

## Tenderization of camel meat by using fresh ginger (*Zingiber officinale*) extract

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### ABSTRACT

This study was conducted to develop a method for improving tenderness and overall qualities of tough aged camel meat using plant proteolytic enzymes from fresh ginger rhizome (*Zingiber officinale*). Samples of camel meat chunks were marinated with different concentrations (0, 15, 30 and 45% v/w) of ginger extract for 48 h at  $4\pm 1^\circ\text{C}$ . The results showed that the treatment with ginger extract induced significant increases in the values of water holding capacity, cooking yield and shear force, solubility of sarcoplasmic and myofibrillar proteins and collagen solubility of aged camel meat chunks. In addition to the electrophoretic patterns of proteins were studied. The results obtained revealed remarkable variations in all the studied parameters in the camel meat using plant proteolytic enzymes from fresh ginger rhizome compared with corresponding control. Sensory evaluation scores revealed a significant improvement in appearance, flavor, tenderness and juiciness of ginger extract treated samples compared to control samples. So, Based on water holding capacity, shear force values, cooking yield and sensory evaluation scores, 30% v/w ginger extract treatment was found to be the optimum level to achieve the best tenderization effect for aged camel meat. Thus, these treatment were select to gamma irradiation at dose levels of 1.5, 3 and 4.5 kGy to extend its shelf-life and improving its hygienic quality at cold storage ( $4\pm 1^\circ\text{C}$ ) compared with control samples (without any treatments, neither ginger extract nor gamma irradiation). The effect of gamma irradiation on microbial load, and sensory characteristics of camel meat has been evaluated. The results indicated that all doses of gamma irradiation reduced the total bacterial count, Psychrophilic bacteria, lactic acid bacteria and Enterobacteriaceae of camel meat. Irradiated at 1.5 kGy reduced the counts of Enterobacteriaceae, *Staphylococcus aureus* and *Bacillus cereus* as well as eliminating and *Salmonella* spp, while irradiated samples at dose levels of 3 and 4.5 kGy completely eliminated these bacteria. Sensory evaluation showed no significant differences between irradiated and non-irradiated camel meats. However, treated samples of camel meat with 30% ginger extract and irradiated at dose levels of 0, 1.5, 3 and 4.5 kGy extended of the refrigerated shelf-life of samples to 9, 18, 27 and 30 days, respectively, compared to 6 days for non-irradiated controls (without any treatments). Therefore, a technology for utilization of easily and cheaply available ginger can be exploited at the industrial or household level for tenderization of tough or aged camel meat.

**Keywords:** Tenderization/ Camel meat/ Ginger/ Extract.

### INTRODUCTION

Most consumers judge quality and overall acceptability of beef products based on tenderness. Therefore it is critical for the consumer acceptance of beef that a commercially applicable method be developed to ensure a consistently tender product (Neely et al. 1998). Meat toughness can be subdivided into actomyosin toughness, which is attributable to changes in myofibrillar proteins, and background toughness, which is attributable to connective tissues. Recently, most studies have focused on clarifying understanding of the role of connective tissues in meat and meat products. It is also found that the structure of collagen and elastin is a significant factor that affects the texture of meat (Takagi et al.1992). There are several means for tenderizing meat, chemically or physically, which mainly reduce the amounts of detectable connective tissues without causing extensive degradation of myofibrillar proteins. Treatment by proteolytic enzymes is one of the popular methods for meat tenderization. At present, most enzymes used are derived from plants: e.g., papa in and bromelain, have been widely used as meat tenderizers in America and Europe (Liu and Tang, 2001). However, these enzymes often degrade the texture of the meat, due to the broad substrate specificity, and develop unfavorable taste due to over-tenderization (Cronlund and Woychik, 1987). Consequently, the ideal meat tenderizer would be a proteolytic enzyme with specificity for collagen and elastin in connective tissues, at the relatively low pH of meat that would act either at the low temperature at which meat is stored or at the high temperature achieved during cooking (Gerelt et al. 2000).

A promising protease "Zingibain" isolated from *Zingiber officinale* roscoe (Ginger rhizome) has been reported to have proteolytic activity. Its proteolytic activity on collagen was found to be many fold greater than on actomyosin, and the combined proteolysis of these two muscle proteins improved the tenderness of meat. Besides tenderizing properties, antioxidant and antimicrobial characteristics of ginger extract have been reported by different workers (Mendiratta et al. 2000). However, ginger rhizome is used primarily as a flavoring agent for bakery products and as a sausage seasoning. Its utilization as a meat tenderizing agent is not fully appreciated and the literature available is limited.

Camel is a unique animal having the ability to survive and produce with low cost of feeding under harsh conditions compared to other livestock. It is a good source of meat in areas where the climate adversely affects other animal's production efficiency (**Kadim et al. 2006**). Traditionally, camel meat comes mostly from old males and females that are primarily kept for milk, racing, and transportation rather than for meat production. General consumers' view is that camel meat is unacceptably tough, but in fact meat from young camels has been reported to be comparable in taste and texture to beef (**Kurtu, 2004**). Chemically camel muscles had been found to have low fat content, high water holding capacity recommending camel meat as a healthy food with good processing properties. However, there is evidence of a great demand for fresh camel meat and for camel meat in blended meat products even in societies not herding camels (**Pérez et al., 2000**). There is also reluctance towards consuming camel meat in general as it is thought to be tough in texture and imparts poor organoleptic characteristics, coarse and watery. This is mainly because camel meat usually comes from old animals that have served other functions in their life or predominantly at the time their labour performance and milk yield declines (**Wilson, 1998**). Thus, plant enzymes with tenderizing capacity are particularly important in applications involving muscles rich in connective tissue. These muscles often make up the cheaper carcass cuts and the tenderizing effect of these enzymes offers a commercially important means of upgrading this tissue.

Therefore, with a view to upgrading low value aged camel meat cuts using easily sourced ingredients, the effect of different concentrations (0, 15, 30 and 45%) of ginger extract on physicochemical, sensory properties of tough aged camel meat chunks were investigated. Then, select the optimum concentration from ginger extract to achieve the best tenderization effect for aged camel meat samples. Then, samples of these treatment were gamma irradiated at dose levels of 0, 1.5, 3 and 4.5 kGy to extend its shelf-life and improving its hygienic quality at cold storage ( $4\pm 1^\circ\text{C}$ ) compared to control samples (without any treatments neither ginger extract nor gamma irradiation).

## **MATERIALS & METHODS**

### **Preparation of ginger extract**

Fresh ginger rhizome (*Zingiber officinale*) from a local market was peeled, sliced and blended with equal quantity of chilled, distilled water for 1–2 min. The homogenate was squeezed with fingers through four layers of muslin cloth.

