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## Effects of different levels of Moringa (*Moringa oleifera*) seed flour on quality attributes of beef burgers

### Efectos de los diferentes niveles de semilla de harina de Moringa (*Moringa oleifera*) en los atributos de calidad de las hamburguesas de ternera

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Meat of beef patties was partially replaced by different levels (0, 2, 4 and 6%) of Moringa seed flour (MSF). The replacement of meat did not significantly ( $p \leq 0.05$ ) affect the chemical composition of the patties. The cooking properties of the patties formulated with MSF improved with the flour level. The thiobarbituric acid value was higher for non-formulated patties than for formulated ones after storage. The addition of MSF slightly reduced the pH of raw patties with the storage time. Patties formulated with MSF had significantly ( $p \leq 0.05$ ) lower aerobic plate counts throughout the storage period. As the level of MSF increased, the patties had high lightness ( $L^*$ ) and yellowness ( $b^*$ ) values but low redness ( $a^*$ ) values. A relatively low sensory acceptability was noted for MSF-formulated patties and was slightly reduced at longer storage times, but that of non-formulated ones decreased significantly ( $p \leq 0.05$ ) at longer storage times. The results indicated that *M. oleifera* seed flour has a high potential as a binder for use in beef patty production in addition to its health and nutritional benefits.

**Keywords:** beef burger; Moringa; chemical composition; cooking properties; texture; colour

La carne de pastelitos de ternera fue parcialmente remplazada por diferentes niveles (0, 2, 4 y 6%) de harina de semilla de Moringa (HSM). La substitución de la carne no afectó significativamente ( $p \leq 0,05$ ) la composición química de los pastelitos. Las propiedades de cocinado de los pastelitos formulados con HSM mejoraron según el nivel de harina. El valor de ácido tiobarbitúrico fue mayor para los pastelitos sin fórmula que aquel de los formulados después de almacenamiento. La adición de HSM redujo ligeramente el pH de los pastelitos crudos con el periodo de almacenamiento. Los pastelitos formulados con HSM obtuvieron un recuento de aerobios en placa significativamente menor ( $p \leq 0,05$ ) durante el periodo de almacenamiento. Según se aumentaba el nivel de HSM, los pastelitos resultaban tener valores mayores de color claro ( $L^*$ ) y amarillento ( $b^*$ ) aunque con un valor menor de color rojizo ( $a^*$ ). Se observó una aceptabilidad sensorial relativamente baja para las formulaciones de pastelitos HSM que se redujo ligeramente con los periodos de almacenamiento más largos, aunque aquella de los pastelitos sin fórmula disminuyó significativamente ( $p \leq 0,05$ ) con los periodos de almacenamiento más largos. Los resultados indicaron que la harina de semilla de *M. oleifera* tiene un gran potencial como aglomerante para su uso en la producción de pastelitos de ternera además de ser beneficiosa para la salud y nutricional.

**Palabras clave:** hamburguesa de ternera; Moringa; composición química; propiedades de cocinado; textura; color

## 1. Introduction

In recent years, the demand for low-fat meat products has increased dramatically, particularly saturated fats, which are associated with a high risk of chronic diseases (Popkin, Adair, & Ng, 2012; Price, Diaz, Banon, & Garrido, 2013). Fat functions as a carrier for flavour compounds and contributes to the texture of the product, and reducing the fat content may alter product quality by causing the product to become firmer, more rubbery, less juicy and darker in colour (Kirchner, Beasley, Harris, & Savell, 2000). The palatability of reduced fat meat products, such as burgers and patties, can be increased with the addition of various fat substitutes. The following non-meat fat substitutes, fillers, binders or extenders are commonly used: wheat flour or soy flour in buffalo meat burgers (Modi, Mahendrakar, Rao, & Sachindra, 2004); ragi or finger millet in chicken patties (Naveena, Muthukumar, Sen, Babji, & Murthy, 2006); bambara groundnut seed flour in beef patties (Alakali, Irtwange, & Mzer, 2010); lupine flour in beef patties (El-Sayed, 2013); oat flour in beef patties (Serdaroglu, 2006); sodium caseinate, milk powder and whey powder in turkey

rolls (Serdaroglu & Deniz, 2003) and common bean flour in beef sausages (Dzudie, Scher, & Hardy, 2002).

The desirable sensory characteristics, such as juiciness and mouth feel, of meat patties are associated with fat content. The reduction in fat adversely affects the textural and sensorial characteristics of meat products (Sarıçoban, Yılmaz, & Karakaya, 2009). To reduce the adverse effects of fat reduction, proteins, modified starches, gums and cereal and legume flours are added (Serdaroglu, 2006), which increase moisture- and fat-retention in the meat products, thereby increasing the juiciness and reducing brittleness.

*Moringa oleifera* is traditionally an important food commodity as the leaves, flowers, fruits and roots of this tree are used locally as highly nutritious vegetables particularly in India, Pakistan, Philippines, Hawaii and many parts of Africa (Anwar & Bhangar, 2003). *M. oleifera* leaves are a rich source of  $\beta$ -carotene, protein, vitamin C, calcium and potassium as well as a good source of other antioxidants. They can be used to extend the shelf-life of foods due to the presence of various

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antioxidants, such as ascorbic acid, flavonoids, phenolics and carotenoids (Siddhuraju & Becker, 2003).

According to Anwar and Bhanger (2003) and studies in our laboratory, *M. oleifera* seeds contain 29.36–34.45% crude protein, 35–40% oil and 5.45–6.60% ash. *M. oleifera* oil is potentially suitable for consumption and industrial purposes, such as developing nutritionally balanced and high-stability blended formulations with other high-linoleic oils (Anwar & Bhanger, 2003). Cereal flours are used widely in ground meat products as binders or extenders. An example is oat flour, which is used to improve the quality of beef patties (Serdaroglu, 2006). Some legumes, such as lentils and chickpeas, are rich in protein and starch (Wang & Daun, 2006).

*M. oleifera* is a legume rich in protein, high quality fat and antioxidants with high activity. Therefore, in this study, we investigated the effect of the *M. oleifera* seed flour level as a binder on the stability and quality attributes of beef burgers.

## 2. Materials and methods

### 2.1. Materials

Four kilograms of boneless beef from a young bull were obtained from a commercial supermarket (Riyadh, Saudi Arabia) within an hour of death. *M. oleifera* whole seeds were obtained from a demonstration farm in Riyadh, Saudi Arabia. Unless otherwise stated, all chemicals used in this study were reagent grade.

