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Effect of *Moringa oleifera* Aqueous Extracts on the Physicochemical Characteristics, Microbiological quality and Biogenic Amines of Semi-dry Fermented Sausage

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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.

Key words: Moringa, extract, sausage, ripening, amines, quality.

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

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(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.

MATERIALS AND METHODS

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.

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.

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

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).

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.

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.

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.

Physicochemical properties

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).

Microbiological examination

Lactic acid bacteria were counted according to the procedures defined by 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).

Biogenic amines

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

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).

RESULTS AND DISCUSSION

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 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.

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 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 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. However, 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.

Table 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

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 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 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.

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). 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.

Table 2: pH, TBARS and TVBN values of semi-dry fermented sausage during ripening and storage period at 4°C for 3months.

	Ripening				Storage			
	1st day	2st day	3st day	4st day	0 time	1st month	2nd month	3rd month
pH								
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

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

	Ripening				Storage			
	1st day	2st day	3st day	4st day	0 time	1st month	2nd month	3rd month
Lactic acid bacteria								
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}
Mould								
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

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.

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 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

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

	Ripening				Storage			
	1st day	2st day	3st day	4st day	0 time	1st month	2nd month	3rd month
Cadaverine								
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.

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.

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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