### **Camel meat chunks**

The chuck muscles of aged camel carcasses were obtained soon after slaughter, from local slaughter units. They were packed in low-density polyethylene bags and stored in refrigerator at  $4\pm 1^\circ\text{C}$  for 24 h. After 24-h chilling, muscles were taken out of refrigerator and cut into small chunks of approximately  $3\text{ cm}^3$  size and were randomly divided into four groups and marinated with different concentrations of ginger extract.

### **Marinating of camel meat chunks**

To prepare the required different concentration of ginger extract, the crude fresh ginger extract was diluted with distilled water to obtain concentrations of 15, 30 and 45% fresh ginger extract. About  $3\times 3\times 3\text{ cm}$  uniform-sized aged camel meat chunks were sprayed with 0, 15, 30 and 45% w/v fresh ginger extract. These concentrations of fresh ginger extract were sprayed at 15% v/w of aged camel meat chunks (15 mL/100 g meat). After thorough mixing by hand, the chunks were placed in polyethylene bags and held at  $4\pm 1^\circ\text{C}$  for 48 h.

### **Proteolytic activity determination**

Protease activity of culture supernatant was determined by the method of **Chopra et al., (1983)**. One ml of the substrate (1% casein in 0.05 M phosphate buffer, pH 7.0) was incubated at  $37^\circ\text{C}$  for 15 min, then 1.0 ml of the culture supernatant which was obtained by centrifugation ( $8000\times g$  at  $4^\circ\text{C}$  for 20 min) was added. After mixing, the reaction mixture was incubated at  $37^\circ\text{C}$  for 20 min. The reaction was terminated by adding 2.0 ml of 0.4 M / trichloroacetic acid (TCA) then filtrated and the mixture was further incubated at the same temperature for 20 min. For the blank, the substrate was precipitated with TCA before adding the enzyme solution and then treated as described above. To 1 ml of the filtrate obtained after TCA precipitation, 5.0 ml of 0.4 M sodium carbonate solution was added followed by 1.0 ml of folins reagent and incubated at  $37^\circ\text{C}$  for 20 min for color development and reading absorbance (A) at 750 nm. A unit of protease activity is defined as the amount of enzyme required to release TCA - soluble fragment giving a blue color equivalent to one  $\mu\text{g}$  of tyrosine under the same condition of the assay.

### **Proximate composition**

Moisture, lipid, protein, ash and carbohydrates contents were determined according to **A.O.A.C. (2000)** official method. pH was assessed using a pH meter on a homogenate consisting of 5g of sample in 50 ml of distilled water as described by **Carballo et al., (1995)**.

### **Physico-chemical analysis**

Water holding capacity (WHC), shear force value and cooking yield were determined according to **Malcolm (2002)**.

### Protein solubility

Protein solubility was determined according to procedures of **Joo et al. (1999)**. Sarcoplasmic proteins were extracted from 2-g minced muscle using 20 ml of ice-cold 0.025 M potassium phosphate buffer (pH 7.2). The samples were homogenized and kept overnight at 4°C with frequent shaking. Samples were centrifuged at 1500g for 20 min and protein concentration in the supernatant was determined by the Biuret method. Total protein (sarcoplasmic + myofibrillar) was extracted from 2-g muscle using 40 ml ice-cold 1.1 M potassium iodide in 0.1 M phosphate buffer (pH 7.2). Homogenization, centrifugation and protein determination were carried out as described above. Myofibrillar protein concentrations were obtained by difference between total and sarcoplasmic protein solubility.

### Hydroxyproline estimation

Hydroxyproline content of the meat sample was determined based on the procedure of **Nueman and Logan (1950)**. Two-gram meat samples were hydrolyzed with 40 ml of 6 N HCl for 18 h. The hydrolysate was filtered and the volume adjusted to 50 ml with distilled water. An aliquot was used for hydroxyproline estimation. Absorbance was measured at 540 nm and the hydroxyproline content was determined by referring to a standard graph. Collagen content was calculated by multiplying by 7.14 and was expressed in mg/g tissue.

### Collagen solubility

Collagen solubility was determined by the method described by **Mahendrakar et al. (1989)**. Five grams of muscle tissue was taken in a 250-ml beaker and immersed in a water bath after covering the beaker with Petri dish. The water bath was then heated to boiling temperature and held for 30 min. The cooked meat was then taken out of the beaker and cut into small pieces and homogenized with 50 ml distilled water at 4±1°C in a blender for 2 min. The extract was then centrifuged at 1500g for 30 min. Aliquots of cooked out juice and centrifuge were hydrolyzed for 18 h and soluble hydroxyproline was calculated. Collagen solubility was calculated as:

% Collagen solubility = 7:14 × % hydroxyproline solubilized.

### SDS – polyacrylamid gel electrophoresis (SDS - PAGE)

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was carried out using the methods of **Laemmli (1970) and See and Jackowski (1993)** with electrophoresis apparatus (Model: Protean Xi Cell, BioRad, USA). Five grams of minced meat was mixed with 50 ml of 0.01 N sodium phosphate buffer (pH 7.0) containing 1% SDS and 1% 2-mercaptoethanol and incubated at 37° C for 2 h. The mixture was centrifuged at 1500g for 30 min. An aliquot of supernatant was dialyzed overnight at room temperature (26° C) against 0.1 N sodium phosphate buffer containing 0.1% 2-mercaptoethanol. About 50  $\mu$ l of dialyzed solution was used for loading the gel. Electrophoresis was performed at a constant voltage mode of 100 V/slab at 30 mA for 5–6 h or until the tracking dye reached the lower end of the gel. The gel was removed and stained with Coomassie blue for 4–5 h. The gels were then detained and photographed. The scanning of polyacrylamide gel and analysis of the results was accomplished using color flatbed scanner (Epson Acer 60p) connected with a computer and printer. Total lab from phortix version 1.11 software was used. The estimation of molecular weights of different protein bands was automatically done by the aid of a protein marker (Albumin bovine 66.000, Albu. Min egg 43.000 and lysozyme 14.3000Da).

### Sensory Evaluation

The sensory evaluation was carried out for both raw and cooked camel meat chunk samples. Raw meat samples were evaluated for their appearance, odor and color. To prepare cooked aged camel meat chunks were washed and drained, then cooked for 20 min in an oven (180°C) to an internal temperature of 75±1°C monitored using a probe thermometer. The cooked meat samples were evaluated for their flavor, tenderness and juiciness. The panel consisted of ten members from our laboratory and scores were obtained as described by **Wierbicki, (1985)** by rating the above quality characteristics using the following rating scale: 9= Excellent, 8= Very good, 7= Good, 6= Below Good-above fair, 5= Fair, 4= Below fair-above poor, 3= Poor, 2= Very poor and 1= Extremely poor.