### 2.2. Sample preparation

The subcutaneous and intramuscular fat of the meat was removed manually. The strips of (3.5 kg) meat were ground for 2 min using a Kenwood kitchen blender under cold conditions (4°C). Moringa seeds were de-coated, carefully cleaned and freed from broken seeds and foreign materials. The de-coated seeds were soaked in water for 12 h, and the water was changed every 2 h. The soaked seeds were freeze dried and ground to a fine powder. Four blends were prepared as shown in Table 1. Each blend was mixed with all ingredients and formed into patties using a burger forming machine (Expro. Co., Shanghai, China).

The patties were cooked for 20 min in a pre-heated hot-air oven at  $180 \pm 1^\circ\text{C}$  to an internal temperature of  $75^\circ\text{C}$  measured at the geometrical centre using a digital probe thermometer

Table 1. Ingredients (%) of four blends of beef burgers formulated with Moringa seed flour.

Tabla 1. Ingredientes (%) de cuatro mezclas de hamburguesas de ternera formuladas con harina de semilla de Moringa.

Ingredients (%)	Moringa seed powder level (%)			
	control	2	4	6
Lean meat	71.0	69.0	67.0	65.0
Added fat	10.0	10.0	10.0	10.0
Cold water	15.4	15.4	15.4	15.4
Salt	1.0	1.0	1.0	1.0
White pepper	0.2	0.2	0.2	0.2
Black pepper	0.2	0.2	0.2	0.2
Garlic powder	0.2	0.2	0.2	0.2
Onion powder	2.0	2.0	2.0	2.0
Moringa seed powder	0.00	2.0	4.0	6.0

(Oakton, Eutech Instruments, China). To ensure uniform cooking, the patties were turned over at 10 min intervals. Three sample replicates from each batch (formulated and non-formulated patties) were analysed on the same day. For storage stability studies, raw non-formulated and formulated patties were separately placed in low-density polyethylene bags and stored for different periods of time (0, 7, 14 and 21 days) at  $4 \pm 1^\circ\text{C}$ . At a pre-specified time interval, raw patties were cooked as indicated above, and both raw and cooked patties were then evaluated for quality attributes.

### 2.3. Chemical composition and pH determination

Moisture, protein, ash and fat contents were determined according to the procedure described in AOAC (1995). The pH was determined by blending a 5.0 g sample in 50 ml of deionised distilled water. The mixture was filtered, and the pH of the filtrate was measured using a pH meter (model: pH meter 240, Corning Scientific Products, New York, USA).

### 2.4. Determination of cooking properties

The cooking yield was determined as reported by Naveena et al. (2006) as follows:

$$\text{Cooking yield} = \frac{\text{Weight of cooked patties}}{\text{Weight of raw patties}} \times 100$$

Fat retention was calculated according to Murphy, Criner, and Gray (1975) as follows:

$$\text{Fat retention} = \text{Cooking yield} \times \frac{\% \text{ fat in cooked patties}}{\% \text{ fat in raw patties}}$$

Based on the method of El-Magoli, Laroja, and Hansen (1996), the moisture retention was determined as follows:

$$\text{Moisture retention} = \text{Cooking yield} \times \frac{\% \text{ moisture in cooked patties}}{\% \text{ moisture in raw patties}}$$

### 2.5. Dimensional shrinkage

The dimensional shrinkage was calculated according to the formula of Murphy et al. (1975) as follows:

$$\text{Dimensional shrinkage} = \frac{(\text{Raw thickness} - \text{cooked thickness}) + (\text{Raw diameter} - \text{cooked diameter})}{\text{Raw thickness} + \text{raw diameter}} \times 100$$

### 2.6. Determination of thiobarbituric acid reactive substances (TBARS)

The thiobarbituric acid reactive substance (TBARS) values were determined as described by Rosmini et al. (1996) where sample extracts were prepared by blending 5 g of burger sample with 20 ml of distilled water followed by filtration. The filtrate (1.0 ml) was placed in a test tube, and 100  $\mu\text{l}$  of 10% butylated hydroxytoluene and 4 ml of 20% thiobarbituric acid were added. The samples were heated for 10 min in a boiling water bath ( $95\text{--}100^\circ\text{C}$ ) to develop a pink colour, and the samples were then cooled under running water. The absorbance of the supernatant obtained by centrifugation at 5500 rpm for 25 min

(Mod. RC5 C, Sorvall Instruments, Kendro LP, Hertfordshire) was measured at 532 nm using a UV spectrophotometer (Mod. 4050, Biochrom, Cambridge, UK). TBARS (mg malonaldehyde  $\text{kg}^{-1}$  sample) values were calculated using a 1,1,3,3-tetraethoxypropane standard curve. Triplicate samples were analysed for each batch.

### 2.7. Microbiological evaluation

For determination of microbial counts, 10.0 g of the meat sample was homogenised with 90 ml of 0.1% sterile peptone water (1.0 g of peptone in 1.0 l of distilled water). Serial 10-fold dilutions were prepared by diluting 1.0 ml of homogenate in 9 ml of 0.1% peptone water. Appropriate serial dilutions were duplicate plated (pour plate method) with plate count agar for aerobic plate counts, and plates were incubated at 37°C for 48 h (Harrigan & McCance, 1976).

### 2.8. Colour measurement

Formulated and non-formulated raw patties before and after storage were subjected to colour measurements, and their colour ( $L^*$ ,  $a^*$  and  $b^*$ ) was measured using a HunterLab colorimeter (Model No. Miniscan<sup>®</sup> XE plus 4500 L, Hunter Associates Laboratory, Inc., VA, USA). The patties were scanned at three different locations to determine the average  $L^*$ ,  $a^*$  and  $b^*$  values during the measurements.

### 2.9. Texture determination

Both raw and cooked patties before and after storage were subjected to texture profile analysis using a texture analyser (Model CT3, Brookfield, Middleboro, Massachusetts, USA) as described by Bourne (1978). Three patties for each parameter were placed on the platform of the texture analyser. The hardness (kg), cohesiveness, springiness (mm) and chewiness (hardness  $\times$  cohesiveness  $\times$  springiness) were measured in triplicate using a 2 cycle test. The test conditions included: a cylindrical probe 6 mm in diameter and 35 mm long; probe speed of 0.5 mm/s; compression of 5% and the sample was approximately 6 cm diameter patties.

### 2.10. Sensory evaluation

The sensory tests were conducted using 5-hedonic scale by a semi-trained panel. Twenty judges were selected who had successfully passed standardised tests for olfactory and taste sensitivities as well as verbal abilities and creativity. The panellists were given a hedonic questionnaire to test the appearance, taste, flavour, juiciness, tenderness and overall acceptability of coded burger samples. The samples were scored on a scale of 1–5 (1 = poor, 2 = fair, 3 = good, 4 = very good and 5 = excellent).

