly perishable foods due to their high protein and moisture contents that favor ideal media for the growth and propagation of microorganisms (**Zhou *et al*., 2010**). Microbial growth and biochemical reactions are the main causative agents of spoilage, resulting in deterioration of meat quality (**Mills, 2004**). The growth of microorganisms and their associated metabolites result in decomposition and obvious deterioration of the organoleptic quality of meat (**Ellis and Goodacre, 2001**), while lipid oxidation is the main non- microbial cause of quality deterioration in meat and its products (**Contini *et al*., 2014**). Both result in color deterioration, off odor and flavors, nutrient losses, poor shelf life, and the development of toxic compounds which can lead to food-borne illnesses (**Faustman *et al*., 2010**).

Fermented sausages are one of the most perishable products where they are produced mainly from about 70-80% meat and 20-30% fat (**Kurćubić *et al*., 2014**). High fat content increase the product susceptibility to oxidative changes and the growth of undesirable bacteria, which reduce the product shelf life stability and may cause serious hazards to the consumers. Although, the fermentation process of the sausages results in low pH and water activity which inhibit the growth of most pathogenic bacteria, many studies have indicated that some pathogens such as *Listeria monocytogenes* and *Escherichia coli* can survive in fermented sausages and may not be completely eliminated (**Lindqvist and Lindblad, 2009**). Several outbreaks of *E. coli* caused by consumption of fermented sausages have been reported in many countries (**Holck *et al*., 2017**) particularly that occurred in Norway in 2006 resulted in 18 food poisoned cases with one case of death (**Ba *et al*., 2016**). Besides the oxidation and microbial growth, the biogenic amine

formation occurs in high concentration in the fermented products. Some biogenic amines, such as tyramine and histamine have the vasoactive and psychoactive effects.

Biogenic amines have been reported as possible indicators of the un-safety of meat products (**Latorre-Moratalla *et al.*, 2012**). In general, the biogenic amines formation is mainly related to the presence of microorganisms but in case of fermented sausages the presence of high amount of the free amino acids as a result of fermentation act as additional cause for the biogenic amines formation (**Cid *et al.*, 2011**). Therefore, the control of undesirable bacterial growth and lipid oxidation and subsequently the biogenic amines formation is necessary for the shelf-life extension and safety of the fermented sausages.

Nitrite and nitrate are commonly used as curing agents and preservatives in fermented sausages production due to their antioxidant and antimicrobial effects (**Ordóñez *et al.*, 1999**). However, there is association between the nitrite and formation of methemoglobin and carninogenic N-nitrosamines which are potentially toxic compounds for human (**Chan, 2011**). Moreover, the use of synthetic antioxidants (e.g., Butylated hydroxytoluene, BHT) has been suspected to cause toxicity problems that negatively affect the consumer's health (**Larsson and Wolk, 2012**). Therefore, a new trend to substitute these synthetic antioxidants with antioxidants from natural sources (e.g., *M. oleifera* and its extracts) have been received the most attention by the consumers and meat processors because the use of natural preservatives provide many benefits to the consumer.

M. oleifera can also be used to improve the nutritional value of meat products where it contain high concentrations of essential fatty acids, amino acids and fiber which found in meat in lower concentrations **(Falowo *et al.*, 2018)**. Essential unsaturated fatty acids have the ability to control the blood cholesterol level and decrease the cardiovascular diseases (**Robiansyah *et al.*, 2014**), while the fiber can enhance the

minerals absorption, delay fat absorption, facilitate protein digestion and reduce the blood cholesterol level (**Aryana *et al.*, 2007**).

To the best of our knowledge, leaves of *M. oleifera* and their extracts have been reported as the most widely used natural antioxidant and antibacterial agent in different meat products which may be attributed to their higher nutritional, polyphenolic and antioxidant compounds (**Moýo *et al.*, 2011; Sreelatha and Padma, 2009; Jayawardana *et al.*, 2015**). Although, seeds of the plant have several and special benefits than the leaves, their application in meat products is still limited. In general, there was not enough information about the effect of addition of *M. oleifera* on the overall quality and safety of fermented sausages. Moreover, the studies that investigated the effect of *M. Oleifera* on the nutritional value, organoleptic quality and biogenic amines content of meat products are rare. Therefore the main objectives of the current study were to:-

1. Study the effect of the use of *M. Oleifera* leaves and seeds aqueous extracts on the physicochemical properties, nutritional value, organoleptic, microbiological quality as well as the biogenic amines content during the processing and chilled storage of the semi-dry fermented sausage at 4°C for 3 months.

2. Compare the antioxidant and antimicrobial effect of *M. Oleifera* leaves and seeds aqueous extracts on the semi-dry fermented either during the ripening process or the chilled storage at 4°C for 3 months.

REVIEW ARTICLE

Review Article

Moringa Oleifera as a natural additive in fermented sausage

Abstract

Fermented sausages are one of the most popular and traditional products throughout the world. However, consumption of these sausages has been associated with serious health hazards due to their high contents of saturated fats, salt, residual nitrite, nitrosamines, biogenic amines and microbial counts. Several studies attempted to overcome these problems by different ways as addition of chemicals and using various starter cultures during their processing. Chemical additives have been reported as hazardous agents to the consumer health and the starter cultures are relatively expensive. Therefore, the natural additives are the ideal solution to overcome the problems of these sausages as they regarded as safe and cheap materials. In this article, we focused on the application of *Moringa oleifera* as a novel additive to improve the nutritional, chemical and microbial safety of the fermented sausage.

Key words: *M. Oleifera*, sausage, oxidation, amines, hazards, additive

Introduction

Consumers are becoming interest and conscious about the safety, types and quantities of the ingredients that added in their foods (**Jongen and Meulenbery, 2005**). To meet the consumer demands for obtaining healthier food, the processors are using special ingredients to add value and safety of their products (**Hsieh and Ofori, 2007**). The use of synthetic ingredients is usually doubtful due to their negative effects on the human health, so new trends towards the natural additives have been increased in the food industry. Application of the phytochemicals from plants as a natural preservative in food products has become an important issue to replace the synthetic ingredients and produce healthier products (**Singh *et al.*, 2015**). Among various plants, *Moringa oleifera* has gained much importance in the recent years, as it has nutritional and pharmaceutical benefits (**Ashfaq *et al.*, 2012**). Different parts of the plant are used as treatment for various diseases as inflammation, cardiovascular, hematological,

gastrointestinal, hepatic and renal disorders (**Morimitsu *et al*., 2000**). Moreover, the leaves are good source of proteins, vitamins, minerals and essential amino acids (**Sanchez-Machado *et al*., 2010**), while the seeds provides mainly the unsaturated fatty acids (**Robiansyah *et al*., 2014**). The plant leaves have been reported as a potent antioxidant and antibacterial agent, while the seeds as an effective antifungal preservative (**Al Husnan and Alkahtani, 2016**; **Ezzat *et al*., 2020**). Therefore, *M. oleifera* leaves and seeds have been widely used in meat industry to increase the nutritional value and the shelf life of meat products (**Siddhuraju and Becker, 2003; Das *et al*., 2012**).

Fermented sausages become the most popular and familiar meat products in the world. The most recent studies reported that about 20-40% of the total produced meat products in Europe are fermented sausages (**Kumar *et al*., 2015**). These sausages are mostly processed with about 60% to 80% raw meat, 20% to 40% fat, spices and starter culture (**Kurćubić *et al*., 2014**). The sausages must be undergone a period of fermentation under controlled conditions to obtain their specific organoleptic features. Although, the fermentation is the main preservation method of these sausages, it may produce undesirable changes and toxic compounds. These changes can be concise as follows, increase in fat, salt contents, lipolytic activities and subsequently lipid oxidation (**Casaburi *et al*., 2007**). Moreover, elevation of protein, free amines contents and proteolytic activities at the end of fermentation process may result in formation and accumulation of the biogenic amines (**Sánchez *et al*., 2017**).

The biogenic amines not only cause many hypersensitive reactions for human but also aid in the formation of carcinogenic nitrosamines in the fermented sausages (**De Mey *et al*., 2014**). Regardless, the problems associated with fermentation process, the contamination of raw materials used in the processing of sausage may magnify these problems as well as may cause the microbiological hazards. Therefore, this type of meat products must be processed under hygienic conditions with addition of preservative to control their hazards. In this review, we gave an overview about the hazards associated with fermented sausages and how using *M. oleifera* as a natural

additive to overcome these hazards with production of healthier and microbiologically safer sausages.

Fermented sausage

Fermented sausages differ greatly from other meat products because of their pronounced tangy flavor as a result from the fermentation process and accumulation of lactic acid. Fermented sausages are processed by blending of coarse ground meat and fat with salt, sugar, spices and starter culture (**Kurćubić *et al.*, 2014**). This mixture are usually filled in medium diameter natural casing and fermented for several days to reach the final pH of 4.7 to 5.2 with a lactic acid content of 0.5 to 1.3%. After the fermentation, the sausage dried to achieve moisture content of approximately 35%, salt content of about 2.5 % and moisture to protein ratio ranging from 2.3:1 to 3.7:1 (**Vignolo *et al.*, 2010**). Traditionally, fermented sausages were considered healthy and safe foods. However, more recent researches reported that the consumption of fermented sausages has been associated with health hazards caused by the high contents of saturated fats and salt, presence of nitrite and degradation products such as nitrosamines. Moreover, the microbial hazards were reported as the major hazards associated with fermented sausages which may be classified as of direct and indirect nature. The direct microbial hazards nature may result from the contamination of sausage with food pathogens, while those of indirect nature originating from the metabolic activity of microorganisms causing presence of biogenic amines and mycotoxins (**Holck *et al.*, 2017**).

Hazards associated with fermented sausages

Fat type, content and oxidation

Fermented sausages are characterized by the marbling appearance which usually achieved by coarse grinding of meat and animal fat. The fat must be firm, white, and fresh, with a high melting point and a low content of polyunsaturated fatty acids to avoid fat exudation and for obtaining a clear cut surface of sausages (**Demeyer, 2004; Lebert *et al.*, 2007**). Fermented sausages undergoes many changes during the fermentation process due to the action of starter culture and production of

lactic acid which leading to acidic pH, decrease in moisture and increase in fat contents (**Con and Gökalp, 2000**). After the fermentation process, the fat content can reach to 50% (**Vignolo *et al*., 2010**). Higher content of saturated fat may cause many health hazards for the consumer as increase in blood pressure, heart rate and fatal coronary heart diseases (**FAO, 2008**). Moreover, the high fat and salt content as well as the propagation of starter culture during the fermentation process may accelerate the fat oxidation (**Campagnol *et al*., 2012**). Fat oxidation is the most important deterioration criteria because it has negative impact on the sensory quality and nutritional value of the products resulting in reduction of their shelf-life stability. Moreover, many studies reported that the oxidation of fat could be responsible for the production of toxic and carcinogenic molecules causing serious hazards for human health (**Zanardi *et al*., 2004**).

Residual nitrite

Nitrite and/or nitrate should be added during the processing of fermented sausages for preservation, development of cured color and flavor as well as prevention of fat oxidation (**Ordóñez *et al*. 1999**). The nitrate is more stable than nitrite, so it is the common form used in meat products (**Daniel and Alan, 1998**). In fermented sausage, the nitrate is reduced to nitrite by nitrate reducing bacteria or reducing agent and then the nitrite is reduced to nitric oxide (NO) which reacts with the myoglobin to produce the typical color of the product (**Sebranek and Bacus, 2007; Parthasarathy and Bryan, 2012**). Subsequently, the concentrations of nitrate and/or nitrite present in the final product become lower than that of their initial concentration and referred as residual nitrite (**Cassens, 1995**). The residual nitrite is affected by several factors such as cooking temperature; salt concentration; presence of reducing agent; pH as well as storage time and temperature. In general, the fermented sausages contain higher residual nitrite than the cooked products. It has been reported that consumption of food containing high levels of residual nitrite may cause several hazards as methemoglobinemia which can occur when nitrite reacts with hemoglobin rendering it incapable of carrying oxygen (**Chan, 2011**). Moreover, the acidic pH of these sausages may aid in the formation of nitrosamine which has carcinogenic effect (**Oostindjer *et***

***al.*, 2014)**. During curing in acidic environment, un-dissociated nitrous acid picks up a hydrogen ion and splits off a water molecule.

The resulting positively charged nitrosonium ion may then react with amino groups to form N-nitrosamines. Formation of these compounds is only possible when secondary amines are present, pH must be <5.5, and temperature must be >130°C or the product must be stored for a long time at room temperature (**Andrèe *et al.*, 2010**). N-nitrosamines can also be formed from the biogenic amines which present in high levels in this type of sausage rather than other meat products as a result of the fermentation process (**De Mey *et al.*, 2014**).

Microbial hazards

The properties of dry fermented sausages can provide the survival and growth of certain pathogens as *E. coli*, *S. typhimurium*, *S. aureus*, *L. monocytogenes*, *C. botulinum*, yeasts and molds (**Holck *et al.*, 2017**). The pathogenic microorganisms can be introduced through contaminated raw materials or through cross-contamination from equipment or personnel during processing and/or at retail.

The pathogenic *E. coli* and *Salmonella* predominantly associated with fermented sausages. Contamination of meat during the slaughtering process acts as the main source of presence of *E. coli* and *Salmonella* in fermented sausages. Although, the combinations of low pH and a_w can inhibit the growth of both pathogens in the finished products (**Incze, 1998**), several studies reported high counts of *E. coli* (**Calicioglu *et al.*, 2002; Lindqvist and Lindblad, 2009**) and *Salmonella* (**Pierre, 2015**) in the finished sausages.

Outbreaks of *E. coli* from consumption of fermented sausages were reported in Australia (**Tilden *et al.*, 1996; Strachan *et al.*, 2005**). Moreover, *Salmonella* have been involved in several outbreaks associated with consumption of fermented sausages (**Gossner *et al.*, 2012; Pierre, 2015**). Conversely to *E. coli*, *Salmonella* and *S. aureus* can grow in a wide range of pH (4 to 10) and temperature (7°C to 48°C) as well as in lower a_W (= 0.86) (**Tatini, 1976**). Although, *S. aureus* can tolerate the high salt, low

pH and a_W, few incidences on bacterial outbreaks from fermented sausages are reported which may be due to its lower ability to grow at anaerobic conditions (**Wieneke *et al.*, 1993**).

Fermented sausages may be also contaminated with *L. monocytogenes* that can cause infections varying from mild flulike symptoms to highly fatal disease (**Thèvenot *et al.*, 2005; Giaouris *et al.*, 2014**). The prevalence of *L. monocytogenes* in fermented sausages was reached up to 40% (**Skandamis and Nychas, 2015**). Therefore, the fermented sausages have been evaluated as products of low moderate incidence of listeriosis outbreaks. The outbreaks of *L. monocytogenes* from fermented sausages were reported in Philadelphia and USA, in 1986 and 1987, respectively. Although, the filling of sausage mix in the casing provide anaerobic conditions, the incidence of *C. botulinum* outbreaks from fermented sausages were rare (**Holck *et al.*, 2017**). This may be explained by the combination of low pH, low a_w, high salt content and addition of nitrite to the sausage mix ensures that *C. botulinum* will not grow in the matured fermented sausage.

Regarding the bacterial hazards, the molds were documented as the major hazards associated with fermented sausage. The processing conditions of these sausages are ideal for mold growth as *Penicillium* species. A safety concern with regard to surface growth of molds on fermented sausages is mycotoxin production. Most *Penicillium* species are capable of producing one or more mycotoxins (**Pitt and Leistner, 1991; Sweeney and Dobson, 1998**). Previous studies were reported that the molds isolated from fermented sausage mainly were toxigenic *Penicillium* strains (**Lòpez *et al.*, 2001; Sunesen and Stahnke, 2003**) and the production of mycotoxins in the sausages also has been reported (**Sweeney and Dobson, 1998**; **Iacumin *et al.*, 2009; Iacumin *et al.*, 2011**).

Biogenic amines

Biogenic amines are basic, non-volatile low-molecular weight, nitrogenous compounds, found in living organisms where they carry out many physiological functions (**BIOHAZ, 2011**). The biogenic amines are considered as an indicator for

food safety because they may be found in various foods and when consumed in huge amounts may cause certain diseases as disturbance in blood pressure, headaches, cardiac disease and nausea (**Gardini *et al*., 2016**).

The most important biogenic amines found in fermented sausages are histamine, putrescine, cadaverine, tyramine, tryptamine, phenylethylamine, spermine, and spermidine. Histamine and tyramine are the most toxic amines and the presence of other amines may enhance their toxic effects (**Shalaby, 1996**). The biogenic amines are formed in foods by the action of amino acid decarboxylases which mainly found in spoilage microorganisms and some desirable starter cultures used in fermentation process of the sausage (**Latorre-Moratalla *et al*., 2012**).

The contamination of fermented sausage by Gram-negative *enterobacteria* is the most important cause in the formation of biogenic amines before the onset of the fermentation. Moreover, during the fermentation of the sausage, the presence of high amounts of protein, the free amino acids and the naturally occurring decarboxylating microflora result in the formation of the biogenic amines (**Cid *et al*., 2011**). It has been reported that the biogenic amines were implicated in several food poisoning outbreaks (**Ekici and Omer 2018**). Moreover, the biogenic amines categorized as a carcinogenic precursor, as some amines may react with nitrite and produce the nitrosamines which are carcinogenic and cause a potential health threat to humans. However, the dangerous effect of biogenic amines, there is no clear standards or guidelines determine the permissible concentrations of biogenic amines in fermented sausages.

These hazards forced the meat processors to seek about a specific preservative technique that maintains the quality and safety of the sausages. The most common way for sausages preservation is addition of synthetic antimicrobial and antioxidant agents during their processing. However, the over use of such agents has been linked to several health hazards (**Singh *et al*., 2015**) so, there are international standard limits for using each additive. Moreover, with increasing the consumer worries about the quantity of chemicals in their foods, the processors are looking for natural ways to preserve the products.

The natural antimicrobial and antioxidant compounds from large numbers of plant sources (fruits, cereals, vegetables, herbs and spices) have been applied in meat and meat products as alternative to synthetic preservatives because of their safety, nutritional and therapeutic value. Among various plants, *Moringa oleifera* has gained much importance in the recent years due to its availability and benefits.