### Irradiation Treatments

For irradiation treatments, frozen camel meat chunk samples treated with 30% ginger extract (frozen at -18°C for 3 days before irradiation) were exposed to gamma irradiation at dose levels of 1.5, 3.0 and 4.5 kGy using <sup>60</sup>Co from unit Gamma Chamber 4000, at the National Center for Radiation Research and Technology, Atomic Energy Authority, Egypt.

### Microbiological Analysis:

Colony forming units for total bacterial count were counted by plating on plate count agar medium and incubation at 30°C for 3-5 days (**APHA, 1992**). Total psychrophilic bacteria were enumerated on plate count agar medium after incubation at 5°C for 7 days as recommended by

**APHA (1992)**. Lactic acid bacteria were counted by the pour plate over layer method on MRS medium **Oxoid manual (1982)**. Enterobacteriaceae were counted on violet red bile glucose agar medium after incubation for 20–24 h at 37°C **Roberts et al., (1995)**. *Staphylococcus aureus* was counted using Baird–Parker medium after incubated at 35 °C for 24–48 h **Oxoid manual (1998)**. *Bacillus cereus* was counted using Mannitol-egg Yolk-Polymyxin (MYP) agar and incubated at 37°C for 16-24 hours as described by **Roberts et al., (1995)**. The detection of *Salmonella* was carried out using the most probable number technique. After enrichment at 37 °C for 24 h in selenite broth, the cultures were streaked on Brilliant green agar and incubated at 37 °C for 24 h, and then colonies were biochemically examined in triple sugar iron agar (**ISO, 1978**)

### Statistical Analysis

The obtained data were exposed to analysis of variance. Duncan's multiple range tests at 5% level was used to compare between means. The analysis was carried out using the PROC ANOVA procedure of Statistical Analysis System (**SAS, 1996**).

## RESULTS AND DISCUSSION

### Proteolytic activity of ginger extract

The Proteolytic activity of ginger extract was determined before starting the experiments of camel meat tenderization. It is apparent the Proteolytic activity of ginger extract was 10 mg /gm

### Proximate composition

The Proximate composition (on dry weight basis) of camel meat affected by different concentrations from ginger extract are tabulated in Table (1). The components of camel meat from moisture, protein, fat, ash and carbohydrates were 75.18, 86.02, 9.07, 4.31 and 0.60%, respectively. The observed results agree with those reported by **Dawood and Alkanhal (1995) & Kadim et al. (2006)**. Therefore, our results are in agreement with previous references. The composition of camel meat varies according to different factors. Age is an important factor in determining meat quality and composition. The percentage of protein decreased and that of fat increased with increasing camel age (**Kadim et al., 2006**). However, the results illustrated that no different significantly between controls and ginger extract treated samples.

### pH values and moisture contents of raw and cooked camel chunk samples

The effect different concentrations of ginger extract on physico-chemical characteristics of camel meat chunk samples were presented in Table (2). The results showed that the pH values no different significantly between raw and cooked control and ginger extract treated samples. Also, the data illustrated that no significant difference between moisture content of raw control and ginger extract treated raw camel meat chunk samples. In cooked camel meat chunk samples the moisture content increase with increasing concentration of ginger extracts of cooked camel meat chunk samples indicates improvement in hydrophilic properties by the enzyme treatment. These results are in agreement with mentioned by **Naveena and Mendiratta (2004)**.

### WHC, share force and cooking yield of camel meat chunks

The effect of ginger extract on water holding capacity (WHC), share force and cooking yield of camel meat chunks are shown in Table (3). The results showed that treating camel meat chunks with different concentrations from ginger extract at levels of 0, 15, 30 and 45% induced significant increases in the values of WHC and cooking yield compared to control samples. While, the share force values were significantly lower in all treated samples compared to control. **Lee et al. (1986)** found a linear decrease in shear force values with increasing amount of GE in beefsteaks. Similar observations were made by **Naveena and Mendiratta (2001)** in spent hen meat treated with different concentrations of ginger extract. **Thompson et al. (1973)** also, reported a decrease in shear force values from 4.27 Kg to 2.8 Kg /cm<sup>2</sup> by ginger treatment in ovine *B. femoris* muscle. Meanwhile, the results obvious that the treated samples of camel meat chunks with 30% ginger extract had significantly higher WHC, share force and cooking yield than control samples and other treated samples with 15 and 45% ginger extract. These may be due to increases in protein and collagen solubility. Hydrolyzed collagen derived from the connective tissues has excellent water binding capacity and is able to improve the tenderness of the cooked meats (**Xiong, 1997 and Badr, 2008**). **Naveena et al. (2004)** they mentioned that Increase in the moisture with increasing concentration of ginger extract indicates improvement in hydrophilic properties by the enzyme treatment.

### Collagen content and solubility of sarcoplasmic, myofibrillar and total protein solubility

Table (4) illustrated that the collagen content and solubility of sarcoplasmic, myofibrillar and total protein solubility of camel meat chunks as affected by different concentrations from ginger extract. The data showed that slightly higher collagen content recorded in treated samples under investigation compared to control samples. Higher significantly collagen solubility values were observed in all treated samples compared to control. The

increased collagen solubility of ginger-treated samples in our experiment was consistent with the findings of **Thompson et al. (1973)**, who reported a significant increase in collagen solubility of ovine *Biceps femoris* muscle with ginger extract treatment. They found that proteolytic activity of ginger protease on collagen was many fold greater than on actomyosin and the combined proteolysis of these two muscle proteins resulted in significantly more tender meat. **Takagi et al. (1992)** also reported significantly higher collagen solubility in beef meat treated with papain compared to water-treated control and alkaline elates-treated sample.

On the other hand, significantly higher myofibrillar and total protein solubility values were observed in all samples compared to control. The sarcoplasmic protein solubility values of treated samples increased only marginally in comparison to control. Increase in solubility of treated samples might be due to increase in permeability of myofibrils, which will disintegrate easily. Lower solubility of sarcoplasmic proteins in treated samples was in agreement with **Kang and Rice (1970)** who reported that water soluble proteins are more resistant to enzyme degradation than other fractions. A promising protease “Zingibain” isolated from *Zingiber officinale* roscoe (Ginger rhizome) has been reported to have proteolytic activity. Its proteolytic activity on collagen was found to be many fold greater than on actomyosin, and the combined proteolysis of these two muscle proteins improved the tenderness of meat (**Thompson et al. 1973**). Increase in protein solubility with ginger and papain treatment was also reported by **Naveena and Mendiratta (2001)**. Also, **Naveena et al (2004)** found that ginger extract increased the collagen solubility, sarcoplasmic and myofibrillar protein solubility compared to controls.