### 2.11. Statistical analysis

Each determination was performed on at least three separate samples and analysed in triplicate, and the figures were then averaged. Data were assessed by the analysis of variance (ANOVA) described by Snedecor and Cochran (1987). Comparisons of means for treatments were made using

Duncan's multiple range tests. Significance was accepted at  $p \leq 0.05$ .

## 3. Results and discussion

### 3.1. Effect of the MSF level on chemical composition of raw and cooked beef patties

The chemical composition (moisture, protein, fat and ash) of the raw and cooked beef patties is shown in Table 2. The results showed that the moisture content of raw patties decreased as the percentage of MSF increased, but the decline rate was found to be not significant. Serdaroglu (2006) reported a decrease in moisture content of beef patties formulated with oat flour due to an increase in solid contents. Moreover, Alakali et al. (2010) stated that an increase in bambara groundnut seed flour decreases the moisture content of beef patties. In contrast, the moisture content of cooked patties increased gradually (but still lower than that of raw) as the percentage of MSF increased, but the decline rate was found to be not significant between the levels of Moringa flour. It has been reported that during cooking, beef burger patties lose moisture through drip and evaporation (Sheridan & Shilton, 2002), which may be because the addition of MSF reduces drip and evaporation resulting in a significant increase in the moisture content of cooked patties with increasing MSF level. The protein, ash and fat contents of raw and cooked patties had a trend similar to that of moisture. Cooking may have caused an increase in dry matter content by leaching water and water soluble constituents with a concomitant increase in protein, ash and fat contents as reported by Alakali et al. (2010) for beef patties formulated with bambara groundnut seed flour. Also, a similar trend has been observed by Dzudie et al. (2002) for beef patties extended with common bean flour and in patties of buffalo meat prepared with different legume flours (Modi et al., 2004).

### 3.2. Effect of the MSF level on cooking properties of cooked beef patties

The cooking characteristics of formulated and non-formulated beef patties are shown in Figure 1. The addition of MSF to beef patties significantly affected cooking characteristics of the patties. The cooking yield was significantly ( $p \leq 0.05$ ) increased in beef patties with an increase in the MSF level compared to non-formulated samples (Figure 1a). The cooking yield was increased from 60.04 for non-formulated patties to 68.52, 72.90 and 81.81% for patties formulated with 2, 4 and 6% MSF, respectively. Alakali et al. (2010) reported similar results for the cooking yield in beef patties formulated with bambara groundnut seed flour. The addition of MSF to beef burgers improves the cooking yield due to the fat and water retention capacity and capability to keep moisture in the patty matrix as reported by Aleson-Carbonell, Fernandez-Lopez, Perez-Alvarez and Kuri (2005) for beef burgers formulated with lemon albedo. The addition of finger millet flour to chicken patties results in better cooking yield (Naveena et al., 2006). The moisture retention of cooked beef patties was significantly ( $p \leq 0.05$ ) increased with the MSF level compared to that of non-formulated patties with values of 48.54 for non-formulated patties and 57.13, 64.16 and 72.19% for patties formulated with 2, 4 and 6% MSF, respectively (Figure 1b). The results showed that MSF addition improves the moisture retention of cooked patties. The

Table 2. Chemical composition of raw and cooked beef burgers formulated with different concentrations of Moringa seed flour.

Tabla 2. Composición química de las hamburguesas de ternera crudas y cocinadas formuladas con diferentes concentraciones de harina de semilla de *Moringa*.

Chemical composition (%)				
Fat	Ash	Protein	Moisture	Moringa concentration (%)
Raw				
10.05 ± 0.92 <sup>a</sup>	2.06 ± 0.02 <sup>a</sup>	17.14 ± 0.12 <sup>a</sup>	70.50 ± 1.22 <sup>a</sup>	0
10.17 ± 0.98 <sup>a</sup>	2.13 ± 0.04 <sup>a</sup>	17.84 ± 0.11 <sup>a</sup>	70.24 ± 2.04 <sup>a</sup>	2
11.27 ± 1.03 <sup>a</sup>	2.23 ± 0.04 <sup>a</sup>	18.11 ± 0.12 <sup>a</sup>	68.57 ± 1.92 <sup>a</sup>	4
11.46 ± 1.12 <sup>a</sup>	2.32 ± 0.06 <sup>a</sup>	18.14 ± 0.17 <sup>a</sup>	68.58 ± 3.02 <sup>a</sup>	6
Cooked				
10.15 ± 0.84 <sup>a</sup>	2.29 ± 0.03 <sup>a</sup>	25.54 ± 0.19 <sup>a</sup>	57.81 ± 0.92 <sup>a</sup>	0
10.89 ± 0.97 <sup>a</sup>	2.34 ± 0.04 <sup>a</sup>	25.84 ± 0.25 <sup>a</sup>	59.40 ± 0.87 <sup>a</sup>	2
11.27 ± 1.05 <sup>a</sup>	2.48 ± 0.07 <sup>a</sup>	26.04 ± 0.31 <sup>a</sup>	60.29 ± 0.89 <sup>a</sup>	4
11.49 ± 1.13 <sup>a</sup>	2.56 ± 0.08 <sup>a</sup>	26.34 ± 0.42 <sup>a</sup>	60.43 ± 1.03 <sup>a</sup>	6

Note: Values are means of triplicate samples (±SD). Means not sharing a common superscript in a column for each treatment are significantly different at  $p \leq 0.05$  as assessed by Duncan's multiple range test.

Nota: Los valores son los promedios de las muestras triplicadas (±SD). Los promedios que no comparten un mismo superíndice en una columna para cada tratamiento son significativamente diferentes a  $p \leq 0,05$  según la evaluación con el Test de rango múltiple de Duncan.