Moringa oleifera as a natural additive in fermented sausage

Moringa Oleifera is a native plant found in Africa and Asia particularly in India, it belong to the family *Moringaceae* (**Iqbal and Bhanger, 2006**). It is categorized as one of the most beneficial tropical trees (**Ashfaq *et al.*, 2012**) and it is also known by various names, as *Horseradish, Benzolive, Kelor, drumstick, Marango, miracle, Mlonge, Saijihan, Mulangay and Sajna ben oil* tree (**Fahey, 2005**). All parts of the plant (leaves, flowers, seeds, fruit and roots) have been consuming by humans throughout the recent years (**Iqbal and Bhanger, 2006**) for its medical and nutritional importance (**Khalafalla *et al.*, 2010**). *Moringa leaves* exhibit anti-tumor, anti-inflammatory, anti-ulcer, anti-atherosclerotic, anti-convulsant, and antioxidant activities (**Chumark *et al.*, 2008**). Moreover, the seeds show antimicrobial, antitumor, anti-inflammatory and antispasmodic activities (**Daljit *et al.*, 2013**). In general, the plant contain high amount of protein, polyunsaturated fatty acids, vitamin A and C, calcium, potassium, iron (**Manzoor *et al.*, 2007**), tocopherols, carotenoids (**Smolin and Grosvenor, 2007**), polyphenols (**Bennett *et al.*, 2004**), alkaloids (**Soliva *et al.*, 2005**) and flavonoids (**Lako *et al.*, 2007**). Therefore, this plant can be applied in fermented sausages to increase their nutritional value and safety by solving the dangerous hazards associated with such products.

Moringa Oleifera as a source of polyunsaturated fatty acids

Moringa oleifera leaves and seeds rich in monounsaturated fatty acids. The fatty acid compositions of *M. oleifera* seeds extract showed 70, 7.8 and 7.6% for oleic, palmitic and stearic acids, respectively (**Abdulkarim *et al.*, 2005**; **2007**; **Robiansyah *et al.*, 2014**). Therefore, the plant seeds oil can be used as a cheaper alternative to more expensive monounsaturated oils as olive oil (**Tsaknis and Lalas, 2002**). Moreover, the

fatty acids analysis of plant leaves contained 44.57, 14.41 and 0.20% for α-Linolenic, heneicosanoic and palmitic acids, respectively (**Moýo *et al.*, 2011**). That mean the plant seeds provides mainly oleic acids, while the leaves provides mainly linolenic acid which have the ability to control the cholesterol level in the blood and reduce the heart diseases.

The quality of meat from different animal species was investigated after inoculation of *M. oleifera* at level ranged from 1 to 5% in their diets. The results revealed that there was obvious elevation in unsaturated fatty acids% without any negative impact on the sensory quality of meat by increasing the level of plant in the diet (**Mukumbo *et al.*, 2014; Najeeb *et al.*, 2014; Nkukwana *et al.*, 2014**). Based on the previous results, we can conclude that *M. oleifera* can be applied in fermented sausage to replace part of saturated fat and subsequently decrease their hazards.

Antioxidant activity

Different parts of *M. oleifera* contain high amounts of proteins, minerals, vitamins and antioxidant compounds (**Anwar *et al.*, 2007**). Among various parts of the plant, *M. oleifera* leaves considered the most potent source of antioxidant due to the presence of different compounds as phenolic, flavonoids (**Dillard and German, 2000**; **Siddhuraju and Becker, 2003**; **Tumer *et al*, 2015; Wang *et al.*, 2017**), the sugar rhamnose, isothicoyanates, the glucosinolates (**Hsu *et al*, 2006; Ashfaq *et al*, 2012**), vitamin A, C and E, carotenoids, caffeoylguinic acids (**Siddhuraju and Becker, 2003; Aslam *et al*, 2005; Mbikay, 2012; Abdull Razis *et al*, 2014**), calcium, arginine and histidine (**Ferrao and Ferrao, 2005**). These compounds have the ability to chelate the free radicals and inhibit the enzymes so, reduce the damage caused by the free radical and oxidation (**Larson, 1988**; **Byme *et al.*, 2002; Sreelatha and Padma, 2009**). This indicates that *M. oleifera* leaves or their extracts can be used as a natural antioxidant to reduce the oxidation and extend the shelf life of the products. It has been reported that the application of *M. oleifera* leaves powder at the level of 1.5% led to improve the quality of beef meat balls by delaying the oxidation throughout the cold storage (**Kenawi and Mohamed, 2017**). **Muthukumar *et al.*** (**2014**) reported that addition of

M. oleifera leaves extract at rate of 600 ppm resulted in reduction of lipid oxidation in pork patties during chilled storage.

Furthermore, several studies reported that *M. oleifera* leaves extract at level of 1-1.5% was more effective than BHT in the reduction of TBARS values of ground buffalo and goat patties during storage at 4 °C (**Hazra *et al.* 2012; Das *et al.*, 2012**). Recently, the effect of *M. oleifera* leaves and seeds extracts in fermented sausage was investigated by **Ezzat *et al.* (2020)**. The authors reported that application of both extracts at level of 1.5% during processing of the sausage led to significant reduction in TBARS values below the safe permissible limit when compared to the control which exceeded this limit at 3rd month of storage 4°C.

Effect of *Moringa oleifera* on the residual nitrite and nitrosamine formation

M. oleifera rich in ascorbic acids, flavonoids and polyphenolic compounds (**Abdull Razis *et al*, 2014; Tumer *et al*, 2015; Wang *et al*., 2017**). The average of ascorbic acid in the plant leaves and seeds was 204 and 130 mg/100 g of fresh weight, respectively (**Siddhuraju and Becker 2003; Koheil *et al*., 2011**). Ascorbic acid was reported as a potent reducing agent that reduced the nitrite to nitric oxide and subsequently, decreased the amount of residual nitrite in the final products (**Sen *et al*., 1976; Rywotycki, 2007; Haak *et al*., 2009; Doolaege *et al*., 2012**). Moreover, the phenolic compounds and flavonoids can react with the nitrite and convert it to different reducing forms leading to reduction of the residual nitrite content (**Balzer *et al*., 2007; Viuda Martos *et al*., 2010**). *M. oleifera* also contain high levels of organic and phenolic acids which may decrease the pH of the products (**Rodríguez *et al*., 2016**) leading to decrease in the level of residual nitrite (**Rahman, 2007)**. The reduction in residual nitrite levels in processed meat is an important issue to decrease the incidence of the carcinogenic N-nitrosamines formation (**Honikel, 2008**). It has been reported that the ascorbic acid had powerful effect in lowering the N-nitrosamines formation (**Fiddler *et al*., 1981; Ahn *et al*., 2004**; **Li *et al*., 2012**). Additionally, in the vitro studies reported that the flavonoid compounds of *M. oleifera* strongly inhibit the production of nitrosamines (**Ferreira *et al*., 2008**). Therefore, *M.*

oleifera can be applied as a natural reducing agent in fermented sausage to produce safe product with lower residual nitrite and nitrosamines.

Antimicrobial activity

M. oleifera possess potent antimicrobial activities even against the organisms that have become resistant to the antibiotics (**Cowan, 1999, Broin *et al.*, 2002; Rahman *et al.*, 2009**, **Sofy *et al.*, 2017**). The antimicrobial activity of the plant may be due to the presence of high content of active compounds as alkaloid, flavonoids, saponins, tannins, quinines, and iridoids (**Nilforoushzadeh *et al.*, 2008**). These active compounds were able to damage DNA, disrupt the cellular membrane, inhibit ATPase activity of various bacteria as *Escherichia coli*, *Salmonella typhi*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Staph aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Clostridium* spp, *Proteus aeruginosa* and *Proteus vulgaris* (**Moreno *et al.*, 2006**; **Raybaudi-Massilia *et al.*, 2009**; **Karim and Azlan, 2012; Vinoth *et al.*, 2012; Daljit *et al.*, 2013, Al Husnan and Alkahtani, 2016)**.

Moreover, the antifungal activities of the plant were reported against *Aspergillus niger*, *Aspergillus flavus*, *Penicillium italicum*, *Fusarium oxysporum*, *Rhizopus stolonifer*, *Alternaria* sp., Candida albicans and Candida parapsilosis (**Al Husnan and Alkahtani, 2016**). Previous studies reported that the plant seeds had the powerful antimicrobial effect than other plant parts where, the recombinant protein of the seeds has the ability to flocculate and damage the bacterial cells (**Casey, 1997**). Moreover, the seeds contain antimicrobial peptides enzymes (**Silvestro *et al.*, 2000; Suarez *et al.*, 2003**) and 4(α-L-rhamnosyloxy) benzyl isothiocynate (**Eilert *et al.*, 1981**) which can inhibit the microbial growth through disruption of the cell membrane and essential enzymes synthesis (**Idris, 2016**).

Application of *M. oleifera* as a natural antimicrobial agent in meat and meat products has been investigated. **Sharaf *et al.*** (**2009**) concluded that addition of *Moringa* meal flour as extender in beef burger patties improved their sensory, physiochemical, microbiological and nutritional quality attributes. Moreover, **Najeeb *et al.* (2014)** found that addition of *Moringa* leaf powders at level of 1% led to

reduction in the microbial counts without any negative impacts on other quality attributes of restructured chicken slices during chilled storage for 20 days.

Improvement of antimicrobial, antioxidant and organoleptic quality of cooked ground buffalo meat by addition of *M. Oleifera* leaves extract at rate of 1.5% was reported by **Hazra *et al.*** (**2012**). Application of *M. Oleifera* in fermented sausages is rare but **Ezzat *et al*.,** (**2020**) reported significant reduction in bacterial and mold counts of semi-dry fermented sausages treated with *M. Oleifera* leaves and seeds extracts in comparison to control group during storage at 4°C for 3 months.

Effect of *Moringa oleifera* on biogenic amines

Biogenic amines are indirect hazards resulted from the metabolic activities of certain bacteria and molds (**Holck *et al*., 2017**). Higher bacterial counts of foods are usually related to higher levels of the biogenic amines particularly putrescine (**Ruiz-Capillas and Jiminez-Colmenero, 2004**). *Enterobacteria* as *Salmonella* and *E. coli* are decarboxylase-positive bacteria that produce huge amounts of cadaverine and histamine (**Komprda, et al., 2009**), while *B. thermosphacta* has been related to the formation of the tyramine (**Nowak *et al*., 2011**). Moreover, it has been reported that the molds growth can elevate the concentrations of the biogenic amines in the foods specially putrescine and histamine (**Montel et al., 1999**). Some studies reported that the presence of biogenic amines in foods also may be related to the incidence of lipid oxidation (**Özyurt *et al*, 2009**; **Hidalgo *et al*., 2010; Zamora *et al*., 2012**). Therefore, *M. oleifera* may decrease the levels of biogenic amines in foods due to its antimicrobial and antioxidant activities (**Al-Juhaimi *et al*., 2016; Sofy *et al*., 2017**).

The studies on the effect of *M. oleifera* on the biogenic amines in meat and meat products are scarce. However, **Ezzat *et al*., (2020)** reported that addition of *M. oleifera* leaves and seeds extracts during the processing of semi-dry fermented sausages led to significant reduction in all examined amines when compared to the control during storage at 4°C for 3 months.

Conclusion

It can be concluded that *Moringa oleifera* is a good source of monounsaturated fatty acids, antimicrobial and antioxidant compounds as well as can decrease the residual nitrite and biogenic amines contents in foods. Therefore, it can be recommended that *Moringa oleifera* may be used as a natural, safe and cheap additive in fermented sausages to prevent the toxic effect of chemical preservative, decrease the hazards and improve the nutritional quality of these sausages.

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Effect of *Moringa oleifera* aqueous extracts on the physicochemical characteristics, microbiological quality and biogenic amines of semi-dry fermented sausage

Abstract

The main objective of the current study was to investigate the effect of *Moringa oleifera* leaves and seeds aqueous extracts on the physicochemical properties, microbiological quality and biogenic amines content of semi-dry fermented sausage during ripening process and storage at 4ºC for 3 months. Semi-dry fermented sausages were formulated by using *M. oleifera* leaves and seeds aqueous extracts at a rate of 1.5% in comparison to control. Incorporation of *M. oleifera* leaves aqueous extracts during the formulation of fermented sausage resulted in a significant ($P<0.05$) decrease in pH, lipid oxidation and total volatile nitrogen content, while significant ($P<0.05$) increase in the lactic acid bacteria when compared with those formulated with seeds and control groups. However, the yeast, mold and biogenic amines content of sausages formulated with *M. oleifera* seeds aqueous extract were significantly ($P<0.05$) lower than those formulated with leaves and the control. From this study, we can conclude that *M. oleifera* leaves aqueous extract exhibited potent antioxidant activity, while that of seeds exhibited potent antimicrobial activity. Therefore, both *M. oleifera* extracts can be used as natural additives to improve the quality and safety of semi-dry fermented sausage.

Keywords: *Moringa*, extract, sausage, ripening, amines, quality.

1. Introduction

Fermented sausages are a unique group of meat products, which characterized by special sensory, physicochemical, and microbial quality attributes. The type of starter culture, added substrates, and conditions during the ripening process determine differences in the quality of the fermented sausage. During ripening, the propagation of starter culture and the consequent production of metabolites, decline in pH and moisture content as well as, increase in fat level give the fermented sausage its desired organoleptic and texture properties. However, the high-fat content may result in rapid

deterioration of the products and the low moisture content may create a favorite condition for the growth of mold and yeast which is the main problem associated with fermented sausage (**Casaburi *et al.*, 2007**). Protein degradation during ripening also determines the quality of fermented sausage. The formation of peptide and free amino acids contributes to the basic flavor of the sausage, while its further degradation by microorganisms results in the formation of biogenic amines (**Sánchez *et al.*, 2017**).

The main contribution of biogenic amines in food is the content of both proteins and amino acids, and the contamination with decarboxylating microorganisms. During the ripening of fermented sausage, the presence of high amounts of the free amino acids and the naturally occurring decarboxylating microflora result in the formation of the biogenic amines particularly tyramine, putrescine, and histamine (**Cid *et al.*, 2011**). Therefore, special additives must be added during the production of fermented sausage to maintain its quality and safety. Synthetic preservatives as nitrite, butylated hydroxytoluene and sorbate have been widely used to control the oxidative and microbial changes and to prolong the storage life of the fermented sausage. However, such additives are associated with many human health risks (**Larsson and Wolk, 2012**). Therefore, the use of natural materials e.g., pharmaceutical plants and their extracts has become a novel trend to produce more safe products.

Moringa Oleifera has gained much importance in the last decades due to its nutritional, health and industrial benefits. The leaves of *M. Oleifera* contain high levels of protein, vitamins, minerals, tocopherols, carotenoids, polyphenols, alkaloids and flavonoids (**Lako *et al.*, 2007**). *M. Oleifera* leaves has been used for the treatment of tumors, ulcers, inflammations, convulsions, and atherosclerosis (**Chumark *et al.*, 2008**). In the food industry, *M.Oleifera* is used successfully to control the lipid oxidation and the control of microbial growth (**Siddhuraju and Becker, 2003**), while the seeds have bacteriostatic activities particularly against *Staph. aureus*, *E. coli*, *S. typhi*, and *S. typhimurium* (**Daljit *et al.*, 2013**).

Previous studies on *M. Oleifera* had focused on the use of leaves extracts in the preservation of meat, burger patties, and fresh sausage; however, its application in

fermented sausage is limited. Moreover, the comparison between *M. Oleifera* leaves and seeds extracts, as natural preservatives in meat products is scarce. Therefore, the foremost objective of this work was to study the effect of the use of *M. Oleifera* leaves and seeds aqueous extracts on the physicochemical, microbiological quality and the biogenic amines contents during the processing of semi-dry fermented sausage.

2. Materials and methods

2.1. Raw materials

Imported frozen Brazilian beef topside blocks were obtained from a local store in Cairo, Egypt within the first third of its shelf life. Fresh mesenteric beef fat was collected from Cairo abattoir within two hours after slaughter, washed and stored at -18 °C. Both meat and fat were minced with a 5 mm grinder plate (Seydelmann, Germany) immediately before processing. The temperature of meat and fat was maintained around -5 ºC. A 30 mm cellulose casing was provided from Podanfol Professional Packaging (Poland). Sodium nitrite, ascorbic acid, lactose, and glucose were obtained from Loba Chemie (Mumbai, India). *Lactobacillus helveticus* (Lh-BO2) and spice oil extracts were purchased from Chr, Hansen (Denmark) and Nubassa (GewürzwerkGmpH, Viernheim, Germany) respectively. Sodium chloride was purchased from a local supplier in Cairo, Egypt. Moreover, *M. oleifera* leaves and seeds were purchased uncrushed from the experimental plant station, Faculty of Pharmacy, Cairo University, Egypt.

2.2. Preparation of *M. oleifera* leaves and seeds aqueous extracts

The leaves and seeds were separately dried at 60 ºC for 24-48 hours in Bionics Scientific hot air oven (India). The dried leaves were ground into a fine powder while the dried seeds were dehusked and ground through 0.2 mm screen before preparation of the extract. Aqueous extracts of *M. oleifera* leaves and seeds were prepared by soaking 2 kg of the dried sample in 6 liters of sterile warm distilled water at 60 °C for 24 hours with frequent shaking. The aqueous extracts of both leaves and seeds were filtered by sterile muslin cloths then by Whatman No.1 filter paper to remove the extractable substances. The aqueous extracts were then concentrated using a rotary

evaporator (HEIDOLF, HEID_31002, Germany) set at 50 °C bath temperature and connected with a vacuum pump (HAHN SHIN, HS-3000, Korea) of 72mbar. The prepared extracts were stored at 4 °C in a sterile glass container until use.

2.3. Examination of *M. oleifera* leaves and seeds aqueous extracts

2.3.1. Qualitative phytochemical analysis

The simple qualitative phytochemical tests were carried out to detect the presence of flavonoids, alkaloids, phenolic compounds, saponins, and volatile oils following the procedures defined by **Evans *et al.*** (**2002**).