#### **Electrophoresis by SDS- PAGE of camel meat chunks as affected by different concentrations from ginger extract**

Electrophoretic separation of protein using polyacrylamide gel in the present of sodium dodecyl sulphate (SDS) and 2-mercapotethanol is widely used for the quantitative analysis of protein sub-units and has shown to be a reliable method for molecular weight determination. In many reports, protein degradation is only judged by visual evaluation of the gel, without the support of quantitative data. The quantification of the separated proteins, however, remains a complex issue. Different approaches have been used to quantify protein bands **Claeys et al., (1995)**.

The results in Table (5) and fig (1) shows that electrophoretic separation of control and aged camel meat chunks treated with different concentrations from ginger protein profiles were different according to the extraction methods. Some changes were detected between control sample proteins among them protein fraction bands with molecular weight (M.W) 72.4 KDa induced change in the protein. In this respect, three bands disappeared from camel meat chunks treated with different concentrations from ginger having molecular weights 59.2, 48.9, and 39.1 KDa. On the hand, five bands appeared with molecular weights 72.4, 59.2, 55.9, 48.9 and 39.1 KDa. The treated with different concentrations from ginger induced increased percentage.

#### **Sensory evaluation**

Table (6) presented that the uncooked and cooked aged camel meat chunks treated with different concentrations from ginger extract received significantly higher scores and acceptable as judged by appearance, odor and color & flavor, tenderness and juiciness compared to control samples, respectively. Also, the results observed that exposed samples of aged camel meat chunks to ascending concentrations of ginger extract caused bright pink-red color compared to dark-red color for control samples. This may be due to the effect of compounds ginger extract on pigment of camel meat samples. Moreover, improvement in flavor, juiciness and tenderness scores with ginger extract treatment in our observed results are consistent with some reports (**Mendiratta et al., 2000** and **Naveena et al., 2004**). **Labell (1987)** reported increase in flavor of microwaved meat and poultry treated with 2% ginger powder. He attributed the increase in flavor of ginger treated sample to flavor producing reactions that occur during cooking. Treatment with ginger extract improved the juiciness scores compared to the control. Improvement in appearance, flavor and juiciness of buffalo meat samples treated with ginger extract and organic acids were also reported by **Syed Ziauddin et al. (1995)**. The observed improvement in eating satisfaction of cooked camel meat chunks treated with ginger extract, especially samples-treated with 30% ginger extract agree with the observed increases in protein and collagen solubility. Hydrolyzed collagen derived from the connective tissues has excellent water binding capacity and is able to improve the tenderness of the cooked meats (**Xiong, 1997 and Badr, 2008**).

Therefore, samples of cooked camel meat treated with 30% ginger extract were rated superior and most preferred by the panelists and appears to be the optimum level to achieve the best tenderization effect, which can be attributed to the desirable ginger flavor and can be effectively utilized at household or industrial level and they can be used as better alternatives to papain for tenderization of tough meat.

#### **Microbiological Aspects**

##### **Microbial flora**

In this study our observed results showed that the samples of camel meat chunks treated with 30% ginger extract were rated superior and highly acceptable by the panelists and appears to be the optimum level to achieve

the best tenderization effect. Thus, these treatment were select to gamma irradiation at dose levels of 0 , 1.5, 3 and 4.5 kGy to extend its refrigeration shelf-life and improving its hygienic quality at cold storage ( $4\pm 1^{\circ}\text{C}$ ) compared to control samples (without any treatments neither ginger extract nor gamma irradiation).

Therefore, the results in Table (7) presented that the effects of combination treatments between ginger extract (30%) and gamma irradiation at cold storage ( $4\pm 1^{\circ}\text{C}$ ) on the microbial load in camel meat chunks. Samples of control (without any treatments) had an initial counts of  $7.8\times 10^5$ ,  $4.3\times 10^4$ ,  $1.9\times 10^3$  and  $8.8\times 10^2$  cfu/g for total bacterial count, Psychrophilic bacteria, lactic acid bacteria and Enterobacteriaceae, respectively. Moreover, treated samples of camel meat chunks with 30% ginger extract reduced the counts of total bacterial count, Psychrophilic bacteria, lactic acid bacteria and Enterobacteriaceae to  $3.1\times 10^5$ ,  $2.3\times 10^4$ ,  $8.7\times 10^2$  and  $4.3\times 10^2$  cfu/g, respectively. While, irradiating-treated samples of camel meat chunks with 30% ginger extract to gamma irradiation at dose levels of 0, 1.5, 3 and 4.5 kGy markedly decrease the counts of total bacterial count, Psychrophilic bacteria and lactic acid bacteria by 89.87, 98.95 and 99.91; 83.02, 97.77 and 99.87 & 74.74, 95.42 and 99.05%, respectively. Moreover, the dose level of 1.5 kGy reduced the counts of Enterobacteriaceae by 98.0%, while the dose levels of 3 and 4.5 kGy completely eliminated these pathogen from samples camel meat chunks as it remained undetectable upon cold storage periods of the treated samples (**Hammad et al., (2000)** and **Rady et al., (2005)**). Besides tenderizing properties, antioxidant and antimicrobial characteristics of ginger extract have been reported by different workers (**Mendiratta et al. 2000**), **Fallah et al. (2008)** they illustrated that irradiation at dose level of 3 kGy reduced the initial counts of aerobic plate bacteria, lactic acid bacteria and Enterococci by 99.5, 93.5 and 93.9% for camel meat samples, respectively, *Pseudomonas*, coliforms, *S. aureus*, *L. monocytogenes* and *E. coli* were not found at dose level of 3 kGy during entire storage period, also Psychrophilic bacteria and molds and yeasts were below the detection levels during 6 days of storage ( $3\pm 1^{\circ}\text{C}$ ). During cold storage, gradual increase in the total bacterial count and psychrophilic bacteria was observed in all samples, but the rate of increase was higher in control samples than irradiated samples. Furthermore, samples were rejected when the count total bacterial count reached  $1\times 10^7$  cfu/g. Thus, the shelf-life samples of control and irradiated–treated camel meat chunks with 30% ginger extract at dose levels 0, 1.5, 3 and 4.5 kGy were 6, 9, 18, 27 and 33 days, respectively. Al-Bachir and Zeinou (2009) reported that gamma irradiation may be used to control TPC in camel meat and increase it's the shelf-life from less than 2 weeks (control) to more than 6 weeks (samples irradiated with 2, 4 or 6 kGy). **Badr (2007)** and **Duong et al. (2008)** they revealed that gamma irradiation with 2–6 kGy and storage under refrigeration reduced the total microorganisms count and increased the shelf-life of ground beef.