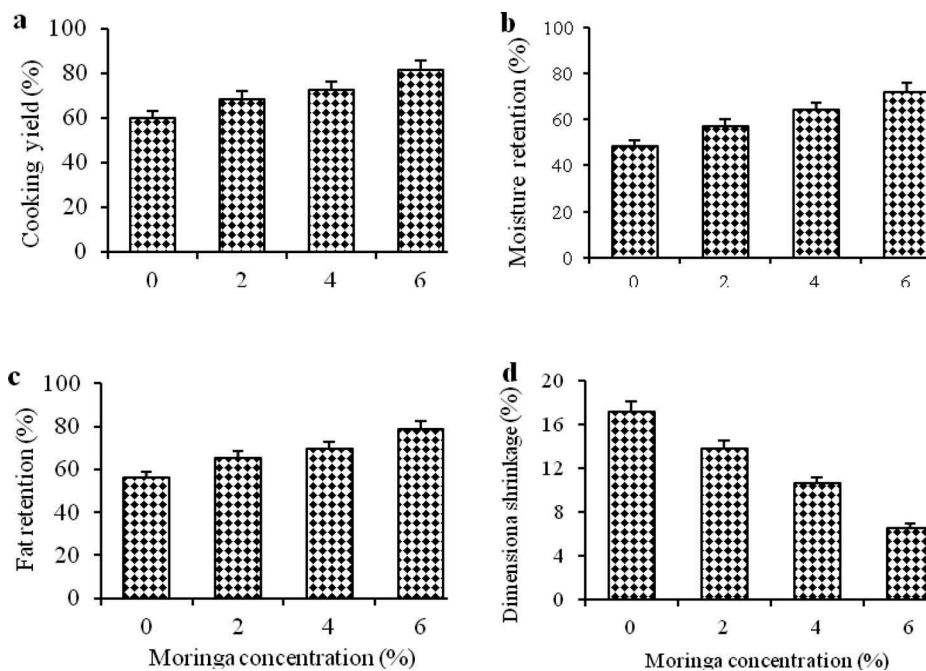


Figure 1. Cooking properties (a, cooking yield; b, moisture retention; c, fat retention and d, dimensional shrinkage) of beef burgers formulated with Moringa seed flour at different levels (0, 2, 4 and 6%). Error bars indicate the standard deviation of three replicates.

Figura 1. Propiedades de cocinado (a, productividad de cocinado; b, retención de humedad; c, retención de grasa y d, encogimiento dimensional) de las hamburguesas de ternera formuladas con harina de semilla de Moringa a diferentes niveles (0, 2, 4 y 6%). Las columnas error indican la desviación estándar de tres réplicas.

improvement in moisture retention of the patties may be attributed to increases in the water absorption capacity of heated protein flours, the heat dissociation of proteins, the gelatinisation of starch in the flour and the swelling of the legume fibre (Modi et al., 2004). The results obtained for fat retention of cooked beef patties (Figure 1c) is similar to that obtained for moisture retention. The fat retention of the patties was 56.17% for non-formulated, and significantly ( $p \leq 0.05$ )

increased to 65.11, 69.40 and 78.29% for the patties formulated with 2, 4 and 6% MSF, respectively. Serdaroglu (2006) reported significant ( $p \leq 0.05$ ) increases in fat retention for Turkish type meat balls formulated with different levels of corn flour. The increase in fat retention may be due to the fact that the swelling of the starch and fibre as well as the fat absorbed by the fibre may interact with the protein of the ground meat matrix to prevent migration of fat from the product (Alakali et al., 2010).

The dimensional shrinkage significantly ( $p \leq 0.05$ ) decreased with the MSF level and differed significantly ( $p \leq 0.05$ ) with the varying levels of MSF in beef patties (Figure 1d). Non-formulated patties had higher shrinkage (17.21) compared to MSF formulated patties, which had a shrinkage range from 13.81 to 6.60. Shrinkage in patties during heating is caused by muscle protein denaturation and partly from the evaporation of water and drainage of melted fat and juices (Alakali et al., 2010). Such changes in the protein structure affect the textural quality of cooked patties. The lower shrinkage observed in MSF-formulated patties compared to the non-formulated ones could be due to the binding and stabilising properties of MSF, which held the meat particles together and prevented changes in product moisture, juice losses and consequently the shape of the product as reported by Naveena et al. (2006) for finger millet flour formulated chicken patties.

### 3.3. Effect of the MSF level on thiobarbituric acid reactive substances (TBARS) and pH of beef patties during storage

Figure 2 shows the effect of MSF formulation on TBARS and pH of raw beef patties during storage at  $4 \pm 1^\circ\text{C}$ . The TBARS values of formulated and non-formulated patties significantly ( $p \leq 0.05$ ) increased with storage with a maximum value of 1.65 for non-formulated patties after 21 days (Figure 2a). The addition of MSF significantly ( $p \leq 0.05$ ) reduced the level of TBARS values during storage of raw patties, and the rate of reduction increased with the MSF level. Generally, there was a significant difference in the TBARS values between formulated and non-formulated patties because *M. oleifera* seed flour is rich in antioxidants with high activity and is unusually resistant to the development of rancidity (Lalas & Tsaknis, 2002). Moreover, the results indicated that MSF is more effective in inhibiting lipid oxidation. It has been reported that low-fat pork patties prepared with carrageenan have low TBARS values compared to the high fat control, which may be because lower fat content reduces the substrate for oxidation (Kumar & Sharma, 2004).

The pH of raw non-formulated beef patties decreased slightly with the storage time from 5.96 at day 0 to 5.68 after 21 days of storage (Figure 2b). However, the addition of MSF slightly increased the pH of raw patties to 6.01, 6.11 and 6.16 for 2, 4 and 6% MSF, respectively, but decreased with the storage time. There was a slight difference between the pH of non-formulated and MSF-formulated samples during the storage period. All levels of MSF formulation in the patties relative to the non-formulated ones showed that the pH values were generally lower than MSF-formulated patties at all levels of MSF formulation. A significant increase in the pH of beef extended with common-bean flour has been observed (Dzudie et al., 2002). The variation in pH among samples shown in this study may be due to putrefaction of protein in the patties by microorganisms. It is generally known that different types of handling methods affect the quality of meat and its products (Narayan, Mendiratta, & Mane, 2013). Protein-containing foods are subject to fermentative processes and degradation by active proteinases released by bacteria during fermentation resulting in rapid amino acid production, which is called "putrefaction" (Ouoba et al., 2003). Naveena et al. (2006) reported an increase in the pH of control and millet flour-treated patties with storage time, and they attributed this result to the deamination of proteins among other factors.