2.3.2. pH and free radical scavenging activity {2, 2-Diphenyl-2-picrylhydrazyl (DPPH)}

The pH value was determined by using pH meter (Lovibond Senso Direct) with a probe-type electrode (Senso Direct Type 330), where three reading for each extract was obtained and the average was calculated. The procedures described by (**Moraes-de-Souza *et al.*, 2008**) were followed for evaluation of the scavenging activity of the plant aqueous extracts.

2.4. Processing of semi-dry fermented sausage

A base batter was prepared according to the General Manufacturing Practices using 80% beef topside, 15% beef fat, 2.0% sodium chloride, 0.02% sodium nitrite, 0.05% ascorbic acid, 1% lactose, 0.50% glucose and 0.05% spice mix. The ground beef and fat were mixed with the dry non-meat ingredients in Seydelmann spiral mixer (Urgstallstraβe, Germany). *L. helveticus* starter culture was added with a dose to achieve 10^5 CFU/g of the sausage mix. The base batter was divided into 3 equal portions; the 1st was used as a control, while the 2nd and 3rd portions were inoculated separately with 1.5% aqueous extract of *M. oleifera* leaves and seeds. The sausage mix was filled into 30 mm cellulose casing (500 g each) using Handtmann VF 628 vacuum filler (Baden-Wurttemberg, Germany) and kept in a ripening chamber at 20ºC and 70% relative humidity for 4 days to achieve a pH value of 5.20. After the end of the ripening period, sausages were cooked up to 72ºC core temperature, then stored at 4ºC

for 3 months. The experiment was repeated three times with three replicates at independent time.

2.5. Examination of semi-dry fermented sausage

For each replicate, three samples were withdrawn for determination of physicochemical, microbiological quality attributes and biogenic amines content throughout the ripening process and storage at 4ºC for 3 months.

2.5.1. Physicochemical properties

2.5.1.1. pH, Thiobarbituric Acid Reactive Substances (TBARS) and Total Volatile Base Nitrogen (TVBN)

For measurement of pH, 5 g sample was homogenized with 20 ml distilled water for 30 seconds and then the pH of the homogenate was determined using a digital pH meter. TBARS value (milligrams of malonaldehyde per kilogram) was evaluated using the method of **Du and Ahn** (**2000**). The TVBN value (milligrams per 100 grams sample) was measured following the procedures established by **Kearsley *et al.*** (**1983**).

2.5.2. Microbiological examination

Lactic acid bacteria were counted according to the procedures defined by De **De Man *et al.*** (**1960**), while those of **Beuchat and Cousin** (**2001**) were followed for enumeration of total yeast and mold counts. The average of each microbial counts of each sample was separately calculated and expressed as colony-forming unit per gram ($\log_{10}$ CFU /g).

2.5.3. Biogenic amines

The amounts of the biogenic amines in the sausages were determined following the procedures of **Sultan and Marrez** (**2014**).

2.6. Statistical analysis

Each analysis was done in three replicates, and the collected results were analyzed using SPSS statistics 23.0 for windows. Results were tabulated as mean ± SE. The

Paired-samples T-test was used to compare the results of pH and between the leaves and seeds aqueous extracts. However, one-way analysis of variance was done by ANOVA procedure to compare the results of physicochemical, microbiological and biogenic amines among the different sausage treatments throughout the ripening and storage period. The least-square difference test (LSD) procedures were used to determine the significances at the level of ($P<0.05$).

3. Results and discussion

3.1. Phytochemical analysis, pH and scavenging activity of the plant extracts

The phytochemical results revealed that aqueous extracts from both *M. oleifera* leave and seeds contained the flavonoids and alkaloids, while the phenolic compounds were detected only in the leaves and the saponins and volatile oils were detected only in the seeds (Table 3.1.1). The results also clarified that the pH value was significantly ($P<0.05$) lower in the leaves extract than that of the seeds with mean values of 4.50±0.01 and 4.68±0.03 respectively. Lower pH value of leaves may be attributed to their high contents of phenolic compounds which constitute mainly from acids as ellagic, tannic, benzoic, and caffiec (**Gaafar *et al.*, 2016**). However, there were non-significant ($P>0.05$) differences in DPPH % between the leaves and seeds extracts with mean values of 82.61±1.22 and 81.80±1.03% respectively. The antioxidant activity of the plant extracts may be related to the presence of the flavonoids in both leaves and seeds; moreover, the presence of the phenolic compounds in the leaves potentiates their scavenging activity than the seeds. These results were in harmony with those of **Siddhuraju and Becker** (**2003**) who reported that the antioxidant properties of plant extracts were directly related to their contents of phenolic and flavonoid. The results also fixed the findings obtained by **Unuigbe *et al.*** (**2014**) who found that *M. oleifera* leaf extract had higher total phenolic compounds and flavonoid contents when compared to those of seeds extract.

Table 3.1.1: Qualitative phytochemical analysis, pH values and DPPH% of *M. oleifera* leaves and seed aqueous extracts

	Leaves extract	**Seeds extract**
Phytochemical analysis		
Flavonoids	+	+
Alkaloids	+	+
Phenolic compounds	+	-
Saponine	-	+
Volatile oils	-	+
pH	4.50 ± 0.01^{a}	4.68 ± 0.03^{b}
DPPH%	82.61 ± 1.22^{a}	81.80 ± 1.03^{a}

*Values represent the mean of three independent replicates ± standard error

* $^{a-b}$: Values with different superscript within the same raw differ significantly at $P<0.05$

3.2. pH, TBARS, and TVBN

Incorporation of *M oleifera* leaves and seeds aqueous extracts during the formulation of fermented sausage resulted in a significant ($P<0.05$) decrease in pH values during the ripening and storage periods in comparison to the control (Table 3.1.2). In general, the ripening process of sausages resulted in a significant ($P<0.05$) decrease in pH values which may be due to the formation of lactic acid content as a result of carbohydrate breakdown by the inoculated starter culture. The addition of plant extracts decreased the ripening time of sausages to reach the desired pH (5.20) from 4 to 3 days (Table 3.1.2). *M. oleifera* had reasonable levels of flavonoids, organic acids, and phenolic acids which are responsible for the acceleration of the ripening process of sausage. Moreover, the leaves contain higher amounts of nutrients that promote the growth of probiotic bacteria, which may explain the significant reduction in pH during the ripening period when compared with the seeds (**Amer *et al.*, 2014**). However, the chilled storage induced a significant ($P<0.05$) increase in pH values in

all formulations which may be related to the growth of spoilage bacteria and the production of ammonia due to the proteolytic activity of bacteria.

The fat oxidation criteria revealed the presence of significant ($P<0.05$) differences between the different sausage treatments. The sausage formulated with *M. oleifera* leaves had the lowest values, while the control samples had the highest values (Table 3.1.2). These results indicated that leaves exhibited obvious antioxidant activities when compared with seeds. The results fixed the finding of **Unuigbe *et al.*** (**2014**), who reported that the *M. oleifra* leaves extract had higher mineral, vitamins, sugars and natural antioxidants in comparison with the seeds. The results also showed that the TBARS values of the control group were significantly ($P<0.05$) increased to reach a value higher than the permissible limit (> 1 mg/kg malonaldehyde, **Zanardi *et al.*, 2004**) at the end of the storage period. However, the sausages formulated with both extracts showed lower values than this limit until the end of the chilled storage. Elevation of TBARS values may be due to dehydration, the elevation of fat content and bacterial growth during the ripening and storage (**Wang *et al.*, 2015**).

TVBN values of sausages treated with *M. oleifera* extracts were significantly ($P<0.05$) lower than those of the control (Table 3.1.2). Incorporation of *M. oleifera* extracts during the processing of fermented sausage led to the acceleration of growth of the starter culture, rapid falling in pH values, control the growth of natural microflora and subsequently decrease TVBN values (**Lorenzo *et al.*, 2000**). The results also clarified that the TVBN contents of control sausage were significantly ($P<0.05$) increased during the ripening process and chilled storage and exceeded the regulatory level (35 mgN/100 g, **Wang *et al.*, 2015**) at the end of chilled storage. However, sausage treated with *M. oleifera* extracts remained below the permissible limit until the end of the storage period.

Table 3.1.2: pH, TBARS and TVBN values of semi-dry fermented sausage during ripening and storage period at 4°C for 3months.

pH								
	Ripening				**Storage**			
	1st day	**2st day**	**3st day**	**4st day**	**0 time**	**1st month**	**2nd month**	**3rd month**
C	5.95±0.00^{a,A}	5.78±0.39^{a,B}	5.42±0.01^{a,C}	5.20±0.02^{a,D}	5.49±0.01^{a,E}	5.68±0.01^{a,F}	5.72±0.01^{a,G}	5.88±0.01^{a,H}
L	5.73±0.01^{b,A}	5.34±0.00^{b,B}	5.20±0.00^{b,C}	5.05±0.01^{b,D}	5.30±0.00^{b,E}	5.35±0.01^{b,E}	5.40±0.01^{b,F}	5.44±0.02^{b,F}
S	5.88±0.01^{c,A}	5.72±0.01^{a,B}	5.22±0.00^{b,C}	5.10±0.01^{b,D}	5.36±0.02^{b,C}	5.39±0.02^{b,C}	5.47±0.00^{c,D}	5.50±0.01^{c,E}
TBA (mg malonaldehyde/kg)								
C	0.39±0.01^{a,A}	0.43±0.01^{a,B}	0.44±0.01^{a,B}	0.48±0.01^{a,C}	0.67±0.01^{a,D}	0.78±0.01^{a,DE}	0.85±0.01^{a,E}	1.20±0.01^{a,F}
L	0.07±0.03^{b,A}	0.13±0.01^{b,B}	0.17±0.01^{b,C}	0.22±0.00^{b,D}	0.33±0.01^{b,E}	0.34±0.01^{b,E}	0.40±0.01^{b,F}	0.47±0.01^{b,G}
S	0.18±0.01^{c,A}	0.23±0.01^{c,B}	0.27±0.01^{c,C}	0.30±0.01^{c,D}	0.40±0.01^{c,E}	0.34±0.02^{b,F}	0.44±0.01^{c,G}	0.54±0.01^{c,H}
TVBN (mg/100g)								
C	8.49±0.24^{a,A}	10.17±0.25^{a,A}	14.26±0.85^{a,B}	18.66±0.09^{a,C}	19.78±0.46^{a,C}	23.99±0.61^{a,D}	32.13±1.26^{a,E}	39.57±1.25^{a,F}
L	7.18±0.33^{b,A}	8.68±0.16^{b,A}	11.38±0.33^{b,B}	13.16±0.32^{b,C}	18.48±0.58^{a,D}	22.68±0.74^{a,E}	29.40±0.42^{b,F}	30.40±0.16^{b,G}
S	7.28±0.16^{b,A}	10.17±0.25^{a,B}	11.94±0.33^{b,C}	14.18±0.18^{c,C}	19.60±0.16^{a,E}	22.58±0.65^{a,F}	31.54±0.18^{ab,G}	31.45±0.51^{ab,H}

C (control), L (sausage with leaves aqueous extract), S (sausage with seeds aqueous extract); *Values represent the mean of three independent replicates ± standard error; * $^{a-c}$: Values with different superscript within the same column differ significantly at $p<0.05$

* $^{A-H}$: Values with different superscript within the same raw differ significantly at $p<0.05$

3.3. Microbiological analysis

Lactic acid bacterial counts were significantly ($P<0.05$) higher in sausages formulated with *M.oleifera* leaves extract when compared with those formulated with seed extract and the control during the ripening and chilled storage (Table 3.1.3). The high content of essential amino acids in *M.oleifera* leaves may be the main reason in the elevation of lactic acid bacteria (**Amer *et al.*, 2014**). However, the yeast and mold counts were significantly ($P<0.05$) lower in sausage formulated with both extracts. The antimicrobial activities of the *M. oleifera* extracts may be due to the presence of high content of alkaloids, tannins, and saponine. These active compounds can inhibit ATPase activity, damage DNA and cellular membrane of the microorganism (**Raybaudi-Massilia *et al.*, 2009**). Previous studies reported that *M. oleifera* seeds exhibited potent antimicrobial actions than the leaves (**Daljit *et al.*, 2013**) because it contains a short polypeptide 4 (ά – L – rhamnosyloxy) benzyl-isothiocyanate which inhibits the microbial growth through disruption of the cell membrane and essential enzymes synthesis (**Idris, 2016**). The results also showed that the ripening period resulted in a significant ($P<0.05$) increase in lactic acid bacterial counts and significant ($P<0.05$) decrease in yeast and mold counts in different sausage treatments. At the end of the ripening period, the yeast and mold contents were below the detectable counts in the sausage formulated with the seed extract. On the contrary, the chilled storage resulted in a significant ($P<0.05$) decrease in lactic acid bacterial counts in all sausage treatments and a significant ($P<0.05$) increase in yeast and mold counts of the control only where the sausage formulated with both plant extracts showed yeast and mold counts below the detectable limits throughout the storage period. These results were in agreement with those of **Anwar and Rashid** (**2007**) who reported that *M oleifera* seeds extracts had more effective antifungal action than leaves extract.

Table 3.1.3: Microbiological counts (log_{10} cfu/g) of semi-dry fermented sausage during ripening period and storage at 4°C for 3months.

	Lactic acid bacteria							
	Ripening				Storage			
	1st day	2st day	3st day	4st day	0 time	1st month	2nd month	3rd month
C	5.24 ±0.08^{a,A}	5.53±0.04^{a,B}	5.77±0.02^{a,AB}	6.48±0.08^{a,B}	3.40±1.21^{a,C}	2.66±0.84^{a,D}	2.05±0.08^{a,E}	1.90±0.22^{a,E}
L	5.95 ±0.02^{b,A}	6.00±0.04^{b,A}	7.16±0.09^{b,B}	7.41±0.01^{b,B}	4.24±0.11^{b,C}	3.35±0.04^{b,D}	3.31±0.16^{b,D}	3.29±0.03^{b,D}
S	4.96^{c,A}±0.01^{c,A}	5.81±0.02^{a,AB}	6.86±0.34^{b,B}	6.59±0.05^{a,B}	4.28±0.05^{b,C}	3.23±1.23^{b,D}	2.76±0.09^{c,E}	2.69±0.06^{c,E}
	Yeast							
C	3.39 ±0.02^{a,A}	3.04±0.07^{a,AB}	2.59±0.06^{a,ABC}	2.40±0.20^{a,ABC}	0.89±0.89^{a,D}	1.44±0.72^{a,CD}	1.63±0.82^{a,BC}	2.53±0.27^{a,ABC}
L	3.23 ±0.06^{ab,A}	1.98±0.99^{a,AB}	0.95±0.95^{ab,B}	0.87±0.86^{ab,B}	<2.00 ±0.00^{a,B}	<2.00 ±0.00^{b,B}	<2.00±0.00^{b,B}	<2.00 ±0.00^{b,B}
S	3.16 ±0.03^{b,A}	1.43±0.72^{a,B}	<2.00±0.00^{b,C}	<2.00 ±0.00^{b,C}	<2.00 ±0.00^{a,C}	<2.00 ±0.00^{b,C}	<2.00 ±0.00^{b,C}	<2.00 ±0.00^{b,C}
	Mold							
C	2.46±0.08^{a,A}	2.60±0.17$^{a.A}$	3.10±0.10^{a,AB}	3.49±0.75^{a,B}	2.10±0.06^{a,A}	2.59±0.10^{a,AB}	2.61±0.08^{a,AB}	2.71±0.07^{a,AB}
L	0.79±0.77^{b,A}	<2.00±0.00^{b,B}	<2.00±0.00^{b,B}	<2.00 ±0.00^{b,B}	<2.00 ±0.00^{b,B}	<2.00±0.00^{b,B}	<2.00±0.00^{b,B}	<2.00±0.00^{b,B}
S	0.66±0.67^{b,A}	<2.00±0.00^{b,B}	<2.00±0.00^{b,B}	<2.00 ±0.00^{b,B}	<2.00 ±0.00^{b,B}	<2.00±0.00^{b,B}	<2.00±0.00^{b,B}	<2.00±0.00^{b,B}

*C (control), L (sausage with leaves aqueous extract), S (sausage with seeds aqueous extract); *Values represent the mean of three independent replicates ± standard error; * $^{a-c}$: Values with different superscript within the same column differ significantly at $p<0.05$

* $^{A-E}$: Values with different superscript within the same raw differ significantly at $p<0.05$

3.4. Biogenic amines

The results revealed that the incorporation of *M. oleifera* aqueous extracts resulted in a significant ($P<0.05$) reduction in all examined biogenic amines, particularly at the end of storage period when compared with the control (Table 3.1.4). The results also clarified that the seeds were more effective in reducing the formation of the biogenic amine in sausage particularly, the cadaverine and tryptamine than the leaves during the ripening and storage periods. These results may be attributed to the higher content of essential amines in the leaves (**Amer *et al.*, 2014**) which act as the main precursors for the formation of the biogenic amine in fermented sausage (**Sánchez *et al.*, 2017**). Furthermore, the higher lactic acid bacterial counts and the rapid falling of pH values due to the addition of *M. oleifera* leaves extract may enhance the accumulation of biogenic amines in fermented sausages (**Papavergou *et al.*, 2012**). In general, the lower biogenic amines content of sausages formulated with both *M. oleifera* extracts may be related to the antibacterial, antifungal and antioxidant activities of the plant (**Sofy *et al.*, 2017**). It has been reported that there was a positive correlation between the putrescine formation and the total aerobic counts, while cadaverine and histamine were usually associated with the presence of the decarboxylase-positive microbiota, as *Enterobacteria* (**Komprda, *et al.*, 2009**). The tyramine formation has been attributed to the action of *B. thermosphacta*. Moreover, there is evidence that the mold can support the formation of biogenic amines especially, putrescine and histamine in meat products (**Gardini *et al.*, 2016**). The results also revealed that cadaverine and -phenylethylamine were not detected in sausages incorporated with seeds extract, while tyramine was not detected in all sausage treatments in the 1st day of ripening. Moreover, tryptamine was not detected in all sausage treatments till the 2nd day of ripening and still under the detectable limit till the end of the ripening period and beginning of the chilled storage in sausage treated with the seeds extract. Spermine and spermidine were found with considerably higher concentrations in different sausage treatments on the 1st day of ripening, which may be due to the natural occurrence of these amines in the living cells (**Cai *et al.*, 2015**). During the ripening process and chilled storage period, all the examined biogenic amines increased significantly ($P<0.05$) in all sausage treatments with the control had

the highest amounts while; the seeds extract formulation showed the lowest concentrations. At the end of the storage period, cadaverine was the dominant biogenic amine followed by spermine, spermidine, putrescine, tyramine, histamine, phenylamine, and tryptamine. These results were in agreement with those reported by **Latorre-Moratalla *et al*.** (**2008**) who established the same order for the amines in fermented meat products.