#### Food borne pathogens

Table (8) showed that the effects of combination treatments between ginger extract (30%) and gamma irradiation at cold storage ( $4\pm 1^{\circ}\text{C}$ ) on food borne pathogens in camel meat chunks. The results show that the initial counts of control samples (without any treatments) were  $8.6\times 10^2$  and  $5.4\times 10^2$  cfu/g for *Staphylococcus aureus* and *B. cereus*, respectively. Moreover, bacteria of *Salmonella* spp was detected in these samples. Subjecting samples of camel meat chunks treated with ginger extract to gamma irradiation at dose levels of 0 and 1.5 kGy reduced the counts of *Staphylococcus aureus* to  $2.5\times 10^2$  and  $1.3\times 10^1$  cfu/g and counts of *B. cereus* to  $2.3\times 10^2$  and  $7.5\times 10^1$ cfu/g, respectively. Meanwhile, bacteria of *Salmonella* spp was detected in control and treated with 30% ginger extract samples, while, these bacteria were not detected in treated samples of camel meat chunks with 30% ginger extract and irradiated at dose level of 1 kGy. On the other hand, treated samples of camel meat chunks with 30% ginger extract and irradiated at dose levels of 3 and 4.5 completely eliminating counts of *Staphylococcus aureus*, *B. cereus* and *Salmonella* spp from these samples. These results are in agreement with previous reports **Zhu et al. (2005)** radiation sensitivity non-sporeforming pathogenic bacteria in meat and meat products is well documented. **Badr (2004)** and **Sedeh et al. (2007)** reported that the optimum dose of gamma irradiation to improve microbial safety of meat and eliminate *Salmonella* spp was observed at 3 kGy. **Fallah et al. (2008)** they found that the dose level of 3 kGy was more effective through its effectiveness in eliminating *Pseudomonas* spp., coliforms, *S. aureus*, *L. monocytogenes* and *E. coli*, with extending refrigerated life camel meat by 21 days. During cold storage, gradual increases of *Staphylococcus aureus* and *B. cereus* were observed during cold storage in samples of control, treated with 30% ginger extract & treated with 30% ginger extract and irradiated at dose level of 1.5 kGy. While, treated samples with 30% ginger extract and irradiated at dose levels of 3 and 4.5 kGy et al. (2004) Found that the combination of aqueous crude extracts of ginger and garlic yielded no inhibition, whereas the combination of their ethanolic extracts inhibited both *S. aureus* and *Bacillus* spp. Also completely eliminated these pathogen from samples camel meat chunks. **Onyeagba, Thongson et al., (2003)** mentioned that the Thai rhizome spices such as ginger (*Zingiber officinale* Rose) had antimicrobial activity against a variety of food-borne pathogens as *Listeria monocytogenes* and *Salmonella Typhimurium* DT104.

### Sensory evaluation

Table (9) showed that the changes in sensory attributes of camel meat chunks as affected by combination treatment between ginger extract (30%) and gamma irradiation at cold storage ( $4\pm 1^\circ\text{C}$ ). The results showed that appearance, flavor and texture characteristics of treated samples of camel meat chunks with 30% ginger extract were unaffected by gamma irradiation treatments compared with control samples (without any treatments). This may be due to the excess ginger flavor imparted by ginger extract at the higher concentration (30%), especially the flavor non-irradiated and irradiated samples treated with ginger extract. The information on the effect of gamma irradiation on sensory quality of camel meat is very limited. But many investigators have studied the effect of gamma irradiation on the sensory characteristics of other meat and meat products. Most of them found no effect of gamma irradiation (2–6 kGy) on sensory quality of tested meat and meat products (Al-bachir, 2007; Badr, 2004 and Badr et al., 2005). Upon cold storage, samples of control were rejected on day 9 of storage, when detected off odor and their total bacterial count more than  $1 \times 10^7$  cfu/g. While treated samples of camel meat chunks and irradiated by gamma irradiation at dose levels of 0, 1.5, 3 and 4.5 kGy were scored as good samples the rejection due to increasing their total bacterial count to more than  $1 \times 10^7$  cfu/g on day 12, 21, 30 and 36 of storage, respectively. These results are in agreement with those of Al-bachir and Zeinou (2009) they found that the microbiological shelf-life of camel meat was significantly extended from less than 2 weeks (control) to more than 6 weeks (samples irradiated with 2, 4 or 6 kGy).

### CONCLUSIONS

This study has shown that a 30% (v/w) ginger rhizome extract can be effectively utilized to tenderize camel meat without adversely affecting other meat quality parameters. Therefore, a technology for utilization of easily and cheaply available ginger can be exploited at the industrial or household level for tenderization of camel meat. An irradiation dose of 4.5 kGy appeared to be the optimum dose for improving the hygienic quality of camel meat treated with 30% ginger extract through its effectiveness in eliminating public health bacteria and extending the shelf-life of samples to 33 days compared to 6 days for control samples without any adverse changes in their sensory characteristics.

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**Table (1): Proximate composition (on dry weight basis) of camel meat as affected by different concentrations from ginger extract.**

Camel meat component	Moisture	Protein	Fat	Ash	Carbohydrates
Control	75.18 <sup>A</sup> ±0.08	86.02 <sup>A</sup> ±0.21	9.07 <sup>A</sup> ±0.17	4.31 <sup>A</sup> ±0.05	0.60 <sup>A</sup> ±0.23
15%	75.15 <sup>A</sup> ±0.14	86.16 <sup>A</sup> ±0.09	9.05 <sup>A</sup> ±0.31	4.17 <sup>A</sup> ±0.07	0.62 <sup>A</sup> ±0.29
30%	75.23 <sup>A</sup> ±0.27	86.12 <sup>A</sup> ±0.24	9.08 <sup>A</sup> ±0.28	4.15 <sup>A</sup> ±0.18	0.65 <sup>A</sup> ±0.14
45%	75.25 <sup>A</sup> ±0.18	86.18 <sup>A</sup> ±0.27	9.06 <sup>A</sup> ±0.15	4.09 <sup>A</sup> ±0.16	0.67 <sup>A</sup> ±0.32

Capital letters were used for comparing between means in the columns.

Means with the same letters are not significantly different (p>0.05).

**Table (2): Effect of different concentrations of ginger extract on physico-chemical characteristics of camel meat chunks.**

Parameters	Control	Concentrations of ginger extract (%)		
		15	30	45
Raw meat pH	6.58 <sup>A</sup> ±0.25	6.26 <sup>A</sup> ±0.19	6.08 <sup>A</sup> ±0.07	6.07 <sup>A</sup> ±0.15
Moisture (%)	75.18 <sup>A</sup> ±0.08	75.15 <sup>A</sup> ±0.14	75.23 <sup>A</sup> ±0.29	75.25 <sup>A</sup> ±0.18
Cooked meat pH	6.64 <sup>A</sup> ±0.21	6.42 <sup>A</sup> ±0.33	6.38 <sup>A</sup> ±0.09	6.35 <sup>A</sup> ±0.22
Moisture (%)	55.72 <sup>A</sup> ±0.35	57.04 <sup>B</sup> ±0.26	59.36 <sup>C</sup> ±0.14	59.82 <sup>C</sup> ±0.16

Capital letters were used for comparing between means in the rows.