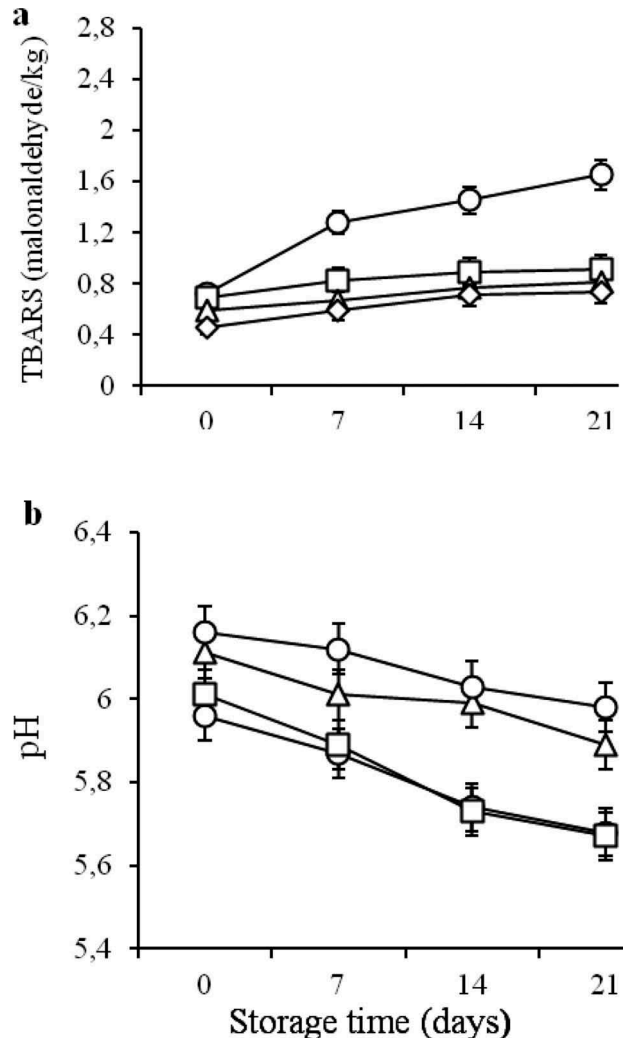


Figure 2. Thiobarbituric acid reactive substances in malonaldehyde/kg samples (a) and pH (b) of raw patties formulated with different levels (0%, O; 2%, □; 4%, Δ and 6%, ◇) of MSF during storage. Error bars indicate the standard deviation of three replicates.

Figura 2. Substancias reactivas de ácido tiobarbitúrico en muestras de malonaldehído/kg (a) y pH (b) de pastelitos crudos con diferentes niveles (0%, O; 2%, □; 4%, Δ y 6%, ◇) de HSM durante almacenamiento. Las columnas error indican la desviación estándar de tres réplicas.

### 3.4. Effect of the MSF level on microbiological characteristics and surface colour of raw beef patties during storage

The effect of the MSF level on microbiological characteristics of raw beef patties during storage is shown in Figure 3. The total plate count (TPC) showed a significant ( $p \leq 0.05$ ) linear increase in non-formulated patties with storage time with a maximum value of 8.25 log cfu/g after 21 days of storage. The addition of MSF to beef patties significantly ( $p \leq 0.05$ ) decreased the aerobic total plate count to 5.45, 4.83 and 3.84 log cfu/g for 2, 4 and 6% MSF, respectively, after 21 days of storage. The variation in the bacterial growth between the non-formulated and MSF-formulated patties during the storage period may be attributed to the differences in pH, which were affected by the level of MSF. Moreover, the *Escherichia coli* test using MacConkey agar medium oxid (CM7) showed that the MSF-formulated patties

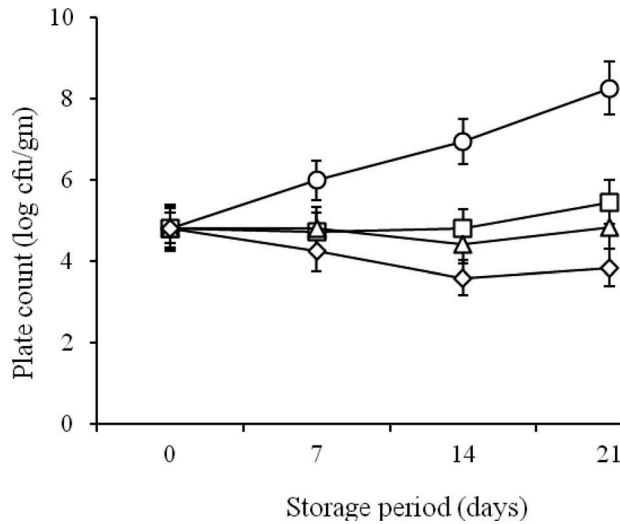


Figure 3. Microbiological characteristics of raw beef burgers formulated with Moringa seed flour at different levels (0%, O; 2%, □; 4%, Δ and 6%, ◇) during storage. Error bars indicate the standard deviation of three replicates.

Figura 3. Características microbiológicas de hamburguesas crudas de ternera formuladas con harina de semilla de Moringa a diferentes niveles (0%, O; 2%, □; 4%, Δ y 6%, ◇) durante almacenamiento. Las columnas error indican la desviación estándar de tres réplicas.

did not have any *E. coli* colonies but that the non-formulated patties had a significant number of colonies during storage (data not provided). The results indicated that MSF has a protective role in meat patties.

The colour scale (lightness, redness and yellowness values) of raw beef patties formulated with MSF is shown in Table 3. The lightness, as measured by the  $L^*$  value of the patties, was significantly ( $p \leq 0.05$ ) increased with the MSF level with a maximum value (50.40) obtained for patties formulated with 6% MSF. The lowest lightness value was obtained in non-formulated beef patties because of the high level of redness in meat and muscle pigment. It has been reported that as wet okara concentration increased, beef patties were lighter (Turhan, Temiz, & Sagir, 2007). Storage of raw patties at  $4 \pm 1^\circ\text{C}$  for 21 days significantly ( $p \leq 0.05$ ) reduced the lightness of non-formulated patties from 43.39 to 41.02 after 21 days and that formulated with 6% MSF reduced from 50.40 to 45.06 after 21 days of storage. Redness, as measured by the  $a^*$  value, of non-formulated patties was 8.95 and decreased to 7.46, 6.75 and 6.15 for patties formulated with 2, 4 and 6% MSF, respectively, probably due to dilution of the myoglobin. Similar results were reported for chicken patties formulated with finger millet flour (Naveena et al., 2006) and low-fat meatballs formulated with whey powder (Serdaroglu, 2006). However, Turhan et al. (2007) reported that increasing the okara level in formulation did not significantly affect the redness of beef patties. Storage of formulated and non-formulated patties at  $4^\circ\text{C}$  significantly ( $p \leq 0.05$ ) increased the redness value with time probably due to accumulation of myoglobin during storage. The values of  $b^*$  (yellowness) were lowest (13.18) in non-formulated beef patties and increased with addition of MSF up to 16.05 in 6% MSF-formulated patties (Table 3). The increase in yellowness in MSF-formulated is likely to be due to the presence of carotenoid pigment in MSF (Siddhuraju & Becker, 2003). Serdaroglu (2006) reported that oat flour appeared to increase yellowness in patties when compared with the control samples. Yilmaz and Dağlıoğlu (2003)

Table 3. Colour scale of raw beef burgers formulated with different concentrations of Moringa seed flour during storage at  $4^\circ\text{C}$  ( $\pm 1.00$ ).