Table 3.1.4: Biogenic amines "mg/kg" of semi-dry fermented sausage during ripening period and storage at 4°C for 3months

Cadaverine								
	Ripening				**Storage**			
	1st day	**2st day**	**3st day**	**4st day**	**0 time**	**1st month**	**2nd month**	**3rd month**
C	17.85±2.02^{a,A}	22.62±2.58^{a,B}	36.50±2.04^{a,C}	42.22±2.05^{a,D}	53.33±2.95^{a,E}	70.73±3.05^{a,F}	93.56±3.21^{a,G}	127.14±5.18^{a,H}
L	15.00±1.36^{b,A}	15.01±2.18^{b,A}	20.10±0.86^{b,B}	27.94±1.92^{b,C}	37.00±1.22^{b,D}	49.89±2.33^{b,E}	69.88±3.78^{b,F}	93.33±3.86^{b,G}
S	ND	3.45±0.21^{c,A}	11.20±1.02^{c,B}	19.67±1.22^{c,C}	22.76±1.23^{c,D}	33.77±2.08^{c,E}	41.90±2.22^{c,F}	53.9±4.28^{c,G}
Putrescine								
C	3.76±0.28^{a,A}	3.95±0.52^{a,A}	4.23±1.12^{a,AB}	5.87±2.05^{a,BC}	6.56±2.81^{a,C}	8.65±1.02^{a,D}	9.22±1.28^{a,D}	9.80±1.28^{a,D}
L	1.34±0.01^{b,A}	1.54±0.23^{b,AB}	1.95±0.22^{b,AB}	2.01±0.05^{b,AB}	2.56±0.33^{b,ABC}	3.11±1.81^{b,BC}	3.89±1.15^{b,C}	5.66±1.21^{b,D}
S	1.28±0.08^{b,A}	1.43±0.21^{b,A}	1.66±1.14^{b,A}	1.83±1.01^{b,A}	2.22±1.16^{b,AB}	2.97±1.55^{b,ABC}	3.65±2.21^{b,BC}	4.50±0.86^{b,C}
Histamine								
C	2.30±0.36^{a,A}	2.38±0.22^{a,A}	2.50±0.36^{a,A}	2.88±0.36^{a,A}	3.59±1.04^{a,AB}	4.66±1.15^{a,B}	5.22±1.44^{a,BC}	6.92±1.21^{a,C}
L	2.11±0.21^{a,A}	2.11±0.14^{a,A}	2.25±0.25^{a,A}	2.37±0.48^{a,A}	2.74±0.21^{a,AB}	3.39±2.11^{a,AB}	4.11±2.23^{a,BC}	5.72±1.28^{ab,C}
S	1.90±0.28^{a,A}	2.11±0.28^{a,A}	2.21±0.20^{a,A}	2.32±0.56^{a,AB}	2.65±0.55^{a,AB}	3.00±2.01^{a,AB}	3.40±2.00^{a,AB}	3.99±1.44^{b,B}
Tyramine								
C	ND	4.53±2.22^{a,A}	5.44±0.36^{a,AB}	5.63±1.14^{a,AB}	6.21±2.10^{a,BC}	6.78±2.00^{a,BC}	7.53±2.78^{a,CD}	8.84±1.36^{a,D}
L	ND	2.88±0.14^{ab,A}	3.28±1.02^{b,AB}	3.72±0.58^{ab,ABC}	4.69±0.86^{ab,BD}	5.00±2.08^{ab,CD}	5.89±1.55^{ab,D}	6.22±1.45^{b,D}
S	ND	1.54±0.23^{b,AB}	2.88±0.14^{b,BC}	3.11±0.34^{b,BCD}	3.89±0.58^{b,CDE}	4.43±0.86^{b,CDE}	4.67±0.94^{b,DE}	4.98±2.54^{b,E}

B-phenyl ethyl amine								
C	0.50±.02^{a,A}	0.92±0.11^{a,AB}	1.21±0.08^{a,ABC}	2.02±0.05^{a,BCD}	2.45±0.11^{a,CDE}	2.76±0.21^{a,DE}	2.96±0.36^{a,DE}	3.88±1.13^{a,E}
L	0.18±.01^{b,A}	0.43±0.02^{b,A}	1.18±0.14^{a,AB}	1.30±0.21^{a,AB}	1.43±0.15^{a,AB}	1.65±0.01^{a,AB}	1.98±0.25^{a,B}	2.24±0.28^{ab,B}
S	ND	0.09±0.00^{c,A}	1.10±0.11^{a,AB}	1.22±0.15^{a,AB}	1.26±0.12^{a,AB}	1.33±0.25^{a,AB}	1.46±1.34^{a,AB}	1.67±0.20^{b,B}
Tryptamine								
C	ND	ND	0.54±0.02^{A}	0.63±0.09^{a,A}	0.75±0.18^{a,A}	0.91±0.15^{a,A}	0.93±0.04^{a,A}	1.03±0.11^{a,A}
L	ND	ND	ND	0.09±0.01^{b,A}	0.13±0.10^{b,B}	0.15±0.11^{b,C}	0.17±0.00^{b,D}	0.59±0.02^{a,E}
S	ND	ND	ND	ND	ND	0.11±0.02^{c,A}	0.13±0.01^{c,B}	0.41±0.17^{a,C}
Spermidine								
C	6.83±0.86^{a,A}	6.91±1.11^{a,A}	7.7±1.14^{a,A}	7.99±3.32^{a,A}	8.55±3.00^{a,A}	10.76±3.44^{a,B}	13.22±2.55^{a,C}	15.35±1.86^{a,D}
L	4.31±1.14^{b,A}	5.83±0.86^{ab,AB}	6.03±2.11^{a,AB}	6.71±2.13^{a,BC}	7.86±2.11^{a,CD}	9.12±1.21^{ab,DE}	10.56±2.43^{b,EF}	11.5±1.58^{b,F}
S	3.74±0.36^{b,A}	4.66±0.86^{b,AB}	5.92±1.02^{a,BC}	6.62±1.08^{a,CD}	7.22±2.22^{a,CD}	7.92±3.11^{b,DE}	8.11±1.44^{c,DE}	9.03±1.36^{c,E}
Spermine								
C	17.68±1.21^{a,A}	19.50±2.01^{a,B}	21.30±2.58^{a,C}	23.97±2.14^{a,D}	26.22±3.04^{a,E}	29.08±3.44^{a,F}	33.09±2.71^{a,G}	36.1±3.04^{a,H}
L	15.00±1.78^{b,A}	16.71±1.33^{b,A}	18.50±2.02^{b,B}	23.30±2.08^{a,B}	23.60±2.28^{b,B}	25.40±2.09^{b,C}	28.12±1.89^{b,C}	30.11±1.58^{b,D}
S	13.76±1.04^{b,A}	15.42±1.25^{b,A}	18.40±1.58^{b,B}	19.93±1.86^{b,B}	20.05±1.28^{c,B}	23.62±1.11^{b,C}	25.00±2.08^{c,C}	28.90±1.98^{b,D}

*C (control), L (sausage with leaves aqueous extract), S (sausage with seeds aqueous extract), ND (not detectable)

*Values represent the mean of three independent replicates ± standard error

* $^{a-c}$: Values with different superscript within the same column differ significantly at $p<0.05$

* $^{A-H}$: Values with different superscript within the same raw differ significantly at $p<0.05$.

Conclusions

The addition of *M. oleifera* leaves and seeds aqueous extract improved the physicochemical and microbiological quality attributes in addition to the reduction of the biogenic amines of semi-dry fermented sausage in comparison to the control. *M. oleifera* leaves aqueous extract was more effective in the reduction of pH, lipid oxidation and total volatile nitrogen values while seeds significantly reduced the yeast, mold and biogenic amine contents during the ripening process and storage at 4ºC for 3 months. In general, the use of *M. oleifera* leaves and seeds aqueous extracts in the production of fermented sausage can give good quality and healthier products with prolonged storage life.

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Improving the nutritional, physicochemical and sensory quality of semi-dry fermented sausage by using *Moringa oleifera* aqueous extracts

Abstract

The main aim of the current research was to study the effect of *Moringa oleifera* leaves and seeds aqueous extracts on the nutritional values, physicochemical and sensory quality of semi-dry fermented sausage during the processing and chilled storage at 4ºC for 3 months. *M. oleifera* leaves and seeds aqueous extracts were added during the formulation of the sausages at a rate of 1.5% in comparison with the control. Addition of both *M. oleifera* aqueous extracts during the processing of fermented sausage resulted in a significant increase in protein, fat, calcium, iron contents, shear force, redness (a*) and lightness (L*) values when compared to the control group. Moreover, incorporation of both plant extracts during the sausages formulations led to significant elevation in the health promoting and functional fatty acids contents. On the other hand, significant reduction in yellowness (b*) values and trans-fatty acids levels were observed by addition of both plant extracts during the sausages formulations. The results also revealed improvement of the overall acceptability with acceptable color and flavor scores of the sausages formulated with both extracts throughout the chilled storage period. From this study we can conclude that the aqueous extracts of *M.oleifera* leaves and seeds improved the nutritional and physicochemical quality of the sausages without any negative impact on their sensory attributes.

Key word: sausages, *M. oleifera*, aqueous extract, fermentation, quality

1. Introduction

Fermented sausages are becoming one of the most popular meat products that are gaining an increased acceptance in the market all over the world. Fermented sausages are composed mainly from meat which characterized by its contents of high biological value proteins, essential amino acids and essential micronutrients (**Pereira, 2013**). However, meat contains high level of saturated fats that can increase the blood

pressure, heart rate and cholesterol level in the blood (**FAO, 2008**). The fat content of meat is ranged from 3 to 25% depending on many factors such as nutrition, species and age of the animal (**Pereira, 2013**). Additionally, in fermented sausage, the fat content may reach to 50% as a result of acidification and moisture loss that occur during the fermentation process (**Vignolo *et al.*, 2010**). It has been reported that meat and meat products provided the human with reasonable amounts of trans-fatty acids which act as carcinogenic promoters (**Scollan *et al.*, 2006**). Therefore, all health organizations have recommended decreasing the consumption of food containing high saturated and trans-fats as a means of preventing the susceptible diseases (**Sari and Sariçoban, 2016**). Moreover, the high fat content may increase the susceptibility to lipid oxidation, which may cause color and flavor deterioration, nutrient losses, short shelf life and the production of toxic compounds causing food-borne illnesses (**Campagnol *et al.*, 2012**). Although, the minerals are recommended as important components in the human nutrition where they play vital roles in different physiological functions, the meat alone is not a sufficient source to meet those requirements (**Peña-Rosas *et al.*, 2014**). Furthermore, meat contains lack amounts of vitamin E, D and C which have important antioxidant activities and aid in the prevention of various diseases incidences (**Nowicka *et al.*, 2018**). For these reasons, meat processors are directed toward addition of functional additives to meat products to improve their nutritional value and provide beneficial health effects to the consumers.

Natural extracts obtained from various plants are becoming the first choice functional additive by the meat producers due to their numerous technological and health benefits (**Zhang *et al.*, 2016**). *M. Oleifera* is the most common plant used not only due to its nutritional, antimicrobial and antioxidant benefits but also its availability in different parts of the world (**Falow *et al.*, 2018**). *M. oleifera* leaves contain higher percentages of vitamin A and C, potassium, calcium, iron and proteins than those of carrots, orange, bananas, milk, yoghurt and spinach, respectively (**Gopalakrishnan *et al.*, 2016**). In addition, *M. oleifera* leaves have contain high amounts of essential amino acids as tyrosine, threonine, valine, methionine, lysine,

phenylalanine, leucine, isoleucine, tryptophan and histidine (**Falow *et al.*, 2018**). Therefore, the plant leaves have been extremely used to compensate the malnutrition among children, pregnant and lactating women (**Saini *et al.*, 2014**). The leaves also were reported as a rich source of unsaturated fatty acids particularly α-Linolenic, while the seeds contains mainly oleic acid that have the ability to control the cholesterol level and decrease the cardiovascular diseases (**Moyo *et al.*, 2011; Robiansyah *et al.*, 2014**). Regardless, the nutritive value of *M. oleifera*, the plant can inhibit the microbial growth (**Anwar *et al.*, 2007**) and lipid oxidation (**Sofy *et al.*, 2017)** and subsequently can improve the sensory quality, shelf life and consumer acceptance of the products. Based on the above mentioned benefits of *M. oleifera*, many researchers applied this plant in the animal diet for improving the nutritional value of meat (**Mukumbo *et al.*, 2014**) however; its application in meat processing area is still limited. Moreover, the studies that investigated the effect of *M. Oleifera* on the organoleptic quality of meat and meat products are rare. Therefore, the aim of the current research was to study the effect of using of *M. Oleifera* leaves and seeds aqueous extracts on the nutritive, physicochemical and sensory quality of semi-dry fermented sausages during storage at 4°C for 3 months.

2. Materials and Methods

2.1. Raw materials

Imported frozen beef topside blocks (MINISTÉRIO DA AGRICULTURA, Brazil) were purchased from a local supplier in Cairo, Egypt within the first third of its shelf life. Fresh mesenteric beef fat was obtained from Cairo slaughter house immediately after animal slaughter, washed and kept frozen at -18 °C until processing. Ascorbic acid, sodium nitrite, lactose and glucose were purchased from Loba Chemie (Mumbai, India), while sodium chloride was obtained from local market in Cairo, Egypt. The Chr, Hansen (Denmark) and Nubassa (GewürzwerkGmpH, Viernheim, Germany) were the main suppliers for *Lactobacillus helveticus* (Lh-BO2) and spice oil extracts, respectively. However, the leaves and seeds of *M. oleifera* were obtained from the experimental plant station, Faculty of Pharmacy, Cairo University, Egypt.

2.2. Preparation of *M. oleifera* leaves and seeds aqueous extracts

M. oleifera leaves and seeds aqueous extracts were prepared following the procedures described by **Ezzat *et al*.** (**2020**).

2.3. Formulation of semi-dry fermented sausages

Immediately before processing of the sausages, the beef and fat were ground through a 5-mm plate grinder (Seydelmann NW 114 E; Stuttgart, Deutschland, Germany). The ground beef and fat should have temperature about of 5°C during the sausage processing.

For production of the control sausage, the sausage dough was formulated using 83% beef meat, 12% beef fat, 2.0% common salt, 200 ppm sodium nitrite, 500 ppm ascorbic acid, 1.5 % lactose and glucose mix, 10^5 CFU/g *L. helveticus* and quantum sufficient of spice mix. The ground beef and fat were mixed with other ingredients using Seydelmann spiral mixer (Urgstallstraβe, Germany). Another two formulas were prepared as control with addition of *Moringa oleifera* leaves and seeds extracts at a rate of 1.5 % to each formula separately. The sausages dough was incorporated into cellulose casing with diameter of 30mm (Podanfol Professional Packaging) using automatic filler (Handtmann VF 628, Baden-Wurttemberg, Germany) and then conditioned for 4 days at 20ºC and 70% RH to reach a pH value of 5.20. After that, the cooking of sausages was conducted to acheive a core temperature of 72ºC. The sausages were packed within polyethylene and stored refrigerated at 4º C for 3 months. The experiment for each trial was replicated three times at different independent times.

2.4. Examination of semi-dry fermented sausage

Three samples from each trial were examined immediately after processing (0-time) and monthly for physicochemical (protein, fat, minerals, fatty acids, shear force and color) and sensory quality parameters. Moreover, three samples each from beef, the extracts of *M. oleifera* leaves and seeds used in the production of the sausages were

analyzed for determination of protein, moisture, minerals and fatty acids contents as presented in Table (3.2.1).

2.4.1. Physicochemical investigations

2.4.1.1. Chemical composition

2.4.1.1.1. Proximate chemical compositions and minerals contents

Proximate composition (protein, fat) and minerals (calcium, iron) contents of the plant extracts were determined according to the procedures of **Oluduro** (**2012**). However, those of the raw meat and sausages were determined according to the methods established by **AOAC** (**2003**). The protein and fat were expressed in percentage (%) while the minerals were expressed as ppm.

2.4.1.1.2. Fatty acids analysis

The fatty acids contents of the raw meat, plant extracts and sausages were extracted according to **Folch *et al.*** (**1957**). After that, the saponification of the lipid extracts of each sample was carried out following the procedures described by **IUPAC** (**1987**). The separation of fatty acids was performed by using gas chromatography (ACME model 6100 GC, Young LTN Instrument Co., Korea) adjusted at temperatures of 110- 210°C. Moreover, a standard of known fatty acids was analyzed by the gas chromatography to confirm the actual content of the fatty acids in the samples. The peaks of standard fatty acids then used to calculate the fatty acids contents in the samples according to the equation established by **Slover and Lanza** (**1979**).

2.4.1.2. Shear force

For shear force measurement, the sausage samples were cut into steaks of 2 cm. A hand-held coring device was used to eliminate six core samples of 1.27 cm diameter from each steak. The shear force of each core was measured using a Warner–Bratzler shear force (WBSF) device attached to an Instron Universial Testing machine (Model 2519 105; Instron Corp., Canton, MA, USA) with a 55-kg tension/compression load cell and a crosshead speed of 200 mm/min (**Shackelford *et al.*, 2004**).

2.4.1.3. Color evaluation

Color of the sausage samples was determined using Croma meter (Konica Minolta, model CR 410, Japan) adjusted with a white plate and light trap provided by the manufacturer. According to Commission International de l'Eclairage (CIE), color was defined as L* (lightness), a* (redness), and b* (yellowness). CIE standard illuminant D65 light source was used to measure L*, a*, and b* values. The bloom time was 30 min and the observation angle was 10°. Three scores were taken from each sample surface at each time of examination and the average was calculated (**Shin *et al*., 2008**).