Means with the same letters are not significantly different (p>0.05).

**Table (3): WHC, share force and cooking yield of camel meat chunks as affected by different concentrations from ginger extract.**

Samples of camel meat treated with ginger extract	WHC (Cm <sup>2</sup> / 0.3gm)	Share force (Newton/cm <sup>2</sup> )	Cooking yield (%)
Control	1.90 <sup>A</sup> ±0.07	833.26 <sup>A</sup> ±0.25	26.02 <sup>A</sup> ±0.16
15%	5.50 <sup>B</sup> ±0.23	809.41 <sup>B</sup> ±0.12	45.07 <sup>B</sup> ±0.08
30%	6.10 <sup>C</sup> ±0.18	785.65 <sup>C</sup> ±0.35	54.14 <sup>C</sup> ±0.27
45%	5.70 <sup>D</sup> ±0.21	779.36 <sup>C</sup> ±0.09	50.93 <sup>D</sup> ±0.34

WHC = Water holding capacity.

Capital letters were used for comparing between means in the columns.

Means with the same letters are not significantly different (p>0.05).

**Table (4): Collagen content and solubility of sarcoplasmic, myofibrillar and total protein solubility of camel meat chunks as affected by different concentrations from ginger extract.**

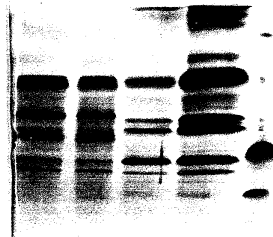
samples of camel mat treated with ginger extract	Collagen content (mg/g tissue)	Collagen solubility (% total collagen)	Sarcoplasmic protein solubility (mg/g)	Myofibrillar protein solubility (mg/g)	Total protein solubility (mg/g)
Control	7.34 <sup>A</sup> ±0.08	7.34 <sup>A</sup> ±0.012	20.34 <sup>A</sup> ±0.23	61.83 <sup>A</sup> ±0.06	82.17 <sup>A</sup> ±0.31
15%	7.92 <sup>A</sup> ±0.12	14.05 <sup>A</sup> ±0.09	21.87 <sup>B</sup> ±0.09	74.46 <sup>B</sup> ±0.24	96.33 <sup>B</sup> ±0.27
30%	8.15 <sup>B</sup> ±0.19	17.69 <sup>A</sup> ±0.35	22.07 <sup>B</sup> ±0.12	75.95 <sup>C</sup> ±0.27	98.02 <sup>C</sup> ±0.19
45%	8.27 <sup>B</sup> ±0.32	20.81 <sup>A</sup> ±0.28	22.53 <sup>BC</sup> ±0.29	77.21 <sup>D</sup> ±0.21	99.74 <sup>D</sup> ±0.13

Capital letters were used for comparing between means in the columns.

Means with the same letters are not significantly different (p>0.05).

**Table (5) Electrophoresis by SDS- PAGE of camel meat chunks as affected by different concentrations from ginger extract**

Bands No.	RF	Molecular weight	Control	Concentrations of ginger extract (%)		
				15	30	45
1	0.025	75.3	2.34	2.51	2.65	3.52
2	0.062	72.4	2.99	3.1	3.3	-
3	0.07	71.6	3.1	3.3	3.5	3.7
4	0.085	70.8	3.6	3.8	4.0	4.4
5	0.92	69.9	3.8	3.9	4.1	4.5
6	0.11	67.5	4.1	4.3	4.5	4.8
7	0.12	63.3	4.2	4.3	4.6	4.7
8	0.164	59.2	4.4	4.5	-	-
9	0.176	57.7	4.7	4.9	5.0	5.3
10	0.211	55.9	4.9	5.0	-	-
11	0.224	52.6	5.0	5.3	5.4	5.7
12	0.227	49.3	5.1	5.4	5.5	5.9
13	0.229	48.9	5.3	-	-	-
14	0.232	45.2	6.0	6.4	6.5	6.7
15	0.235	39.1	6.3	-	-	-



**Fig(1) Protein pattern of camel meat chunks treated with different concentration from ginger extract.**

**Table (6): The sensory attributes of camel meat chunks treated with different concentration from ginger extract.**

Quality attributes	Control	Concentrations of ginger extract (%)		
		15	30	45
Appearance *	8.5 <sup>A</sup> ±0.09	8.9 <sup>A</sup> ±0.32	9.4 <sup>B</sup> ±0.24	9.3 <sup>B</sup> ±0.17
Odor *	8.6 <sup>A</sup> ±0.18	9.1 <sup>B</sup> ±0.21	9.7 <sup>C</sup> ±0.09	9.8 <sup>C</sup> ±0.24
Color *	8.9 <sup>A</sup> ±0.37	9.2 <sup>B</sup> ±0.35	9.8 <sup>C</sup> ±0.32	9.8 <sup>C</sup> ±0.07
Flavor **	8.7 <sup>A</sup> ±0.15	9.1 <sup>B</sup> ±0.08	9.7 <sup>B</sup> ±0.28	8.9 <sup>AB</sup> ±0.12
Tenderness **	7.2 <sup>A</sup> ±0.27	8.4 <sup>B</sup> ±0.12	9.5 <sup>C</sup> ±0.31	9.4 <sup>C</sup> ±0.07
Juiciness **	7.4 <sup>A</sup> ±0.21	8.6 <sup>B</sup> ±0.25	9.6 <sup>C</sup> ±0.18	9.7 <sup>C</sup> ±0.35

Capital letters were used for comparing between means in the rows.

Means with the same letters are not significantly different ( $p>0.05$ ).

\* = Raw or uncooked camel meat chunks.

\*\*= Cooked camel meat chunks.