Tabla 3. La escala de color de las hamburguesas de ternera formuladas con diferentes concentraciones de harina de semilla de Moringa durante almacenamiento a  $4^\circ\text{C}$  ( $\pm 1.00$ ).

	Moringa concentration (%)				Storage period (days)
	0	2	4	6	
$L^*$					
$43.39 \pm 0.72^{\text{aR}}$	$46.58 \pm 0.76^{\text{aQ}}$	$49.26 \pm 2.05^{\text{aP}}$	$50.40 \pm 1.83^{\text{aP}}$	0	
$42.42 \pm 1.12^{\text{aR}}$	$45.78 \pm 2.01^{\text{abQ}}$	$46.55 \pm 0.97^{\text{bPQ}}$	$47.97 \pm 1.45^{\text{bP}}$	7	
$41.68 \pm 0.91^{\text{bR}}$	$44.47 \pm 0.32^{\text{bcQ}}$	$45.23 \pm 1.45^{\text{bcPQ}}$	$46.31 \pm 0.99^{\text{bcP}}$	14	
$41.02 \pm 0.87^{\text{bR}}$	$43.14 \pm 1.13^{\text{cQ}}$	$44.45 \pm 1.32^{\text{cPQ}}$	$45.06 \pm 0.89^{\text{cP}}$	21	
$a^*$					
$8.95 \pm 0.31^{\text{acP}}$	$7.46 \pm 0.81^{\text{bPQ}}$	$6.75 \pm 0.87^{\text{bQ}}$	$6.15 \pm 0.31^{\text{cQ}}$	0	
$9.47 \pm 0.42^{\text{abcP}}$	$8.13 \pm 1.02^{\text{bPQ}}$	$7.23 \pm 0.47^{\text{bQ}}$	$6.94 \pm 0.46^{\text{bcQ}}$	7	
$10.11 \pm 0.56^{\text{abP}}$	$9.34 \pm 0.95^{\text{aP}}$	$7.75 \pm 0.38^{\text{bQ}}$	$7.24 \pm 0.57^{\text{abQ}}$	14	
$11.02 \pm 0.61^{\text{a}}$	$10.21 \pm 1.67^{\text{a}}$	$8.34 \pm 0.36^{\text{a}}$	$8.09 \pm 0.83^{\text{a}}$	21	
$b^*$					
$13.18 \pm 2.13^{\text{cQ}}$	$13.89 \pm 0.46^{\text{cQ}}$	$15.51 \pm 0.73^{\text{cPQ}}$	$16.05 \pm 0.74^{\text{cP}}$	0	
$14.75 \pm 1.72^{\text{bcQ}}$	$15.05 \pm 0.72^{\text{bQ}}$	$16.43 \pm 0.82^{\text{bcPQ}}$	$17.29 \pm 0.45^{\text{bcP}}$	7	
$15.26 \pm 0.89^{\text{abQ}}$	$16.49 \pm 0.86^{\text{abQ}}$	$17.31 \pm 0.89^{\text{abPQ}}$	$18.37 \pm 0.38^{\text{abP}}$	14	
$16.23 \pm 1.32^{\text{aQ}}$	$17.15 \pm 0.88^{\text{aQ}}$	$18.31 \pm 0.76^{\text{aPQ}}$	$19.14 \pm 0.59^{\text{aP}}$	21	
$a^*/b^*$					
$0.68 \pm 0.05^{\text{aP}}$	$0.54 \pm 0.02^{\text{aPQ}}$	$0.43 \pm 0.04^{\text{aQ}}$	$0.38 \pm 0.04^{\text{aQ}}$	0	
$0.64 \pm 0.08^{\text{aP}}$	$0.54 \pm 0.06^{\text{aPQ}}$	$0.44 \pm 0.06^{\text{aQ}}$	$0.40 \pm 0.05^{\text{aQ}}$	7	
$0.66 \pm 0.03^{\text{aP}}$	$0.57 \pm 0.09^{\text{aPQ}}$	$0.45 \pm 0.02^{\text{aQR}}$	$0.39 \pm 0.07^{\text{aR}}$	14	
$0.68 \pm 0.12^{\text{aP}}$	$0.59 \pm 0.03^{\text{aQ}}$	$0.46 \pm 0.08^{\text{aR}}$	$0.42 \pm 0.02^{\text{aR}}$	21	

Note: Values are means of three replicates ( $\pm$  SD). Means not sharing a common superscript(s), a, b or c in a column for each colour parameter or P, Q or R in a row are significantly different at  $p \leq 0.05$  as assessed by Duncan's multiple range test.

Nota: Los valores son los promedios de tres réplicas ( $\pm$  SD). Los promedios que no comparten un mismo superíndice a, b o c en una columna para cada parámetro de color P, Q o R en una fila son significativamente diferentes a  $p \leq 0.05$  según la evaluación con el Test de rango múltiple de Duncan.

observed that all beef control meatballs had lower  $b^*$  values than meatballs containing oat bran. The increase in the  $b^*$  value compared to the  $a^*$  value resulted in an overall decrease in the  $a/b$  value of formulated patties (Table 3). The storage of beef patties with and without MSF had no significant ( $p \leq 0.05$ ) effect on the  $a^*/b^*$  value. The  $a^*/b^*$  value is commonly used as an index to report the colour quality (brightness of red colour). The result indicated that the formulation of patties with MSF had a significant ( $p \leq 0.05$ ) effect on colour quality (brightness of red colour) of the patties as indicated by low  $a^*/b^*$  values.

### 3.5. Effect of the MSF level on textural properties and sensory characteristics of beef patties during storage

Figure 4 shows the effects of MSF formulation on the textural properties of the raw and cooked beef patties. The addition of MSF to beef patties significantly ( $p \leq 0.05$ ) affected the textural properties of both raw and cooked beef patties. The hardness (Figure 4a) of raw patties was 0.10 kg for the non-formulated patties, and it increased to 0.11, 0.16 and 0.24 kg for patties formulated with 2, 4 and 6% MSF, respectively. Cooking significantly ( $p \leq 0.05$ ) increased the hardness of both formulated and non-formulated patties to 1.03 kg for non-formulated and to 1.13, 1.17 and 1.23 kg for the patties formulated with 2, 4 and 6% MSF, respectively. Friction and/or binding among meat particles may be increased by adding MSF. The hardness of both formulated and non-formulated raw and cooked patties significantly ( $p \leq 0.05$ )