2.4.2. Sensory investigations

Sensory parameters (color, flavor, tenderness and overall acceptability) of all sausage trials were evaluated according to the guidelines of **AMSA** (**1995**). The evaluation was carried out by nine qualified panelists from the staff members of the Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Cairo University, Egypt. The panelists were from both sexes and their ages ranged from 30–45 years old. The analysis was performed in specific room provided with good lightning and controlled temperatures. Nine different samples (3 samples for each trial) in the form of slices were randomly coded and served to the panelists. Worm water was provided to rinse the palate between the samples. All panelists were requested to evaluate the samples at the same time during the same session and ranked each sensory parameter using 1–9 hedonic scale, where 9 mean extremely like and 1 mean extremely dislike. These procedures were repeated 2 times again at other 2 different sessions and the average of the panel scores for each parameter were taken as a final result.

2.5. Statistical analysis

One-way analysis of variance (ANOVA) was carried out to determine the statistical differences between the mean values of protein, fat, minerals and fatty acids contents obtained for the raw meat, leave and seeds extracts once before the sausage processing using the SPSS statistics 23.0 for windows. Moreover, ANOVA was

performed to analyze the differences between the mean values of protein, fat, minerals, fatty acids, shear force, color and sensory scores obtained for the different sausage treatments at each examination time (at 0-time and monthly for 3 months). The results were tabulated in terms of mean values and standard error of the mean. Least squares differences (LSD) were used to compare the mean values between the three sausage formulations at significant differences of $P < 0.05$.

3. Results and discussion

Table 3.2.1: Chemical analysis of raw materials

	Meat	Leaves extract	Seeds extract
Proximate analysis (%)			
Protein	19.97±2.18^{a}	16.98±2.45^{b}	10.56±1.45^{c}
Fat	7.05±0.26^{a}	1.88±0.25^{b}	3.55±0.55^{b}
Mineral analysis (ppm)			
Ca	245.66±23.00^{a}	12564.87±99.65^{b}	2612.37±133.00^{c}
Fe	44.523±12.82^{a}	220.56±11.56^{b}	117.76±12.76^{c}
Fatty acid analysis (%)			
C14:0	2.73±0.35^{a}	3.56±0.98^{a}	N.D±0.00^{b}
C14:1	0.47±0.11^{a}	0.50±0.02^{b}	N.D±0.00^{c}
C15:0	0.56±0.04^{a}	0.63±0.01^{b}	N.D±0.00^{c}
C15:1	0.27±0.02^{a}	0.29±0.01^{b}	N.D±0.00^{c}
C16:0	23.43±2.11^{a}	10.66±1.15^{b}	6.27±2.25^{c}
C16:1	1.23±0.43^{a}	1.20±0.88^{a}	1.01±.55^{a}
C17:0	1.43±0.15^{a}	1.11±0.54^{a}	0.09±0.01^{a}
C17:1	0.42±0.26^{a}	0.08±0.02^{b}	0.05±0.00^{c}
C18:0	30.48±2.76^{a}	5.59±1.29^{b}	6.62±1.11^{b}
C18:1	33.55±2.15^{a}	58.78±2.25^{b}	69.87±2.11^{c}
C18:2	1.93±0.32^{a}	1.85±0.65^{a}	1.80±0.50^{a}
C18:3	0.04±0.01^{a}	0.13±0.01^{b}	0.14±0.02^{b}
C18:4	0.24±0.03^{a}	N.D±0.00^{b}	N.D±0.00^{b}
C20:0	0.30±0.01^{a}	4.00±1.57^{b}	4.35±1.66^{b}
C20:1	0.17±0.01^{a}	2.65±0.50^{b}	2.71±0.65^{b}
C22:0	0.05±0.00^{a}	6.98±2.11^{b}	7.08±2.76^{b}
TSFA	57.61±5.42^{a}	32.53±5.54^{b}	24.32±3.37^{c}
PUFA	3.27±0.36^{a}	1.98±0.66^{a}	1.94±0.52^{a}
MUFA	36.11±2.98^{a}	63.50±3.18^{b}	73.64±3.31^{c}
TUFA	39.38±3.34^{a}	65.47±3.84^{b}	75.58±3.38^{c}
Trans F.A	N.D±0.00^{a}	ND±0.00^{a}	N.D±0.00^{a}
Total unknown	1.54±0.03^{a}	0.03±0.00^{b}	0.05±0.00^{b}

*Values represent the mean of three independent replicates ± standard error

*TSFA (Total saturated fatty acid), PUFA (poly unsaturated fatty acid), MUFA (mono unsaturated fatty acid), TUFA (total unsaturated fatty acid), TFA (trans-fatty acids)

*$^{a-c}$:Values with different superscript within the same raw differ significantly at $p<0.05$.

3.1. Chemical composition

The protein, fat, calcium and iron contents of the sausages formulated with *M. oleifera* leaves and seeds aqueous extracts were significantly (P<0.05) higher than those of the control group at 0-time and during storage at 4°C for 3 months (Table 3.2.2). These results may be related to the high protein and minerals contents of the plant leaves and seeds (**Khalafalla *et al*., 2010; Salama *et al*. 2017**) used in the sausage production (Table 3.2.1). These results were in agreement with those of **Al-Juhaimi *et al*.** (**2016**) **and Abd El-Rahman *et al*.** (**2019**) who reported that there were significant (P<0.05) elevation in the protein and fat levels of beef burger and traditional Egyptian luncheon with increasing the rate of *M. oleifera* addition during their processing. Moreover, **Ibrahim *et al*.** (**2017**) found that the meat-rice kofta formulated with *M. oleifera* leaves had higher minerals contents than the control group. The results also may be explained by the significant reduction in pH values of the sausages processed by *M. oleifera* leaves and seeds extracts in comparison with the control (**Ezzat *et al*., 2020**).

M. oleifera leaves and seeds contain huge amounts of flavonoids, organic acids, and phenolic acids that can decrease the pH value of the products formulated with them (**Rodríguez *et al*., 2016**).The reduction in pH values may be related to significant decrease in moisture and significant increase in protein, fat, ash and minerals contents of the sausages (**Mauriello *et al*., 2004**). The results also showed that the calcium content of the sausages formulated with leaves extract was significantly (P<0.05) higher than those of formulated with seeds extract at 0-time and during the chilled storage.

On the base of chemical analysis of *M. oleifera* leaves and seeds extracts, the leaves showed significantly (P<0.05) higher calcium content than the seed with mean values of 12564.87and 2612.37 respectively (Table 3.2.1). Several studies reported higher calcium and iron contents of *M. oleifera* leaves and recommended using them as a main food for treatment of malnutrition and bone diseases (**Sahay *et al*., 2020**).

Table 3.2.2: Chemical composition of semi-dry fermented sausages during storage at 4°C for 3months

Proximate analysis (%)								
	Protein				Fat			
Treatments	**0 time**	**1st month**	**2nd month**	**3rd month**	**0 time**	**1st month**	**2nd month**	**3rd month**
C	$15.23\pm0.27^{a,A}$	$17.93\pm0.18^{a,B}$	$34.18\pm0.50^{a,C}$	$39.43\pm1.62^{a,D}$	$19.42\pm0.59^{a,A}$	$26.36\pm0.63^{a,B}$	$29.07\pm0.62^{a,BC}$	$30.33\pm2.03^{a,C}$
L	$17.51\pm0.10^{b,A}$	$22.38\pm0.11^{b,B}$	$42.76\pm0.52^{b,C}$	$50.29\pm0.96^{b,D}$	$21.80\pm0.70^{b,A}$	$30.48\pm0.94^{b,B}$	$32.76\pm0.34^{b,BC}$	$34.96\pm1.98^{b,C}$
S	$16.59\pm0.28^{c,A}$	$21.54\pm0.41^{b,B}$	$39.78\pm0.84^{b,C}$	$45.50\pm0.40^{c,D}$	$21.63\pm0\ .08^{b,A}$	$27.63\pm0.91^{ab,B}$	$29.40\pm1.20^{b,BC}$	$31.23\pm1.82^{a,C}$
Mineral analysis (ppm)								
	Ca				Fe			
C	$565.05\pm27.32^{a,A}$	$767.44\pm23.94^{a,B}$	$838.04\pm20.04^{a,C}$	$932.04\pm30.04^{a,D}$	$51.51\pm11.04^{a,A}$	$54.04\pm6.92^{a,B}$	$56.77\pm12.25^{a,C}$	$58.09\pm7.93^{a,C}$
L	$708.12\pm30.05^{b,A}$	$927.29\pm24.86^{b,B}$	$1002.40\pm30.01^{b,C}$	$1074.81\pm31.11^{b,D}$	$53.60\pm7.87^{b,A}$	$57.57\pm14.87^{b,B}$	$58.57\pm10.32^{b,C}$	$61.11\pm16.25^{b,D}$
S	$608.05\pm27.21^{c,A}$	$827.267\pm20.09^{c,B}$	$968.88\pm26.86^{c,C}$	$1004.24\pm29.44^{c,D}$	$55.02\pm13.87^{b,A}$	$56.24\pm13.33^{b,B}$	$57.08\pm9.01^{ab,C}$	$60.60\pm13.07^{b,D}$

C (control), L (sausage with leaves aqueous extract), S (sausage with seeds aqueous extract)
*Values represent the mean of three independent replicates ± standard error
* $^{a-c}$:Values with different superscript within the same column differ significantly at $p<0.05$
* $^{A-D}$: Values with different superscript within the same raw differ significantly at $p<0.05$

Addition of *M. oleifera* leaves and seeds extracts during processing of the sausages led to significant ($P<0.05$) changes in the fatty acids profile when compared to the control group either at 0-time or during the chilled storage (Table 3.2.3). It has been observed that the sausages formulated with seeds extract showed significantly ($P<0.05$) higher arachidic acid (C20:0) contents when compared with those formulated with leaves extract and the control group at 0-time and during the chilled storage. However, the highest ($P<0.05$) contents of pentadecanoic acid (C15:0, C15:1) were observed in the sausages treated with the leaves extract at 0-time of processing. Both plant extracts formulated sausages showed significantly ($P<0.05$) higher concentrations of linolenic (C18:3) and eicosenoic (C20:1) acids in comparison with the control group at 0-time and during the chilled storage. Moreover, decosanoic acid (C22:0) levels were significantly ($P<0.05$) higher in the sausages treated with both plant extracts than those of the control group at 1st month of the chilled storage.

On the other hand, heptadecanoic acid (C17:1) levels were significantly ($P<0.05$) lower in sausages treated with both plant extracts than those of the control group at 2nd and 3rd months of the chilled storage. The results also showed that stearidonic acid (C18:4) not appeared in the sausages treated with both extracts till 3rd month of the chilled storage with those treated with seeds extract had significantly ($P<0.05$) lower content.

The differences of fatty acid contents of the experimentally formulated sausages may be related to the variations in fatty acids profile of raw beef, leaves and seeds extracts used in their processing. The leaves extracts showed the highest ($P<0.05$) contents of myristoleic (C14:1) and pentadecanoic (C15:0, C15:1) acids, while the seeds extracts had the highest ($P<0.05$) values of oleic acid (C18:1) (Table 3.2.1). However, the highest ($P<0.05$) contents of palmitic (C16:0), heptadecanoic (C17:1), stearic (C18:0) and stearidonic (C18:4) acids were observed in the beef. The results also revealed that the total saturated fats were the highest ($P<0.05$) in beef, while the monounsaturated fatty acids were the highest ($P<0.05$) in seeds extract. There was similarity in the obtained results of the fatty acids profile of the plant extracts with

those reported by **abdulkarim *et al.* (2005); Moyo *et al.* (2011) and Nadeem *et al.* (2014)**.

Incorporation of leaves and seeds extracts during the processing of sausages led to significant ($P<0.05$) elevation in health promoting fatty acids contents. It has been reported that pentadecanoic (**Warensjö *et al.*, 2010**; **Forouhi *et al.*, 2014**), linolenic and eicosenoic acids (**Gerhard *et al.* 2004**) had the ability to decrease the incidence of cardiovascular disease. In addition, linolenic and eicosenoic acids were reported as potent acids in reduction of the blood cholesterol level (**Ogunlesi *et al.*, 2010**). *M. oleifera* aqueous extracts also increased the functional fatty acids contents in the sausage where, monounsaturated fatty acids particularly oleic acid were known to have potent antifungal and antibacterial properties (**Riveros *et al.* 2010; Ogunlesi *et al.*, 2010**).

However, trans-fatty acids were not appeared in all raw materials used, they appeared in the processed sausages with the control group had the highest values at 0-time and throughout the chilling storage (Table 3.2.1, 3.2.3). The results also clarified that the seeds were more effective in reducing the formation of the trans-fatty acids in the sausage. Lower trans-fatty acids contents of the sausages treated with both plant extracts may be attributed to the antibacterial, antifungal and antioxidant activities of the plant (**Sofy *et al.*, 2017**; **Ezzat *et al.*, 2020**), so it can prevent the conversation of unsaturated fatty acids into the trans-form. Generally, the reduction of trans-fatty acids in the sausages by the application of both *M. oleifera* extracts was an important issue where, there was a possible relationship between trans- fatty acids intake and cardiovascular disease, elevation of low-density lipoproteins as well as reduction in the level of high density lipoproteins in the blood (**Fritsche and Steinhart, 1997; Lichtenstein, 1998**). The results also showed that the chilled storage led to significant ($P<0.05$) increase in the protein, fat, mineral and fatty acids contents of all sausage treatments (Table 3.2.2, 3.2.3) which may due to the occurrence of dryness and moisture loss (**Vesković *et al.*, 2013**).

Table 3.2.3: Fatty acids analysis (%) of semi-dry fermented sausages during storage at 4°C for 3months

Fatty acids analysis (%)												
Fatty acids	**0 Time**			**1st M**			**2st M**			**3st M**		
	C	**L**	**S**	**C**	**L**	**S**	**C**	**L**	**S**	**C**	**L**	**S**
C14:0	2.49±0.11^{a,A}	2.57±0.15^{a,A}	2.45±0.09^{a,A}	2.52±0.13^{a,A}	2.65±0.12^{a,A}	2.55±0.11^{a,A}	2.56±0.05^{a,A}	2.65±0.12^{a,A}	2.56±0.03^{a,A}	2.61±0.16^{a,A}	2.72±0.19^{a,A}	2.60±0.11^{a,A}
C14:1	0.26±0.02^{a,A}	0.26±0.03^{a,A}	0.24±0.01^{b,A}	0.26±0.09^{a,A}	0.26±0.07^{a,A}	0.25±0.00^{a,AB}	0.26±0.02^{a,A}	0.27±0.04^{a,A}	0.26±0.01^{a,B}	0.26±0.05^{a,A}	0.27±0.08^{a,A}	0.26±0.01^{a,B}
C15:0	0.48±0.00^{a,A}	0.51±0.01^{b,A}	0.48±0.03^{a,A}	0.50±0.02^{a,BC}	0.51±0.04^{a,A}	0.48±0.01^{b,A}	0.51±0.00^{ab,B}	0.52±0.02^{a,A}	0.49±0.01^{b,AB}	0.52±0.01^{a,C}	0.52±0.05^{a,A}	0.50±0.01^{b,B}
C15:1	0.25±0.04^{a,A}	0.27±0.01^{b,A}	0.24±0.03^{a,A}	0.27±0.01^{a,B}	0.27±0.03^{a,A}	0.27±0.01^{a,B}	0.27±0.02^{a,B}	0.27±0.00^{a,A}	0.27±0.05^{a,B}	0.28±0.01^{a,B}	0.28±0.05^{a,A}	0.27±0.00^{a,B}
C16:0	22.56±2.00^{a,A}	22.46±1.15^{a,A}	22.46±2.04^{a,A}	22.59±1.98^{a,A}	23.01±1.25^{a,A}	22.71±1.25^{a,A}	23.06±1.45^{a,A}	23.13±1.66^{a,A}	22.76±1.25^{a,A}	23.23±2.01^{a,A}	23.23±1.11^{a,A}	23.05±1.09^{a,A}
C16:1	1.17±0.00^{a,A}	1.18±0.01^{a,A}	1.18±0.40^{a,A}	1.18±0.05^{a,A}	1.19±0.01^{a,A}	1.18±0.02^{a,A}	1.21±0.06^{a,A}	1.22±0.01^{a,A}	1.18±0.02^{a,A}	1.22±0.05^{a,A}	1.28±0.10^{a,A}	1.20±0.05^{a,A}
C17:0	1.41±0.03^{a,A}	1.41±0.05^{a,A}	1.28±0.01^{a,A}	1.44±0.04^{a,A}	1.42±0.04^{a,A}	1.43±0.05^{a,A}	1.48±0.08^{a,A}	1.48±0.05^{a,A}	1.48±0.02^{a,A}	1.52±0.02^{a,A}	1.50±0.01^{a,A}	1.48±0.01^{a,A}
C17:1	0.41±0.02^{a,A}	0.41±0.01^{a,A}	0.34±0.03^{b,A}	0.42±0.01^{a,A}	0.41±0.00^{a,A}	0.41±0.04^{a,B}	0.47±0.06^{a,AB}	0.45±0.03^{b,B}	0.45±0.00^{b,C}	1.41±0.05^{a,B}	0.44±0.01^{b,B}	0.44±0.01^{b,C}
C18:0	30.89±2.19^{a,A}	30.81±3.15^{a,A5}	30.17±1.17^{a,A}	31.34±2.12^{a,A}	31.17±2.00^{a,A}	31.17±1.15^{a,A}	31.66±2.25^{a,A}	31.25±1.11^{a,A}	31.44±1.09^{a,A}	32.38±4.01^{a,A}	31.54±3.11^{a,A}	31.56±1.25^{a,A}
C18:1	33.74±1.11^{a,A}	33.78±2.76^{a,A}	34.20±1.25^{a,A}	34.05±1.15^{a,A}	34.17±1.45^{a,A}	34.28±2.11^{a,A}	34.37±1.66^{a,A}	34.67±1.34^{a,A}	34.81±2.01^{a,A}	34.42±2.04^{a,A}	34.56±1.25^{a,A}	35.68±1.11^{a,A}
C18:2	1.59±0.05^{a,A}	1.62±0.03^{a,A}	1.67±0.24^{a,A}	1.73±0.05^{a,A}	1.79±0.33^{a,A}	1.78±0.50^{a,A}	1.76±0.01^{a,A}	1.81±0.01^{a,A}	1.79±0.00^{a,A}	1.78±0.11^{a,A}	2.01±0.13^{a,A}	2.08±0.27^{a,A}
C18:3	0.13±0.02^{a,A}	0.15±0.01^{b,A}	0.16±0.01^{b,A}	0.14±0.00^{a,A}	0.16±0.02^{b,A}	0.17±0.03^{b,A}	0.17±0.00^{a,B}	0.20±0.01^{b,B}	0.28±0.00^{c,B}	0.26±0.04^{a,C}	0.30±0.05^{b,C}	0.32±0.05^{c,C}
C18:4	N.D±0.00^{a,A}	N.D±0.00^{a,A}	N.D±0.00^{a,A}	N.D±0.00^{a,A}	N.D±0.00^{a,A}	N.D±0.00^{a,A}	0.16±0.02^{a,B}	N.D±0.00^{b,A}	N.D±0.00^{b,A}	0.25±0.02^{a,C}	0.22±0.00^{b,B}	0.17±0.01^{c,B}
C20:0	0.25±0.00^{a,A}	0.26±0.01^{a,A}	0.28±0.02^{b,A}	0.27±0.01^{a,B}	0.27±0.00^{a,A}	0.29±0.02^{b,A}	0.28±0.03^{a,B}	0.30±0.00^{b,B}	0.31±0.00^{b,B}	0.30±0.01^{a,C}	0.31±0.03^{a,B}	0.33±0.05^{b,C}
C20:1	0.16±0.01^{a,A}	0.18±0.01^{b,A}	0.19±0.05^{b,A}	0.17±0.04^{a,AB}	0.20±0.01^{b,A}	0.22±0.03^{c,B}	0.18±0.02^{a,BC}	0.21±0.00^{b,A}	0.24±0.01^{c,C}	0.19±0.03^{a,C}	0.25±0.02^{b,C}	0.28±0.03^{c,D}
C22:0	N.D±0.00^{a,A}	N.D±0.00^{a,A}	N.D±0.00^{a,A}	0.02±0.00^{a,B}	0.04±0.01^{b,A}	0.05±0.00^{b,AB}	0.05±0.01^{a,C}	0.08±0.00^{a,A}	0.10±0.02^{a,B}	0.05±0.00^{a,C}	0.10±0.00^{a,B}	0.13±0.03^{a,B}
TSFA	58.08±4.33^{a,A}	58.02±4.25^{a,A}	57.12±3.36^{a,A}	58.68±4.30^{a,A}	59.07±3.46^{a,AB}	58.68±4.30^{a,AB}	59.60±3.86^{a,A}	59.70±2.96^{a,AB}	59.70±2.42^{a,B}	59.14±6.22^{a,A}	59.92±4.4^{a,B}	59.89±2.55^{a,B}
PUFA	1.72±0.07^{a,A}	1.77±0.04^{a,A}	1.83±0.25^{a,A}	1.87±0.05^{a,A}	1.95±0.35^{a,A}	1.88±0.53^{a,A}	2.09±0.03^{a,A}	2.01±0.02^{a,A}	2.01±0.00^{a,A}	2.92±0.17^{a,A}	2.53±0.18^{a,A}	2.57±0.33^{a,A}
MUFA	35.99±1.18^{a,A}	35.82±2.80^{a,A}	36.15±1.76^{a,A}	36.09±1.26^{a,A}	36.24±1.50^{a,AB}	36.36±2.21^{a,A}	36.50±1.82^{a,A}	36.82±1.38^{a,AB}	36.95±2.08^{a,A}	37.52±2.11^{a,A}	37.81±1.43^{a,B}	37.88±1.20^{a,A}
TUFA	37.71±1.25^{a,A}	37.59±2.84^{a,A}	37.98±2.01^{a,A}	37.96±1.31^{a,A}	38.19±1.85^{a,A}	38.24±2.74^{a,A}	38.59±1.85^{a,AB}	38.83±1.40^{a,AB}	38.96±2.08^{a,AB}	40.44±2.28^{a,B}	40.34±1.61^{a,B}	40.45±1.50^{a,B}
TFA	0.66±0.05^{a,A}	0.65±0.00^{a,A}	0.60±0.00^{b,A}	0.71±0.03^{a,B}	0.66±0.04^{b,A}	0.61±0.01^{c,A}	0.76±0.02^{a,B}	0.69±0.04^{b,A}	0.61±0.04^{c,A}	0.77±0.06^{a,B}	0.70±0.01^{b,A}	0.65±0.02^{c,B}