**Table (7): Effects of combination treatments between ginger extract (30%) and gamma irradiation at cold storage (4±1°C) on the microbial load in camel meat chunks.**

Microbial determinations (cfu/g)	Storage (days)	Control	Ginger extract (30%) and gamma irradiation			
			0 kGy	1.5 kGy	3 kGy	4.5 kGy
Total aerobic count	0	7.8x10 <sup>5</sup>	3.1x10 <sup>5</sup>	7.9x10 <sup>4</sup>	8.2x10 <sup>3</sup>	6.9x10 <sup>2</sup>
	3	5.2x10 <sup>6</sup>	7.9x10 <sup>5</sup>	9.6x10 <sup>4</sup>	9.2x10 <sup>3</sup>	8.5x10 <sup>2</sup>
	6	8.9x10 <sup>6</sup>	4.2x10 <sup>6</sup>	2.4x10 <sup>5</sup>	3.2x10 <sup>4</sup>	2.3x10 <sup>3</sup>
	9	6.8x10 <sup>7</sup> R	9.6x10 <sup>6</sup>	5.8x10 <sup>5</sup>	6.7x10 <sup>4</sup>	5.5x10 <sup>3</sup>
	12		3.7x10 <sup>7</sup> R	8.4x10 <sup>5</sup>	9.1x10 <sup>4</sup>	7.8x10 <sup>3</sup>
	15			2.4x10 <sup>6</sup>	3.7x10 <sup>5</sup>	2.9x10 <sup>4</sup>
	18			8.7x10 <sup>6</sup>	6.2x10 <sup>5</sup>	5.8x10 <sup>4</sup>
	21			2.8x10 <sup>7</sup> R	9.8x10 <sup>5</sup>	1.2x10 <sup>5</sup>
	24				3.9x10 <sup>6</sup>	6.5x10 <sup>5</sup>
	27				9.7x10 <sup>6</sup>	9.9x10 <sup>5</sup>
	30				5.3x10 <sup>7</sup> R	3.6x10 <sup>6</sup>
	33					7.8x10 <sup>6</sup>
	36					4.7x10 <sup>7</sup> R
Psychrophilic bacteria	0	4.3x10 <sup>4</sup>	2.3x10 <sup>4</sup>	7.3x10 <sup>3</sup>	9.6x10 <sup>2</sup>	5.4x10 <sup>1</sup>
	3	6.9x10 <sup>4</sup>	4.9x10 <sup>4</sup>	8.6x10 <sup>3</sup>	2.3x10 <sup>2</sup>	7.8x10 <sup>1</sup>
	6	8.7x10 <sup>4</sup>	7.3x10 <sup>4</sup>	9.1x10 <sup>3</sup>	4.7x10 <sup>3</sup>	9.2x10 <sup>1</sup>
	9	3.7x10 <sup>5</sup> R	9.5x10 <sup>4</sup>	2.1x10 <sup>4</sup>	6.3x10 <sup>3</sup>	1.9x10 <sup>2</sup>
	12		2.6x10 <sup>5</sup> R	4.3x10 <sup>4</sup>	7.2x10 <sup>3</sup>	5.1x10 <sup>2</sup>
	15			7.2x10 <sup>4</sup>	8.9x10 <sup>3</sup>	7.2x10 <sup>2</sup>
	18			9.6x10 <sup>4</sup>	1.8x10 <sup>4</sup>	9.1x10 <sup>2</sup>
	21			3.3x10 <sup>5</sup> R	5.9x10 <sup>4</sup>	2.7x10 <sup>2</sup>
	24				8.3x10 <sup>4</sup>	4.3x10 <sup>3</sup>
	27				2.1x10 <sup>4</sup>	6.5x10 <sup>3</sup>
	30				4.4x10 <sup>5</sup> R	9.4x10 <sup>3</sup>
	33					5.2x10 <sup>4</sup>
	36					8.7x10 <sup>4</sup> R
Lactic acid bacteria	0	1.9x10 <sup>3</sup>	8.7x10 <sup>2</sup>	4.8x10 <sup>2</sup>	8.7x10 <sup>1</sup>	1.8x10 <sup>1</sup>
	3	5.5x10 <sup>3</sup>	<10	<10	<10	<10
	6	9.7x10 <sup>3</sup>	<10	<10	<10	<10
	9	6.8x10 <sup>4</sup> R	<10	<10	<10	<10
	12		5.2x10 <sup>2</sup> R	<10	<10	<10
	15			<10	<10	<10
	18			3.1x10 <sup>2</sup>	<10	<10
	21			6.5x10 <sup>2</sup> R	<10	<10
	24				1.6x10 <sup>2</sup>	<10
	27				7.7x10 <sup>2</sup>	<10
	30				1.2x10 <sup>3</sup>	5.0x10 <sup>1</sup>
	33				7.7x10 <sup>3</sup> R	17x10 <sup>2</sup>
	36					6.3x10 <sup>2</sup> R
Enterobacteriaceae	0	8.8x10 <sup>2</sup>	4.3x10 <sup>2</sup>	1.5x10 <sup>1</sup>	Nil	Nil
	3	3.1x10 <sup>3</sup>	<10	<10	Nil	Nil
	6	7.9x10 <sup>3</sup>	<10	<10	Nil	Nil
	9	1.9x10 <sup>4</sup> R	1.2x10 <sup>2</sup>	<10	Nil	Nil
	12		5.9x10 <sup>2</sup> R	<10	Nil	Nil
	15			7.0x10 <sup>1</sup>	Nil	Nil
	18			4.4x10 <sup>2</sup>	Nil	Nil
	21			8.2x10 <sup>2</sup> R	Nil	Nil
	24				Nil	Nil
	27				Nil	Nil
	30				Nil R	Nil
	33					Nil
	36					Nil R

R= Rejected

**Table (8): Effects of combination treatments between ginger extract (30%) and gamma irradiation at cold storage (4±1°C) on food borne pathogens in camel meat chunks.**

Microbial determinations (cfu/g)	Storage (days)	Control	Ginger extract (30%) and gamma irradiation			
			0 kGy	1.5 kGy	3 kGy	4.5 kGy
Staphylococcus aureus	0	8.6x10 <sup>2</sup>	2.5x10 <sup>2</sup>	1.3x10 <sup>1</sup>	Nil	Nil
	3	2.4x10 <sup>3</sup>	5.1x10 <sup>2</sup>	3.8x10 <sup>1</sup>	Nil	Nil
	6	6.7x10 <sup>3</sup>	7.2x10 <sup>2</sup>	6.2x10 <sup>1</sup>	Nil	Nil
	9	8.9x10 <sup>3</sup> R	8.4x10 <sup>2</sup>	7.7x10 <sup>1</sup>	Nil	Nil
	12		3.9x10 <sup>3</sup> R	9.1x10 <sup>1</sup>	Nil	Nil
	15			1.9x10 <sup>2</sup>	Nil	Nil
	18			4.3x10 <sup>2</sup>	Nil	Nil
	21			9.4x10 <sup>2</sup> R	Nil	Nil
	24				Nil	Nil
	27				Nil	Nil
	30				Nil R	Nil
	33					Nil
	36					Nil R
Bacillus cereus	0	5.4x10 <sup>2</sup>	2.3x10 <sup>2</sup>	7.5x10 <sup>1</sup>	N.D	N.D
	3	8.7x10 <sup>2</sup>	3.5x10 <sup>2</sup>	8.9x10 <sup>1</sup>	N.D	N.D
	6	2.4x10 <sup>3</sup>	4.9x10 <sup>2</sup>	1.7x10 <sup>2</sup>	N.D	N.D
	9	6.1x10 <sup>3</sup> R	6.3x10 <sup>2</sup>	2.9x10 <sup>2</sup>	N.D	N.D
	12		2.8x10 <sup>3</sup> R	4.5x10 <sup>2</sup>	N.D	N.D
	15			5.8x10 <sup>2</sup>	N.D	N.D
	18			7.1x10 <sup>2</sup>	N.D	N.D
	21			2.6x10 <sup>3</sup> R	N.D	N.D
	24				N.D	N.D
	27				N.D	N.D
	30				N.DR	N.D
	33					N.D
	36					N.DR
Salmonella spp	0	+	+	N.D	N.D	N.D
	3	+	+	N.D	N.D	N.D
	6	+	+	N.D	N.D	N.D
	9	+ R	+	N.D	N.D	N.D
	12		+ R	N.D	N.D	N.D
	15			N.D	N.D	N.D
	18			N.D	N.D	N.D
	21			N.D R	N.D	N.D
	24				N.D	N.D
	27				N.D	N.D
	30				N.DR	N.D
	33					N.D
	36					N.DR