increased with the storage time. The formulation of raw patties had no significant effect on cohesiveness, but a slight increase was observed after cooking (Figure 4b). The springiness (Figure 4c) of raw patties was 3.30 mm for the non-formulated patties, and it increased to 3.55, 3.70 and 4.00 mm for the patties formulated with 2, 4 and 6% MSF, respectively. Cooking slightly increased the springiness of both the formulated and non-formulated patties. Storage of raw patties increased the springiness with time and even after cooking. According to Li, Carpenter, and Cheney (1998), less water tends to increase hardness, springiness and cohesiveness of smoked sausage made with technically separated poultry meat and wheat protein. The chewiness of raw patties increased with increasing MSF levels (Figure 4d) from 0.19 kg mm for the non-formulated to 0.22, 0.34 and 0.57 kg mm for patties formulated with 2, 4 and 6% MSF, respectively. Cooking of raw patties before storage led to a dramatic increase in chewiness with values of 2.17 kg mm for the non-formulated and 2.72, 3.15 and 3.88 kg mm for the patties formulated with 2, 4 and 6% MSF, respectively. Storage of patties significantly ( $p \leq 0.05$ ) increased chewiness, and a further increase was observed after cooking for both the formulated and non-formulated patties. Desmond, Troy, and Buckley (1998) found that carrageenan improves the overall texture of low-fat beef burgers. Hsu and Chung (2001) reported that j-carrageenan significantly increases hardness, chewiness and gumminess of low emulsified meatballs.

The mean sensory scores of formulated and non-formulated beef patties after cooking are shown in Table 4. The addition of non-meat ingredient to meat usually decreases its quality and

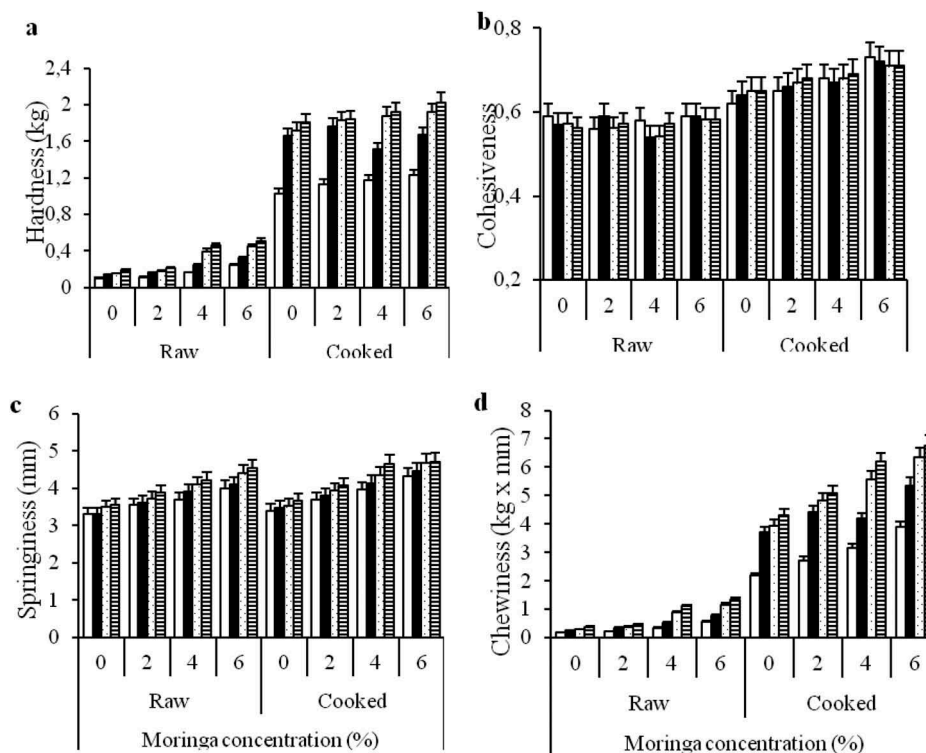


Figure 4. Textural properties (a, hardness; b, cohesiveness; c, springiness; d, chewiness) of beef burgers formulated with Moringa seed flour of different levels during storage for 0 day (blank bars), 7 days (solid bars), 14 days (dotted bars) or 21 days (dashed bars).

Figura 4. Propiedades texturales (a, dureza; b, cohesión; c, ligereza; d, masticabilidad) de las hamburguesas de ternera formuladas con harina de semilla de Moringa de diferentes niveles durante almacenamiento de 0 días (columnas blancas), 7 días (columnas sólidas), 14 días (columnas con topos) o 21 días (columnas ralladas).



Table 4. Sensory characteristics of cooked beef burgers formulated with different concentrations of Moringa seed flour during storage at  $4 \pm 1.00^\circ\text{C}$ .Tabla 4. Características sensoriales de las hamburguesas de ternera cocinadas formuladas con diferentes concentraciones de harina de semilla de *Moringa* durante almacenamiento a  $4 \pm 1,00^\circ\text{C}$ .