C (control), L (sausage with leaves aqueous extract), S (sausage with seeds aqueous extract), TSFA (Total saturated fatty acid), PUFA (poly unsaturated fatty acid), MUFA (mono unsaturated fatty acid), TUFA (total unsaturated fatty acid), TFA (trans-fatty acids)

*Values represent the mean of three independent replicates ± standard error

* $^{a-c}$: Values with different superscript within the same column differ significantly at $p<0.05$

* $^{A-D}$: Values with different superscript within the same raw differ significantly at $p<0.05$

3.2. Shear force and color

The shear force values of the sausages formulated with the seeds extracts were not significantly (P>0.05) higher than those of the control group at 0-time and throughout the storage period (Table 3.2.4). However, the addition of the leaves extract led to significant (P<0.05) increase in shear force values of the sausages when compared with the two other treatments at 0 time and 1st month of the chilled storage. The high protein content of *M.oleifera* leaves (**Salama *et al.* 2017)** and the high fiber content of the seeds (**Falowo *et al.*, 2018**) may be the main factors which determine the texture of the products. The shear force results indicated that addition of the seeds extracts during processing of the sausages was preferred than the leaves extracts where that of seeds did not alter the texture of the products when compared with the control one. However, **Muthukumar *et al.*** (**2014**) and **Shah *et al.*** (**2015**) reported that the direct incorporation of *M.oleifera* leave aqueous extract in fresh meat and pork patties had no significant effect on their shear force values. The results also clarified that the chilling storage led to significant (P<0.05) increase in shear force values of different sausages treatments which may be attributed to their higher protein contents at the end of chilled storage.

Meat color is one of the most important meat quality parameters that determine the consumer acceptance for purchasing meat products. The lightness (L*) values of the sausages treated with *M. oleifera* leaves extract were significantly ($P < 0.05$) higher than those of formulated with seeds extract and the control groups at 1st month till the end of chilled storage period (Table 3.2.4). The lightness (L*) values of meat and meat products depend mainly on the fat and moisture contents (**Fischer, 2007**), therefore, higher lightness (L*) values of sausages formulated leaves extract may be related to its higher fat content.

The results also revealed that there were significant (P<0.05) increase in redness values (a*) by incorporation of the leaves extracts during processing of the sausages when compared to the other two formulations particularly at 0-time and 3rd month of the chilled storage period (Table 3.2.4). On the other hand, the lowest yellowness (b*) values were observed in the sausages treated with leaves extracts

when compared to those treated with the seeds and control groups at 0-time and throughout the chilled storage period. It has been reported that redness (a*) and yellowness (b*) values of meat can be affected by the level of lipid oxidation **(Phillips *et al.*, 2001)**, therefore the higher values (a*) and lower (b*) values of leaves extract incorporated sausages may be closely related to the antioxidant activity of the plant leaves (**Sofy *et al.*, 2017**; **Ezzat *et al.*, 2020**). Previous studies reported improvement of color with high redness (a*) value by increasing the rate of *M. oleifera* leaves aqueous extracts addition in fresh meat (**Shah *et al.*, 2015**) and pork patties (**Muthukumar *et al.*, 2014**) during the chilled storage. On the other hand, chilling storage of different sausages treatments resulted in significant ($P<0.05$) decrease in (L*) and (a*) values while significant ($P<0.05$) increase in (b*) values which may be due to increase in lipid oxidation level.

Table 3.2.4: Shear force and color of semi-dry fermented sausages during storage at 4°C for 3months

Shear force				
	Storage			
Treatments	**0 Time**	**1st M**	**2nd M**	**3rd M**
C	0.47±0.01^{a,A}	1.28±0.07^{a,B}	3.87±0.30^{a,C}	4.18±0.32^{a,C}
L	0.62±0.03^{b,A}	2.06±0.11^{b,B}	4.80±0.21^{b,C}	4.81±0.38^{a,C}
S	0.49±0.04^{a,A}	1.56±0.08^{a,B}	4.27±0.08^{ab,C}	4.29±0.08^{a,C}
		L*		
C	55.37±0.01^{a,A}	50.63±0.06^{a,B}	48.44±0.03^{a,C}	48.64±0.17^{a,C}
L	55.97±0.01^{b,A}	51.36±0.02^{b,B}	51.26±0.14^{b,B}	49.52±0.24^{b,C}
S	54.89±0.30^{b,A}	51.26±0.01^{c,B}	48.58±0.02^{a,C}	48.74±0.10^{a,C}
		a*		
C	14.77±0.05^{a,A}	13.65±0.13^{a,B}	13.30±0.07^{a,B}	10.36±0.11^{a,C}
L	17.08±0.00^{b,A}	14.44±0.22^{b,B}	13.81±0.01^{a,B}	10.90±0.02^{b,C}
S	15.59±0.02^{c,A}	13.98±0.10^{ab,B}	13.73±0.18^{a,B}	10.50±0.10^{c,C}
		b*		
C	4.18±0.05^{a,A}	5.98±0.04^{a,B}	7.20±0.03^{a,C}	9.02±0.03^{a,D}
L	3.83±0.03^{b,A}	3.88±0.01^{b,A}	3.93±0.04^{b,AB}	4.11±0.02^{b,B}
S	4.10±0.01^{c,A}	4.25±0.05^{c,A}	4.55±0.02^{c,B}	4.98±0.04^{c,C}

C (control), L (sausage with leaves aqueous extract), S (sausage with seeds aqueous extract)
*Values represent the mean of three independent replicates ± standard error
* $^{a-c}$: Values with different superscript within the same column differ significantly at $p<0.05$
* $^{A-D}$: Values with different superscript within the same raw differ significantly at $p<0.05$

3.3. Sensory analysis

The sensory scores of the sausages formulated with *M. oleifera* leaves and seeds aqueous extracts as well as those of control sausages are presented in Table 3.2.5. There were non-significantly (P>0.05) differences in the sensory panel scores between the different sausage formulas at 0-time and during the chilled storage period.. The sensory analysis results revealed that addition of both *M. oleifera* aqueous extracts particularly that of seeds during the formulation of the sausages did not alter the sensory characteristics of the products and improved their overall acceptability during the storage period. Improvement of overall acceptability of the sausages by addition of both plant extracts during the chilled storage period may be explained by their antioxidant and antimicrobial effects (**Sofy *et al.*, 2017**; **Ezzat *et al.*, 2020**) that prevent fat oxidation and microbial growth which are the main reasons that alter all sensory attributes of meat and meat products. However, slightly lower tenderness scores of both plant extracts formulated sausages may be related to their ability to reduce pH of the products (**Rodríguez *et al.*, 2016**; **Ezzat *et al.*, 2020**) and consequently increase the moisture loss which considered the main sensory feature of this type of sausage. The same results were obtained by using *M. oleifera* leaves and seeds in the formulation of different meat products, where there were non- significant (p<0.05) differences in all sensory panel scores between products formulated with or without the plant (**Das *et al.*, 2012; Hazra *et al.*, 2012; Al-Juhaimi *et al.*, 2016; Ganguly *et al.*, 2017; Abd El-Rahman *et al.* 2019; Bishnoi *et al.*, 2019**). However, **Jayawardana *et al.*** (**2015**) reported that incorporation of *M. oleifera* leaves extract had a significant negative impact on the sensory attributes and texture of the chicken sausages.

Table 3.2.5: Sensory attributes of semi-dry fermented sausages during storage at 4°C for 3months

Sensory attributes				
Treatments	**0-time**	**1st month**	**2nd month**	**3rd month**
Color				
C	7.50±0.12^{a,A}	6.53±0.11^{a,A}	6.23±0.12^{a,A}	6.00±0.21^{a,A}
L	8.00,±0.00^{a,A}	7.67±0.15^{a,A}	7.57±0.39^{a,A}	7.50±0.23^{a,A}
S	7.86,±0.25^{a,A}	7.63±0.13^{a,A}	7.52±0.22^{a,A}	7.41±0.21^{a,A}
Flavor				
C	8.10±0.03^{a,A}	7.11±0.03^{a,AB}	5.49±0.33^{a,B}	5.00±0.30^{a,B}
L	6.76±0.29^{a,A}	6.33,±0.39^{a,A}	5.00±0.40^{a,A}	4.98±0.22^{a,A}
S	8.00±0.33^{a,A}	7.33,±0.20^{a,A}	6.11±0.10^{a,A}	6.01±0.25^{a,A}
Tenderness				
C	6.11±0.15^{a,A}	5.22±0.15^{a,A}	5.13±0.39^{a,A}	4.78±0.30^{a,A}
L	5.24±0.09^{a,A}	4.98±0.12^{a,A}	4.81±0.21^{a,A}	4.50±0.12^{a,A}
S	5.98±0.05^{a,A}	5.00±0.00^{a,A}	4.87±0.33^{a,A}	4.66±0.15^{a,A}
Overall acceptability				
C	7.23±0.27^{a,A}	6.28±0.16^{a,A}	5.61±0.10^{a,A}	5.26±0.27^{a,A}
L	6.67±0.32^{a,A}	6.33±0.24^{a,A}	5.79±0.20^{a,A}	5.66±0.34^{a,A}
S	7.28±0.30^{a,A}	6.65±0.47^{a,A}	6.17±0.09^{a,A}	6.02±0.3^{a,A}

C (control), L (sausage with leaves aqueous extract), S (sausage with seeds aqueous extract)

*Values represent the mean of three independent replicates ± standard error

* ^{a} Values with different superscript within the same column differ significantly at $p<0.05$

* $^{A-B}$: Values with different superscript within the same raw differ significantly at $p<0.05$

Conclusion

Addition of *M. oleifera* leaves and seeds extracts to the semi-dry fermented sausages resulted in higher protein, fat and iron contents when compared with those of the control sausages. Significant elevation in calcium content, shear force and significant reduction in yellowness (b*) values were observed by addition of the leaves extracts to the sausages formulations. However, significant reduction of trans-fatty acids concentrations with improvement of the sensory panel scores of the sausages were achieved by incorporation of the seeds extracts. Therefore, these results may encourage the meat processors to incorporate the aqueous extracts of *M. oleifera* leaves and seeds during the formulation of fermented sausages to improve the nutritional values and all quality attributes of the products.

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DISCUSSION

DISCUSSION

Effect of *Moringa oleifera* aqueous extracts on the physicochemical characteristics, microbiological quality and biogenic amines of semi-dry fermented sausage

Phytochemical analysis, pH and scavenging activity of the plant extracts

Flavonoid and alkaloids were found in both *M. oleifera* leaves and seeds aqueous extracts, while the phenolic compounds were detected only in the leaves and the saponins and volatile oils were detected only in the seeds (Table 3.1.1). The results also revealed that *M. oleifera* leaves extract had significantly ($p<0.05$) lower pH value than the seeds extract which may be explained by the presence of higher proportion of phenolic compounds as ellagic, tannic, benzoic, and caffiec in *M. oleifera* leaves (**Gaafar *et al*., 2016**). The free radical scavenging results showed that there were non-significant ($p>0.05$) differences in DPPH % between the leaves and seeds extracts with mean values of 82.61±1.22 and 81.80±1.03% respectively. Higher scavenging activities of both extracts may be related to their higher contents of flavonoids and phenolic compounds **(Wu *et al*., 2004**)

pH, TBARS and TVBN

The results indicated that there were significant ($p<0.05$) reduction in pH values of the sausage by addition of both *M oleifera* leaves and seeds aqueous extracts during their formulation in comparison to the control group throughout the ripening process and chilled storage at 4°C for 3months (Table 3.1.2). Reduction of pH values during the ripening process of the sausage may be related to the elevation of lactic acid content as a result of carbohydrate breakdown by the inoculated starter culture (**Bozkurt and Erkmen 2002; Aleson-Carbonell *et al*. 2005; Fernández-López *et al*. 2008**). The results also revealed that the ripening time was significantly ($p<0.05$) shorter in sausages treated with both *M. oleifera* extracts than that of the control. Where, the control group reached to the desired pH (5.20) after 4 days while, the sausages treated with *M. oleifera* leaves and seeds extracts reached to this pH after 3 days from the beginning of the ripening process. The presence of flavonoids, organic acids, and phenolic acids in *M. oleifera* leaves and seeds may be responsible for the

acceleration of the ripening process of sausage (**Rodríguez *et al*., 2016**). Moreover, *M. oleifera* leaves contain higher amounts of vitamins, iron, calcium, proteins and essential amino acids as arginine, histidine, leucine, isoleucine and phenylalnine which can promote the growth of probiotic bacteria as lactobacilli causing rapid drop in pH value than the plant seeds (**Ritcher *et al*., 2003; Elkhalifa *et al*., 2007**; **Amer *et al*., 2014**). On the contrary, the chilling storage resulted in significant ($p<0.05$) elevation in pH values of all sausage treatments which may be attributed to the growth of spoilage bacteria and production of ammonia that was aroused from the proteolytic activity (**Mauriello *et al*., 2004; Ahmad and Srivastava, 2007**).