R= Rejected    + = Positive    N.D= not detected

**Table (9): Changes in sensory attributes of camel meat chunks as affected by combination treatment between ginger extract (30%) and gamma irradiation at cold storage (4±1°C).**

Storage period (days)	Appearance					Flavor					Texture				
	Control	Ginger extract (30%) and gamma irradiation				Control	Ginger extract (30%) and gamma irradiation				Control	Ginger extract (30%) and gamma irradiation			
		0	1.5	3	4.5		0	1.5	3	4.5		0	1.5	3	4.5
0	8.8 <sup>Ba</sup>	9.3 <sup>Aa</sup>	9.3 <sup>Aa</sup>	9.2 <sup>Aa</sup>	9.3 <sup>Aa</sup>	8.4 <sup>Aa</sup>	9.6 <sup>Aa</sup>	9.5 <sup>Aa</sup>	9.5 <sup>Aa</sup>	9.3 <sup>Ba</sup>	9.3 <sup>Ba</sup>	9.6 <sup>Aa</sup>	9.7 <sup>Aa</sup>	9.6 <sup>Aa</sup>	9.7 <sup>Aa</sup>
3	8.3 <sup>Cb</sup>	9.1 <sup>Aa</sup>	9.3 <sup>Aa</sup>	9.2 <sup>Aa</sup>	9.3 <sup>Aa</sup>	8.2 <sup>Bb</sup>	9.5 <sup>Aa</sup>	9.5 <sup>Aa</sup>	9.5 <sup>Ba</sup>	9.3 <sup>Ba</sup>	8.8 <sup>Cb</sup>	9.6 <sup>Aa</sup>	9.7 <sup>Aa</sup>	9.6 <sup>Aa</sup>	9.7 <sup>Aa</sup>
6	7.8 <sup>Dc</sup>	8.9 <sup>Aa</sup>	9.2 <sup>Aa</sup>	9.2 <sup>Aa</sup>	9.3 <sup>Aa</sup>	8.2 <sup>Bb</sup>	9.5 <sup>Aa</sup>	9.5 <sup>Aa</sup>	9.5 <sup>Ba</sup>	9.3 <sup>Ba</sup>	8.7 <sup>Db</sup>	9.5 <sup>Ca</sup>	9.6 <sup>Ca</sup>	9.5 <sup>Aa</sup>	9.78 <sup>Aa</sup>
9	3.2 <sup>DdR</sup>	8.5 <sup>Aa</sup>	9.2 <sup>Aa</sup>	9.2 <sup>Aa</sup>	9.3 <sup>Aa</sup>	3.3 <sup>DcR</sup>	9.4 <sup>Aa</sup>	9.5 <sup>Aa</sup>	9.5 <sup>Ca</sup>	9.3 <sup>Ba</sup>	3.4 <sup>CcR</sup>	9.4 <sup>Bb</sup>	9.6 <sup>Aa</sup>	9.5 <sup>Aa</sup>	9.6 <sup>Aa</sup>
12		4.7 <sup>CcR</sup>	9.1 <sup>Aa</sup>	9.2 <sup>Aa</sup>	9.3 <sup>Aa</sup>		8.8 <sup>AaR</sup>	9.2 <sup>Ba</sup>	9.2 <sup>Ba</sup>	9.2 <sup>Ba</sup>		4.2 <sup>DdR</sup>	9.5 <sup>Ac</sup>	9.5 <sup>Aa</sup>	9.6 <sup>Aa</sup>
15			9.1 <sup>Ba</sup>	9.1 <sup>Ba</sup>	9.3 <sup>Aa</sup>			9.2 <sup>Aa</sup>	9.2 <sup>Aa</sup>	9.2 <sup>Ba</sup>			9.4 <sup>Bc</sup>	9.2 <sup>Cb</sup>	9.6 <sup>Aa</sup>
18			8.9 <sup>Bc</sup>	9.1 <sup>Aa</sup>	9.2 <sup>Aa</sup>			9.2 <sup>Ab</sup>	9.2 <sup>Aa</sup>	9.1 <sup>Bb</sup>			8.6 <sup>Cd</sup>	9.2 <sup>Bb</sup>	9.4 <sup>Ab</sup>
21			4.3 <sup>CdR</sup>	9.1 <sup>Aa</sup>	9.2 <sup>Aa</sup>			8.7 <sup>CbR</sup>	8.9 <sup>Bb</sup>	9.1 <sup>Bb</sup>			3.1 <sup>CcR</sup>	9.1 <sup>Bc</sup>	9.4 <sup>Ab</sup>
24				8.9 <sup>Bb</sup>	9.2 <sup>Aa</sup>				8.9 <sup>Bb</sup>	9.1 <sup>Bb</sup>				9.1 <sup>Bc</sup>	9.2 <sup>Ac</sup>
27				8.9 <sup>Bb</sup>	9.2 <sup>Aa</sup>				8.9 <sup>Ab</sup>	8.8 <sup>Cc</sup>				8.5 <sup>BeR</sup>	9.2 <sup>Ac</sup>
30				3.8 <sup>BdR</sup>	9.2 <sup>Aa</sup>				8.6 <sup>AcR</sup>	8.7 <sup>Cc</sup>					9.1 <sup>Ac</sup>
33					8.9 <sup>Ab</sup>					8.7 <sup>Cc</sup>					9.1 <sup>Ac</sup>
36					4.2 <sup>dR</sup>					8.6 <sup>dR</sup>					8.8 <sup>dR</sup>

Capital and small letters were used for comparing between means in the columns and rows, respectively.

Means with the same letters are not significantly different ( $p>0.05$ ).

R = Rejected

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