Storage period (days)	Moringa concentration (%)			
	0	2	4	6
<i>Appearance</i>				
0	3.95 $\pm$ 0.03 <sup>ap</sup>	3.37 $\pm$ 0.32 <sup>ap</sup>	3.15 $\pm$ 0.20 <sup>ap</sup>	3.01 $\pm$ 0.78 <sup>ap</sup>
7	2.92 $\pm$ 0.12 <sup>ap</sup>	2.79 $\pm$ 0.25 <sup>ap</sup>	2.30 $\pm$ 0.61 <sup>abp</sup>	2.24 $\pm$ 0.63 <sup>ap</sup>
14	0.00 $\pm$ 0.00 <sup>bq</sup>	2.50 $\pm$ 0.33 <sup>ap</sup>	2.23 $\pm$ 0.43 <sup>abp</sup>	2.21 $\pm$ 0.09 <sup>ap</sup>
21	0.00 $\pm$ 0.00 <sup>bq</sup>	0.00 $\pm$ 0.00 <sup>bq</sup>	2.16 $\pm$ 0.35 <sup>bp</sup>	2.13 $\pm$ 0.11 <sup>ap</sup>
<i>Juiciness</i>				
0	3.77 $\pm$ 0.21 <sup>ap</sup>	3.11 $\pm$ 0.32 <sup>ap</sup>	2.97 $\pm$ 0.46 <sup>ap</sup>	2.83 $\pm$ 0.41 <sup>ap</sup>
7	2.58 $\pm$ 0.18 <sup>ap</sup>	2.66 $\pm$ 0.57 <sup>ap</sup>	2.54 $\pm$ 0.74 <sup>ap</sup>	2.39 $\pm$ 0.44 <sup>ap</sup>
14	0.00 $\pm$ 0.00 <sup>bq</sup>	2.57 $\pm$ 0.69 <sup>ap</sup>	2.36 $\pm$ 0.23 <sup>ap</sup>	2.25 $\pm$ 0.27 <sup>ap</sup>
21	0.00 $\pm$ 0.00 <sup>bq</sup>	0.00 $\pm$ 0.00 <sup>bp</sup>	2.26 $\pm$ 0.07 <sup>apq</sup>	2.17 $\pm$ 0.06 <sup>ap</sup>
<i>Flavour</i>				
0	3.29 $\pm$ 0.63 <sup>ar</sup>	2.68 $\pm$ 0.55 <sup>arq</sup>	2.37 $\pm$ 0.19 <sup>apq</sup>	2.26 $\pm$ 0.47 <sup>ap</sup>
7	2.53 $\pm$ 0.14 <sup>ap</sup>	2.25 $\pm$ 0.36 <sup>ap</sup>	2.31 $\pm$ 0.31 <sup>ap</sup>	2.50 $\pm$ 0.54 <sup>ap</sup>
14	0.00 $\pm$ 0.00 <sup>bq</sup>	2.09 $\pm$ 0.66 <sup>ap</sup>	2.14 $\pm$ 0.72 <sup>ap</sup>	2.17 $\pm$ 0.22 <sup>ap</sup>
21	0.00 $\pm$ 0.00 <sup>bq</sup>	0.00 $\pm$ 0.00 <sup>bq</sup>	2.09 $\pm$ 0.52 <sup>ap</sup>	2.17 $\pm$ 0.24 <sup>ap</sup>
<i>Taste</i>				
0	3.79 $\pm$ 0.33 <sup>ap</sup>	2.85 $\pm$ 0.29 <sup>ap</sup>	2.81 $\pm$ 0.24 <sup>ap</sup>	2.77 $\pm$ 0.31 <sup>ap</sup>
7	2.53 $\pm$ 0.18 <sup>ap</sup>	2.59 $\pm$ 0.04 <sup>ap</sup>	2.34 $\pm$ 0.37 <sup>ap</sup>	2.31 $\pm$ 0.77 <sup>ap</sup>
14	0.00 $\pm$ 0.00 <sup>bq</sup>	2.15 $\pm$ 0.08 <sup>ap</sup>	2.08 $\pm$ 0.21 <sup>ap</sup>	2.23 $\pm$ 0.07 <sup>ap</sup>
21	0.00 $\pm$ 0.00 <sup>bq</sup>	0.00 $\pm$ 0.00 <sup>bq</sup>	2.01 $\pm$ 0.13 <sup>ap</sup>	2.21 $\pm$ 0.45 <sup>ap</sup>
<i>Tenderness</i>				
0	3.09 $\pm$ 0.09 <sup>aq</sup>	3.23 $\pm$ 0.12 <sup>ap</sup>	3.27 $\pm$ 0.49 <sup>ap</sup>	3.48 $\pm$ 0.38 <sup>ap</sup>
7	2.48 $\pm$ 0.65 <sup>ap</sup>	2.65 $\pm$ 0.23 <sup>ap</sup>	2.72 $\pm$ 0.37 <sup>ap</sup>	2.78 $\pm$ 0.71 <sup>ap</sup>
14	0.00 $\pm$ 0.00 <sup>bq</sup>	2.80 $\pm$ 0.16 <sup>ap</sup>	2.81 $\pm$ 0.25 <sup>ap</sup>	2.83 $\pm$ 0.65 <sup>ap</sup>
21	0.00 $\pm$ 0.00 <sup>bq</sup>	0.00 $\pm$ 0.00 <sup>bq</sup>	2.65 $\pm$ 0.38 <sup>ap</sup>	2.69 $\pm$ 0.09 <sup>ap</sup>
<i>Overall acceptability</i>				
0	3.50 $\pm$ 0.24 <sup>ap</sup>	2.97 $\pm$ 0.57 <sup>ap</sup>	2.83 $\pm$ 0.91 <sup>ap</sup>	2.85 $\pm$ 0.28 <sup>ap</sup>
7	2.69 $\pm$ 0.09 <sup>ap</sup>	2.77 $\pm$ 0.36 <sup>ap</sup>	2.79 $\pm$ 0.36 <sup>ap</sup>	2.82 $\pm$ 0.39 <sup>ap</sup>
14	0.00 $\pm$ 0.00 <sup>bq</sup>	2.64 $\pm$ 0.80 <sup>ap</sup>	2.51 $\pm$ 0.42 <sup>ap</sup>	2.48 $\pm$ 0.32 <sup>ap</sup>
21	0.00 $\pm$ 0.00 <sup>bq</sup>	0.00 $\pm$ 0.00 <sup>bq</sup>	2.36 $\pm$ 0.59 <sup>ap</sup>	2.47 $\pm$ 0.72 <sup>ap</sup>

Note: Values are means of 20 samples ( $\pm$ SD). Means not sharing a common superscript(s) a, b or c in a column or p, q or r in a row are significantly different at  $p \leq 0.05$  as assessed by Duncan's multiple range test.

Nota: Los valores son los promedios de 20 muestras ( $\pm$ SD). Los promedios que no comparten un superíndice en común a, b o c en una columna o p, q o r en una fila son significativamente diferentes a  $p \leq 0,05$  según la evaluación con el Test de rango múltiple de Duncan.

the main problem is to keep it at the level as close as possible to the full-meat product. The sensory attributes (appearance, juiciness, flavour, taste, tenderness and overall acceptability) of the cooked patties decreased (except tenderness) with increasing MSF levels but the decline rate is not significant. It was observed that the non-formulated patties after 7 days of storage started to develop some sign of deterioration and that formulated with 2% MSF started to develop deterioration after 14 days. However, patties formulated with 4 and 6% MSF did not develop any kind of deterioration even after 21 days and had a reasonable score for sensory attributes. A significant decrease in sensory attributes during cold storage for 21 days has been reported in chicken patties formulated with ragi flour (Naveena et al., 2006). Kumar and Sharma (2004) also reported a decrease in sensory values during storage of low-fat ground pork patties formulated with carrageenan, and these authors attributed the decrease in appearance, juiciness and tenderness scores to moisture loss and surface dehydration of beef patties during aerobic storage. The sensory characteristics of cooked beef patties revealed that MSF-formulated beef patties, especially those formulated with 4 and 6% MSF have acceptable sensory scores. The stability of MSF-formulated patties may be attributed to the fact that MSF had a preservative characteristic due to the presence of antioxidants.

#### 4. Conclusions

The *M. oleifera* seed flour used in this study improved the physicochemical properties, cooking characteristics, shelf life, consumer preference and aerobic refrigerated storage stability of beef patties. The results indicated that *M. oleifera* seed flour has high potential as a binder for use in beef patty production in addition to its health and nutritional benefits.

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No potential conflict of interest was reported by the authors.

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