TBARS values of the sausages formulated with *M. oleifera* leaves extract were significantly ($P<0.05$) lower than those of formulated with seeds and control group during the ripening process and chilled storage (Table 3.1.2). These results indicated that *M. oleifera* leaves exhibited obvious antioxidant activities when compared with *M. oleifera* seeds. This result was in good agreement with that reported by **Sreelatha and Pedma (2009)**, **Ashfaq *et al*. (2012)** and **Unuigbe *et al*. (2014)**, who reported that the *M. oleifra* leaves extract had higher contents of natural antioxidants such as quercetin and kaempferol (**Tumer *et al*, 2015; Wang *et al*., 2017**), the sugar rhamnose, isothicoyanates, the glucosinolates (**Hsu *et al*, 2006; Ashfaq *et al*, 2012**), vitamin A, C and E, carotenoids, caffeoylguinic acids (**Siddhuraju and Becker, 2003; Aslam *et al*, 2005; Abdull Razis *et al*, 2014**), calcium, arginine and histidine (**Ferrao and Ferrao, 2005**) when compared with other parts of the plant. The results also revealed that the TBARS values of control group were significantly ($p<0.05$) increased during the ripening process and chilled storage to reach a value higher than the permissible limit (>1mg/kg malonaldehyde, **Zanardi *et al*., 2004**) at the end of storage period. However, the sausages formulated with both *M. oleifera* leaves and seeds extract showed TBARS values lower than this limit during the ripening process and till the end of chilled storage period. Previous studies reported lower TBARS values of meat and course ground meat products formulated with different forms and levels of *M. oleifera* during the chilled storage (**Sharaf *et al*., 2009; Ayssiwede *et al*., 2011; Hazra *et al*., 2012; Muthukumar *et al*., 2014**). Elevation of TBARS values of the

sausages during ripening and storage may be explained by the occurrence of dehydration and elevation of fat content and bacterial counts of sausage during the ripening and chilled storage (**Fan *et al*. 2015; Wang *et al*., 2015**).

TVBN values of the sausage formulated with *M. oleifera* leaves and seeds extracts were significantly ($p<0.05$) lower than those of the control group during the ripening process and chilled storage (Table 3.1.2). These results may be explained by the acceleration of starter culture growth, rapid falling in pH values and control the growth of natural microflora by addition of *M. oleifera* extracts during the processing of fermented sausage (**Lorenzo *et al*., 2000)**. The results also clarified that the ripening and chilled storage resulted in significant ($p<0.05$) increase in TVBN values the control group that exceeded the regulatory level (35 mgN/100 g, **Wang *et al*., 2015**) at the end of chilled storage. However, sausage treated with *M. oleifera* extracts were remained below the permissible limit till the end of chilled storage period.

Microbiological analysis

The microbiological counts of semi-dry fermented sausages during ripening and chilled storage were presented in Table 3.1.3. Lactic acid bacterial counts were significantly ($p<0.05$) higher in sausages formulated with *M. oleifera* leaves extract when compared with those formulated with seed extract and the control during the ripening and chilled storage. The result may be attributed to the higher contents of essential amino acids in *M.oleifera* leaves that act as growth promoters of lactic acid bacteria (**Ritcher *et al*., 2003; Elkhalifa *et al*., 2007**; **Amer *et al*., 2014**). However, the yeast and mold counts were significantly ($p<0.05$) lower in sausage formulated with both *M.oleifera* extracts than those of the control during the ripening and chilled storage. It has been reported that *M. oleifera* can be used as a natural antimicrobial material in the preservation of meat and meat products (**Najeeb *et al*., 2014; Singh *et al*., 2015**).

The antimicrobial activities of the plant leaves and seeds extract may be related to the presence of high content of alkaloids, tannins and saponine (**Nilforoushzadeh *et al*., 2008**). These active compounds of the plant aqueous extracts are able to inhibit

ATPase activity, damage DNA and cellular membrane of the microorganism **(Moreno *et al.*, 2006; Raybaudi-Massilia *et al,* 2009)**. Previous studies reported that *M. oleifera* seeds exhibited potent antimicrobial actions than the plant leaves (**Daljit *et al.*, 2013**) where, the seeds contain a short polypeptide named 4 (ά – L – rhamnosyloxy) benzyl-isothiocyanate which may inhibit the microbial growth though disruption of cell membrane and essential enzymes synthesis (**Idris, 2016**).

The results also showed that the ripening period resulted in significant ($p<0.05$) increase in lactic acid bacterial counts and significant ($p<0.05$) decrease in yeast and mold counts in different sausage treatments. At the end of the ripening period the yeast and mold contents were below the detectable counts in the sausage formulated with plant seed extract. In contrary, chilled storage resulted in significant ($p<0.05$) decrease in lactic acid bacterial counts in all sausage treatments and significant ($p<0.05$) increase in yeast and mold counts of the control only where the sausage formulated with both plant extracts showed yeast and mold below the detectable limits throughout the storage period. These results were in agreement with those of **Lockett *et al.*, (2000)**; **Tetsuji Okuda *et al.*, (2001)**; **Kebreab *et al.*, (2005)**; **Anwar and Rashid, (2007)**; **Jamil *et al.*, (2007)** who reported that *M oleifera* seeds extracts had more effective antifungal action than leaves extract.

Biogenic amines

The biogenic amines can be used as important quality and safety indicators of meat and meat products (**Delgado-Pando *et al.*, 2011**). Cadaverine, putrescine, histamine, tyramine, B-phenyl ethyl amine, tryptamine, spermidine and spermine have been reported as the most important biogenic amines in meat products, especially in fermented sausages (**Papavergou *et al.*, 2012**). The biogenic amines content of the semi-dry fermented sausages during the ripening and storage periods were shown in Table 3.1.4. The results revealed that the addition of *M. oleifera* leaves and seeds aqueous extracts during the production of semi- dry fermented sausage resulted in significant ($p<0.05$) reduction in all examined biogenic amines, especially at the end of storage period when compared to the control group. The results also clarified that *M. oleifera* seeds extract was more effective in the reduction of the biogenic amines

formation in sausage particularly, the cadaverine, and tryptamine than the leaves extract either throughout the ripening and storage periods These results may be attributed to the higher content of essential amines in the plant leaves (**Ritcher *et al*., 2003; Elkhalifa *et al*., 2007**; **Amer *et al*., 2014**) which act as the main precursors of the biogenic amines formation in fermented sausage (**Mora *et al*., 2015; Sánchez *et al*., 2017**).

Furthermore, the higher lactic acid bacterial counts and the rapid falling of pH values by addition of *M. oleifera* leaves extract may enhance the accumulation of biogenic amines in fermented sausages (**Suzzi and Gardini, 2003**; **Papavergou, 2011; Papavergou *et al*., 2012)**. In general the lower biogenic amines content of sausages formulated with both *M. oleifera* extracts may be related to the antibacterial, antifungal (**Sofy *et al*., 2017**) and antioxidant (**Al-Juhaimi *et al*., 2016**) activities of the plant. It has been reported that there was a positive relation between the putrescine formation and the total aerobic counts (**Ruiz- Capillas and Jiminez-Colmenero, 2004**), while cadaverine and histamine were usually associated with the presence of the decarboxylase-positive microbiota, as *Enterobacteria* (**Komprda, *et al*., 2009**). The tyramine formation has been attributed to the action of *B. thermosphacta* (**Nowak *et al*., 2011**). Moreover, there is evidence that the mold can be aid in the formation of biogenic amines especially, putrescine and histamine in meat products (**Montel *et al*., 1999**).

The results also revealed that cadaverine and -phenyl ethyl amine were not detected in sausages incorporated with *M. oleifera* seeds extract, while tyramine was not detected in all sausage treatments at the 1st day of ripening. In addition, tryptamine was not detected in all sausages treatments till the 2nd day of ripening and still under the detectable limit till the end of ripening period and at 0 time of the chilled storage in sausage treated with plant seeds extract. Spermine and spermidine were found with considerable higher concentrations in different sausage treatments at the 1st day of ripening, which may be due to the natural occurrence of these amines in the living cells (**Cai *et al*., 2015**). During the ripening and storage periods, all examined biogenic amines increased significantly ($P<0.05$) in all sausage treatments with the control had

the highest amounts while; the sausages treated with seeds extract showed the lowest concentrations. At the end of storage period, cadaverine was the dominant biogenic amine followed by spermine, spermidine, putrescine, tyramine, histamine, phenylamine and tryptamine. These results were in agreement with those reported by **Latorre-Moratalla *et al*. (2008)** who established the same order of the amines in fermented meat products. The formation of biogenic amines during the ripening or storage of fermented sausage is a very complex phenomenon that depending on several factors, such as the microbial growth, acidity, protein content, proteolytic and decarboxylase activities (**Latorre-Moratalla *et al.*, 2008**).

The reduction of biogenic amines formation in fermented sausage by the addition of *M. oleifera* leaves and seeds aqueous extracts is important to produce more healthy product with prolonged storage life. It has been reported that cadaverine, histamine and putrescine can be used as spoilage indicators, moreover, histamine was regarded as a toxic agent and the presence of putrescine and cadaverine can potentiate the histamine toxicity (**Cai *et al*., 2015**). **Ruiz-Capillas and Jiminez-Colmenero (2004)** reported that tyramine may cause hypertension, headaches and hormonal disturbance. Moreover; putrescine, cadaverine and spermidine have been reported as potent precursors in the formation of carcinogenic *N*-nitrosamines (**Mah and Hwang, 2009**). Therefore, the formation of biogenic amines in food should be controlled and not exceeded their established permissible limits.

Improving the nutritional, physicochemical and sensory quality of semi-dry fermented sausage by using *Moringa oleifera* aqueous extracts

Chemical composition

Incorporation of *M. oleifera* leaves and seeds aqueous extracts during the formulation of semi-dry fermented sausages resulted in significant ($P<0.05$) increase in protein, fat, calcium and iron contents when compared to the control group at 0-time and during storage at 4°C for 3 months (Table 3.2.2). These results may be related to the high protein and minerals contents of the plant leaves and seeds **(Khalafalla *et al*., 2010; Salama *et al*. 2017**) used in the sausage production (Table 3.2.1). These results were in agreement with those of **Al-Juhaimi *et al*. (2016) and Abd El-Rahman *et al*.**

(2019) who reported that there were significant (P<0.05) elevation in the protein and fat levels of beef burger and traditional Egyptian luncheon with increasing the rate of *M. oleifera* addition during their processing. Moreover, **Ibrahim *et al*. (2017)** found that the meat-rice kofta formulated with *M. oleifera* leaves had higher minerals contents than the control group. The results also showed that the calcium content of the sausages formulated with leaves extract was significantly (P<0.05) higher than those of formulated with seeds extract at 0-time and during the chilled storage which may be related to the higher calcium content of the plant leaves (**Sahay *et al*., 2017**).

Fatty acids profile showed that arachidic acid (C20:0) was significantly (P<0.05) higher in the sausages formulated with the seeds extract when compared with those formulated with leaves extract and the control group at 0-time and during the chilled storage (Table 3.2.3). However, the highest (P<0.05) contents of pentadecanoic acid (C15:0, C15:1) were observed in the sausages formulated with the leaves extract at 0-time of processing. Linolenic (C18:3) and eicosenoic (C20:1) acids were significantly (P<0.05) higher in the sausages formulated with both plant extracts in comparison with the control group at 0-time and during the chilled storage. Moreover, decosanoic acid (C22:0) levels were significantly (P<0.05) higher in the sausages treated with both plant extracts than those of the control group at 1st month of the chilled storage.

On the other hand, the control group showed significantly (P<0.05) higher content of heptadecanoic acid (C17:1) than the sausages formulated with both plant extract at 2nd and 3rd months of the chilled storage. The results also showed that stearidonic acid (C18:4) not appeared in the sausages treated with both extracts till 3rd month of the chilled storage with those treated with seeds extract had significantly (P<0.05) lower content. The differences of fatty acid contents of the experimentally formulated sausages may be related to the variations in fatty acids profile of raw beef, leaves and seeds extracts used in their processing (Table 3.2.1).

Incorporation of leaves and seeds extracts during the processing of sausages led to significant (P<0.05) elevation in health promoting and functional fatty acids while

significant (P<0.05) reduction in trans-fatty acids content. It has been reported that pentadecanoic (**Warensjö *et al*., 2010**; **Forouhi *et al*., 2014**), linolenic and eicosenoic acids **(Gerhard *et al*. 2004)** had the ability to decrease the incidence of cardiovascular disease. In addition, linolenic and eicosenoic acids were reported as potent acids in reduction of the blood cholesterol level (**Ogunlesi *et al*., 2010**). Moreover, monounsaturated fatty acids particularly oleic acid were known to have potent antifungal and antibacterial properties (**Riveros *et al*. 2010; Ogunlesi *et al*., 2010**). However, trans-fatty acids have been reported as a main cause of cardiovascular disease, elevation of low-density lipoproteins as well as reduction in the level of high density lipoproteins in the blood (**Fritsche and Steinhart, 1997; Lichtenstein, 1998**). The results also showed that the chilled storage led to significant (P<0.05) increase in the protein, fat, mineral and fatty acids contents of all sausage treatments which may due to the occurrence of dryness and moisture loss (**Vesković *et al*., 2013**).

Shear force and color

The shear force values of the sausages formulated with the seeds extracts were not significantly (P>0.05) higher than those of the control group at 0-time and throughout the storage period (Table 3.2.4). However, the addition of the leaves extract led to significant (P<0.05) increase in shear force values of the sausages when compared with the two other treatments at 0 time and 1st month of the chilled storage. The high protein content of *M.oleifera* leaves (**Salama *et al*. 2017)** and the high fiber content of the seeds (**Falowo *et al*., 2018**) may be the main factors which determine the texture of the products.

The lightness (L*) values of the sausages treated with *M. oleifera* leaves extract were significantly (P < 0.05) higher than those of formulated with seeds extract and the control groups at 1st month till the end of chilled storage period (Table 3.2.4). The lightness (L*) values of meat and meat products depend mainly on the fat and moisture contents **(Fischer, 2007)**, therefore, higher lightness (L*) values of sausages formulated leaves extract may be related to its higher fat content.

The results also revealed that addition of *M. oleifera* leaves extracts during the formulation of the sausage led to significant (P<0.05) increase in redness values (a*) when compared to those formulated with the seeds and control group particularly at 0-time and 3rd month of the chilled storage period (Table 3.2.4). However, the lowest yellowness (b*) values were observed in the sausages formulated with leaves extracts when compared to those treated with the seeds and control groups at 0-time and throughout the chilled storage period. It has been reported that redness (a*) and yellowness (b*) values of meat can be affected by the level of lipid oxidation **(Phillips *et al.*, 2001)**, therefore the higher values (a*) and lower (b*) values of leaves extract incorporated sausages may be closely related to the antioxidant activity of the plant leaves (**Sofy *et al.*, 2017**; **Ezzat *et al.*, 2020**). Chilling storage of different sausages treatments resulted in significant (P<0.05) decrease in (L*) and (a*) values while significant (P<0.05) increase in (b*) values which may be due to increase in lipid oxidation level.

Sensory analysis

There were non-significantly (P>0.05) differences in the sensory panel scores between the different sausages formulas at 0-time and during the chilled storage period (Table 3.2.5). The sensory analysis results revealed that addition of both *M. oleifera* aqueous extracts particularly that of seeds during the formulation of the sausages did not alter the sensory characteristics of the products and improved their overall acceptability during the storage period. Improvement of overall acceptability of the sausages by addition of both plant extracts during the chilled storage period may be explained by their antioxidant and antimicrobial effects (**Sofy *et al.*, 2017**; **Ezzat *et al.*, 2020**) that prevent fat oxidation and microbial growth which are the main reasons that alter all sensory attributes of meat and meat products.

CONCLUSION

CONCLUSION

It could be concluded from the present study that

1. Incorporation of *M. oleifera* leaves aqueous extracts during the formulation of semi-dry fermented sausage resulted in significant reduction in the ripening period by rapid falling of pH values in comparison with the control group.
2. *M. oleifera* leaves aqueous extracts exhibited potent antioxidant activities which appeared in the product by significant reduction in TBARS, TVBN and yellowness (b*) values while significant elevation in redness (a*) value when compared to the control group immediately after processing and during the chilled storage.
3. Significant elevation in protein, fat, calcium, iron contents, lactic acid bacterial counts, lightness (L*) and shear force values were noted by the addition of *M. oleifera* leaves aqueous extracts during the formulation of semi-dry fermented sausage when compared to the control group.
4. Aqueous extract of *M. oleifera* seeds showed potent antifungal and antibacterial effects by significant reduction in yeast and mold counts as well as the biogenic amines content of the sausages when compared to the control group during the ripening process and chilled storage.
5. Significant elevation in healthy and functional fatty acids while significant reduction in trans-fatty acids contents were observed by the incorporation of *M. oleifera* seeds aqueous extracts during the formulation of semi-dry fermented sausage when compared to the control group immediately after processing and during the chilled storage.
6. There were non-significant changes in the sensory panel scores of the sausages formulated with both extracts in comparison with the control group immediately processing and during the chilled storage.

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Abstract

Key words: *M. oleifera*, extract, fermentation, sausages, quality

The main objective of the current work was to investigate the effect of *Moringa oleifera* leaves and seeds aqueous extracts on the physicochemical, microbiological, nutritional and sensory quality attributes in addition; the biogenic amines content of semi-dry fermented sausage during ripening process and storage at 4ºC for 3 months. To achieve these objectives, semi-dry fermented sausages were formulated by using *M. oleifera* leaves and seeds aqueous extracts at a rate of 1.5% in comparison to the control. Different sausage formulations were ripened till reach the desired pH value of 5.2, cooked at 72ºC core temperature and stored at 4ºC for 3 months. Incorporation of both plant aqueous extracts during the formulation of fermented sausage resulted in a significant (P<0.05) decrease in pH, TBARS, TVBN values, yeast and mould counts, biogenic amines, trans-fatty acids contents as well as yellowness (b*) value when compared to the control group. However, the sausages formulated with both extracts exhibited significantly (P<0.05) higher lactic acid count, protein, fat, calcium, iron, beneficial fatty acids contents, shear force value, lightness (L*) and redness (a*) values in comparison to the control group. However, there were non-significant (P>0.05) changes in the sensory panel scores among various sausage formulations. The results also revealed that the chilled storage had negative impact on all examined parameters of the sausages with the control group had unacceptable values while those treated with plant extracts maintained their overall quality till the end of the chilled storage period.

RECOMMENDATIONS

RECOMMENDATIONS

1. It is highly recommended to apply *M. oleifera* aqueous extracts as a natural additive during the processing of fermented sausages to improve their nutritional, physicochemical, microbiological and sensory quality attributes.
2. Using *M. oleifera* leaves and seeds aqueous extracts as natural antioxidant and antimicrobial preservatives, respectively to substitute the synthetic preservatives which may cause serious health hazards to the consumer.
3. Application of *M. oleifera* leaves aqueous extracts during the formulation of fermented meat products can provide beneficial health benefits to the consumer through their prebiotic effect that stimulate the growth of health promoting bacteria (probiotics) after the product ingestion so, the fermented products formulated with *M. oleifera* leaves can be categorized as functional foods.
4. Decrease the incidences of food poisoning outbreaks that may cause either by the presence of pathogenic microorganisms and or biogenic amines can be achieved by the incorporation *M. oleifera* seeds aqueous extracts during the processing of meat products.
5. Using *M. oleifera* seeds aqueous extracts as a functional additive in meat products can reduce the conversion of unsaturated fatty acids to trans-fats that may cause serious health hazards to the consumer.

SUMMARY

SUMMARY

Nowadays, new preservation techniques are being developed to extend the storage time with maintaining both the quality and safety of meat products. Synthetic preservatives as nitrite, sorbate and BHT have been widely used for preserving meat products such as sausages. However, there is an association between consumption of the processed meat containing these preservatives and increase the risk of different diseases. Unlike synthetic compounds, the natural extracts obtained from plants are rich in phenolic compounds and flavonoids which can enhance the overall quality of the products by decreasing the lipid oxidation and microbial growth. Moreover, the plant extracts are considered rich source of vitamins, minerals, proteins, essential fatty and amino acids so they can improve the nutritional value of the products treated with them. *M. Oleifera* aqueous extract is a multifunctional compound as it exhibits potent antioxidant and antimicrobial activities in addition; it can provide various health benefits to the consumers.

Application of *M. Oleifera* leaves as a natural preservative in meat processing area has been progressively increased but still limited on few numbers of the products such as fresh sausages and burger patties. Moreover, there was not enough information about the functional effects of *M. Oleifera* seeds on the overall quality of the meat products. In general, the studies that investigated the effect of using of *M. Oleifera* on the nutritional value and sensory quality of meat products are scarce. Therefore, the main objective of the current work was to study the effect of using *M. Oleifera* leaves and seeds aqueous extracts on the physicochemical, microbiological, sensory and nutritional quality attributes as well as on the biogenic amines contents of semi-dry fermented sausage.

The research work of this study has been divided into two parts. In the first part, effect of *Moringa oleifera* aqueous extracts on the physicochemical characteristics, microbiological quality and biogenic amines of semi-dry fermented sausage has been studied. Semi-dry fermented sausages were formulated with *M. oleifera* leaves and seeds aqueous extracts at the rate of 1.5% separately in comparison with the control.

Different sausage formulations were ripened till reach the desired pH value of 5.2 , cooked at 72ºC core temperature and stored at 4ºC for 3 months. The analysis of the sausages for different parameters was performed daily during the ripening period and monthly during the chilled storage for 3 months.

The results revealed that incorporation of *M oleifera* leaves and seeds aqueous extracts during the formulation of fermented sausage resulted in a significant ($P<0.05$) decrease in pH values during the ripening and storage periods in comparison to the control. Moreover, the lowest TBARS and TVBN values were observed in the sausage formulated with *M. oleifera* leaves when compared to the other two formulations. The results also revealed that the chilled storage led to significant ($P<0.05$) increase in pH, TBARS and TVBN values of different sausage formulations where at the end of the storage period, the control group exceeded the established regulatory levels while the sausages formulated with both extracts showed lower values than this levels.

Microbiological analysis of the sausages showed that the sausages formulated with the plant leaves extract had the highest lactic acid bacterial counts than those formulated with seed extract and the control during the ripening and chilled storage. However, the yeast and mold counts were significantly ($P<0.05$) lower in sausage formulated with both extracts in comparison to the control group. The results also showed that the ripening period resulted in a significant ($P<0.05$) increase in lactic acid bacterial counts and significant ($P<0.05$) decrease in yeast and mold counts in different sausage treatments. On the contrary, the chilled storage resulted in a significant ($P<0.05$) decrease in lactic acid bacterial counts in all sausage treatments and a significant ($P<0.05$) increase in yeast and mold counts of the control only where the sausage formulated with both plant extracts showed yeast and mold counts below the detectable limits throughout the storage period.

Incorporation of both *M. oleifera* leaves and seeds aqueous extracts resulted in significant ($P<0.05$) reduction in all examined biogenic amines when compared with the control particularly at the end of the chilled storage period. However, the ripening and chilled storage periods led to significant ($P<0.05$) elevation in all the examined

biogenic amines with the control had the highest amounts while; the seeds extract formulation showed the lowest concentrations.

In the second part of the study, the effect of using *Moringa oleifera* aqueous extracts at level of 1.5% on the nutritional, physicochemical and sensory quality attributes of semi-dry fermented sausage has been compared with the control through a three trials based experiment. The analysis of the sausages for different parameters was carried out immediately after processing (0-time) and during the chilled storage at 4 ºC for 3 months. The results revealed that addition of both plant extracts during the sausages processing led to significant ($P<0.05$) elevation in protein, fat, calcium and iron contents when compared to the control at 0-time and during the chilled storage. The results also clarified that formulation of the sausages with both extracts resulted in significant increase ($P<0.05$) in the functional and health promoting fatty acids and significant ($P<0.05$) reduction in trans-fatty acids in comparison to the control at 0-time and during the chilled storage. The results also showed that the chilled storage led to significant ($P<0.05$) increase in the protein, fat, mineral and fatty acids contents of all sausage treatments.

Addition of the leaves extract during the processing of the sausage led to significant ($P<0.05$) increase in shear force values when compared with the two other treatments at 0 time and 1st month of the chilled storage. Moreover, the lightness (L*) values of the sausages treated with *M. oleifera* leaves extract were significantly ($P < 0.05$) higher than those of formulated with seeds extract and the control groups at 1st month till the end of chilled storage period. The results also revealed that there were significant ($P<0.05$) increase in redness values (a*) by incorporation of the leaves extracts during processing of the sausages when compared to the other two formulations particularly at 0-time and 3rd month of the chilled storage period. On the other hand, the lowest yellowness (b*) values were observed in the sausages treated with leaves extracts when compared to those treated with the seeds and control groups at 0-time and throughout the chilled storage period. Chilled storage led to significant ($P<0.05$) increase in shear force and b* values while significant ($P<0.05$) decrease in L*and a* values in all sausages formulations. However, addition of both extracts and

the chilled storage resulted in non-significant ($P>0.05$) changes in the sensory panel scores between the different sausage formulations.

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الملخص العربي

الملخص العربى

في الوقت الحاضر ، تم استخدام تقنيات جديدة للحفظ وذلك بغرض اطالة فترة الصلاحيه مع الحفاظ على جودة وسلامة منتجات اللحوم باستخدام المواد الحافظة الاصطناعية مثل النتريت والسوربات و بيوتيليتت هيدروكسي طولوين على نطاق واسع للحفاظ على منتجات اللحوم مثل السجق. بالرغم من أن هناك ارتباط وثيق بين استهلاك اللحوم المصنعة التي تحتوي على هذه المواد الحافظة وزيادة خطر الإصابة بالأمراض المختلفة. وعلى عكس المركبات الاصطناعية، فإن المستخلصات الطبيعية التي يتم الحصول عليها من النباتات الطبيعية الغنية بالمركبات الفينولية والفلافونويد التي يمكن أن تعزز الجودة الإجمالية للمنتجات عن طريق تقليل أكسدة الدهون والنمو الميكروبي. علاوة على ذلك ، تعتبر هذه المستخلصات النباتية مصدرًا غنيًا بالفيتامينات والمعادن والبروتينات والأحماض الأمينية الأساسية والأحماض الدهنية لذلك فانها تقوم بتحسين القيمة الغذائية للمنتجات المعالجة بها.

ويعتبرالمستخلص المائي لنبات المورينجا اولفيرا مركب متعدد الوظائف نظرا لما يتمتع به من فاعليه قوية كمضاد للأكسدة ومضاد للميكروبات كما أنه له فوائد صحية مختلفة للمستهلكين.

وفى الأونة الأخيرة قد تم استخدام أوراق المورينجا اولفيرا بشكل متزايد كمادة حافظة طبيعية في مجال تصنيع وحفظ اللحوم ولكن لا يزال استخدامها محدود في عدد قليل من المنتجات مثل السجق الشرقى والبرجر. وعلاوة على ذلك ، لم تكن هناك معلومات كافية حول التأثيرات الوظيفية لبذور المورينجا اولفيرا على الجودة الكلية لمنتجات اللحوم. وبشكل عام فان الدراسات التي بحثت في تأثير استخدام المورينجا اولفيرا على القيمة الغذائية والجودة الحسية لمنتجات اللحوم نادرة. لذلك، فقد كان الهدف الرئيسي من العمل الحالي هو دراسة تأثير استخدام المستخلصات المائية لأوراق وبذور المورينجا اولفيرا على الخصائص الفيزيائية والكيميائية والميكروبيولوجية و الجودة الحسية والقيمة الغذائية وكذلك على محتويات الأمينات الحيوية للسجق المتخمر شبه الجاف.

فقد تم تقسيم الجزء العملى لهذه الدراسة إلى قسمين: في الجزء الأول، كان الهدف الرئيسى من الدراسة هو دراسة تأثير المستخلص المائي لأوراق وبذور نبات المورينجا أوليفيرا على الخواص الفيزيوكيمائية، الميكروبيولوجية ومحتوى الأمينات الحيوية في السجق المتخمر نصف الجاف أثناء عملية التخمر والتخزين عند درجة حرارة ٤° م لمدة ثلاثة أشهر. حيث تم الانتاج التجريبي لمنتج السجق المتخمر نصف الجاف باضافة المستخلص المائي لكل من البذور والأوراق بمعدل ١.٥ ٪ مقارنة بالعينة الضابطة التى تم انتاجها بدون اصافة المستخلصات المائية للنبات. وقد أظهرت النتائج أن إضافة تلك المستخلصات قد أدى إلى انخفاض معنوي في تركيز الأس الهيدروجيني مما أدي للوصول لدرجة الحموضه المرغوبه لهذا النوع من السجق خلال ثلاثة ايام مقارنة باربعة ايام في العينة الضابطة. كما أدت اضافة تلك المستخلصات إلى إنخفاض معدلات اكسدة الدهون والبروتينات

وزيادة في بكتيريا حمض اللاكتيك وإنخفاض الفطريات والخمائر. كما أدي استخدام المستخلصات المائية لنبات المورينجا أوليفيرا إلى إنخفاض معدل تكون الأمينات الحيوية أثناء فترة التخمر والتخزين مقارنة بالعينة الضابطة. وبناءا علي هذه النتائج يمكن استنتاج أن استخدام هذه المستخلصات في منتجات اللحوم قد يؤدي الي انتاج منتج امن لصحه المستهلك.

وفى الجزء الثانى، فقد تم دراسة تاثيراضافة المستخلص المائي لأوراق وبذور نبات المورينجا أوليفيرا على القيمة الغذائية والجودة الحسية والخواص الفيزيائية والكيميائية للسجق المتخمر نصف الجاف بعد التصنيع وخلال فترة التخزين بالتبريد عند درجة حرارة ٤درجه مئويه ولمدة ثلاثة اشهر بالمقارنة مع العينه الضابطة. وقد خلصت الدراسة الى أن اضافة تلك المستخلصات المائيه لأوراق وبذور نبات المورينجا أوليفيرا أدى الى زيادة القيمة الغذائية للمنتج عن طريق زيادة معنوية فى نسب البروتينات والدهون بالاضافة الى زيادة معنوية لمحتوى المنتج من المعادن الهامة والضروريه للنمو مثل الحديد والكالسيوم وكذلك زيادة معنوية فى العديد من الاحماض الدهنية الضروريه التى لها العديد من الفوائد المختلفة على الصحة العامة كما ادت الى تحسين الخواص الحسية للمنتج خاصة فى المجموعة المعاملة بمستخلص بذور المورينجا. ومن ناحيه أخري أدي اضافه هذه المستخلصات الى تقليل نسب الدهون المتحولة التى لها اضرار بالغة على صحة المستهلكين وبالتالى تقليل الاصابة بأمراض القلب وتصلب الشرايين وغيرها وذلك مقارنة بالمجموعة الضابطة بعد التصنيع مباشرة واثناء التخزين بالتبريد. أوضحت النتائج أيضا أن التخزين بالتبريد أدى إلى زيادة معنوية في محتوى البروتين والدهون والمعادن والأحماض الدهنية لجميع معالجات السجق.

كما توصلت الدراسة الى أن اضافة مستخلص الأوراق قد أدت الى زيادة معنوية فى قوة الشد للمنتج بالمقارنة بالمعاملتين الأخرتين وذلك بعد التصنيع مباشرة وخلال الشهر الاول من التخزين بالتبريد. وعلاوة على ذلك، كانت قيم (L*) للسجق المعالج بمستخلص أوراق المورينجا أعلى بشكل ملحوظ من تلك التي تم معالجتها بمستخلص البذور والعينه الضابطة وذلك بداية من الشهر الأول وحتى نهاية فترة التخزين بالتبريد. كما أوضحت النتائج أن هناك زيادة معنوية في قيم (a*) نتيجة اضافة مستخلصات الأوراق أثناء تصنيع السجق مقارنة بالمعاملتين الأخريين خاصة بعد التصنيع مباشرة واثناء الشهر الثالث من الحفظ بالتبريد. ومن ناحية أخرى ،فقد لوحظ انخفاض معنوي في قيم (b*) لدي السجق الذى تم معالجته بمستخلصات الأوراق عند مقارنتها مع تلك التي تمت معالجتها بالبذور و المجموعة الضابطة بعد التصنيع مباشرة وطوال فترة التخزين بالتبريد. بالاضافة الى ذلك فقد أدى التخزين بالتبريد إلى زيادة معنوية في قوة الشد وقيم (b*) بينما ادى الى انخفاض كبير في قيم (L*) و (a*) في جميع المعاملات المختلفه. وقد اوضحت النتائج أيضا ان كلا من إضافة المستخلصات المائيه والتخزين المبرد للسجق المتخمر نصف الجاف أدي إلى تغييرات غير معنويه في خواص الجودة الحسية للمعاملات المختلفه.

جامعة القاهرة
كلية الطب البيطري
قسم الرقابة الصحية على الأغذية

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تاريخ الميلاد: ١٩٨٦/٧/١٩

الدرجة: دكتوراه الفلسفة في العلوم الطبية البيطرية "الرقابة الصحية على اللحوم ومنتجاتها"
عنوان الرسالة: "تأثير نبات المورينجا على سلامة وجودة السجق المتخمر النصف الجاف"
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المستخلص:
الكلمات الدالة:الجودة، مستخلص المورينجا اوليفيرا، التخمر، السجق

الهدف الرئيسى من العمل الحالي هو دراسة تأثير المستخلص المائي لأوراق وبذور نبات المورينجا أوليفيرا على الخواص الفيزيوكيمائية، الكيميائية، الميكروبيولوجية ، القيمة الغذائية والجودة الحسية بالإضافة إلى محتوى الأمينات الحيوية في السجق المتخمر نصف الجاف أثناء عملية التخمر والتخزين عند درجة حرارة ٤°م لمدة ثلاثة أشهر. ولتحقيق هذه الأهداف تم الانتاج التجريبي لمنتج السجق المتخمر نصف الجاف باضافة المستخلص المائي لكلا من البذور والأوراق بمعدل ١.٥ ٪ مقارنة بالعينة الضابطة التى تم انتاجها بدون اضافة المستخلصات المائية للنبات وتم تخزين المعاملات المختلفه عند ٤°م لمدة ثلاثة أشهر. وقد أظهرت النتائج أن إضافة تلك المستخلصات قد أدت إلى انخفاض معنوي في تركيز الأس الهيدروجيني مما أدي للوصول لدرجة الحموضة المناسبة لهذا النوع من السجق خلال ثلاثة ايام مقارنه باربعة أيام في العينة الضابطة. كما أدي استخدام تلك المستخلصات إلى إنخفاض معنوي في معدل اكسدة الدهن و تحلل البروتينات وزيادة في بكتيريا حمض االلاكتيك وإنخفاض نمو الفطريات والخمائر. كما أدي استخدامها أيضا إلى إنخفاض معنوي في معدل تكون الأمينات الحيوية أثناء فترة التخمر والتخزين مقارنة بالعينة الضابطة .وأوضحت النتائج أيضا انخفاض معنوى فى محتويات الأحماض الدهنية المتحولة و قيم (b*) باستخدام تلك المستخلصات عند مقارنتها بالمجموعة الضابطة بعد التصنيع مباشرة. ولكن اضافه كلا من المستخلص الأوراق والبذور اثناء تصنيع السجق أدي الي زيادة معنوية فى العد الكلى لبكتريا حمض اللاكتيك ، نسب محتوى البروتين، الدهون، الكالسيوم، الحديد، الأحماض الدهنية المفيدة ، قيمة قوة الشد، (L*) و (a*) مقارنة بالعينة الضابطة. ومع ذلك ، كانت هناك تغييرات غير معنويه في خواص الجودة الحسية بين معاملات السجق المختلفة. وأوضحت النتائج أيضا أن التخزين بالتبريد كان له تأثير سلبي على جميع معايير الجوده التي تم فحصها خاصة في العيته الضابطه. وكذلك فقد حافظت المجموعات المعالجة بالمستخلصات النباتية على جودتها الشاملة حتى نهاية فترة التخزين بالتبريد. وبالتالى فقد خلصت الدراسة الى امكانية استخدام هذا النبات للحصول على منتج آمن مع تحسين معايير الجودة المختلفة للمنتج حتى نهاية صلاحيته.

جامعة القاهرة
كلية الطب البيطرى
قسم الرقابة الصحية على الأغذية

تأثير نبات المورينجا على سلامة وجودة السجق المتخمر النصف الجاف

رسالة مقدمه من

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للحصول على
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٢٠٢٠

To my mother and my father

To my husband and my family

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First of all, gratitude and prayerful to my ALLAH who gives me the power not only to carry out this work but also during my whole life.

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LIST OF ABBREVIATIONS

DPPH	2,2 Diphenyle-2-picrylhydrazyle
MUFA	Monounsaturated Fatty Acids
PUFA	Polyunsaturated Fatty Acids
SFA	Saturated Fatty Acids
TBARS	Thiobarbituric acid reactive substance
TVBN	Total volatile base nitrogen
UFA	Unsaturated Fatty Acids
WBSF	Warner-Bratzler shear force

INTRODUCTION