

## IMPROVING THE LIPID STABILITY AND SENSORY CHARACTERISTICS OF IRRADIATED MINCED BEEF BY USING NATURAL HERBAL EXTRACTS

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### SUMMARY

The objective of the present study was designed to use natural herbal extracts that help meat industry to minimize lipid oxidation and improve sensory characteristics of irradiated minced meat. Beef *Longissimus dorsi* was minced and packed in polyethylene bags (50 g each) then received one of the following treatments: (1) non irradiated control, (2) irradiated control (2 or 4.5 kGy), (3) herbal extracts of marjoram, rosemary or sage (0.04 % of non irradiated meat), (4) irradiated at 2 or 4.5 kGy plus herbal extracts at 0.04 %. Aerobically packaged samples were stored at 5°C. Samples were withdrawn periodically to be analyzed for thiobarbituric acid reactive substances (TBARS), sensory characteristics and psychrotrophic bacterial counts. Addition of marjoram and rosemary extracts resulted in significant reduction of TBARS ( $P \leq 0.05$ ) in both irradiated and non-irradiated samples. However, sage

caused significant reduction ( $P \leq 0.05$ ) of TBARS in samples treated with 2 kGy only. Marjoram and sage significantly reduced ( $P \leq 0.05$ ) flavor scores in both samples treated with 2 or 4.5 kGy, meanwhile, rosemary induced a significant reduction in flavor scores for samples treated with 4.5 kGy only. Addition of herbal extracts before irradiation resulted in significant ( $P \leq 0.05$ ) increase in the acceptability scores of samples after irradiation and during storage period. Addition of herbal extracts to minced beef before irradiation resulted in non-significant reduction ( $P \geq 0.05$ ) in Psychrotrophic counts. The combination effect of herbal extracts plus irradiation extended the shelf life of samples treated with 2 kGy and 4.5 kGy for 2 weeks and one week, respectively more than samples irradiated alone. In conclusion the addition of herbal extracts can minimize lipid oxidation, improve color and decrease the off-odor production.

## INTRODUCTION

Irradiation is one of the newly emerging technologies that can effectively ensure the microbiological safety and extend the shelf life of meat. Food and Drug Administration (FDA) has approved the use of ionizing radiation at levels of 4.5 kGy for fresh meat and 7 kGy for frozen meat (Olson, 1998). However, a major problem associated with irradiation of raw meat is the development of off-odors due to free radical induced lipid oxidation and radiolytic breakdown of proteins and lipids (Patterson and Stevenson, 1995; Murano et al., 1998; Kim et al., 2000). This lipid oxidation and off-flavor reduce the meat quality and consumer acceptance of irradiated meat. Therefore, the interest of industry and researchers is directed towards the use of additives which can minimize lipid oxidation and off-odors with consequent increase of the acceptability of irradiated meat.

Antioxidants are added to fresh and further processed meats to prevent oxidative rancidity, retard development of off-flavors, and improve color stability (Xiong et al., 1993). The process of fatty acid degradation induced by irradiation follow a mechanism similar to that of lipid oxidation especially when oxygen is available. Therefore, antioxidants can effectively prevent the quality changes and off-flavor in irradiated meat through the same mechanism by which they prevent lipid oxidation in untreated meat (Du and Ahn, 2002).

Chen et al. (1999) found that sesamol, quercetin and butylated hydroxytoluene were effective in preventing off-odor in irradiated raw and cooked pork during 7 days of storage, whereas rosemary oleoresin and rutin were effective in irradiated raw pork during 3 days of storage. Moreover, Shahidi and Wanasundara (1992), Kanatt et al. (1998), Nam and Ahn (2003) reported that the addition of antioxidants minimized lipid oxidation in irradiated meat. Meanwhile the development of effective natural antioxidant has been investigated to retard lipid oxidation in meat products (Barbut et al., 1985; Buckley et al., 1995).

Although, there is an extensive work on the effect of irradiation on the quality of meat, the information on the use of herbal extracts to minimize quality changes in meats further processed by irradiation still lacking. Therefore, the objective of the present study was to determine the effect of selected herbal extracts on the sensory characteristics, lipid oxidation and bacterial count of beef irradiated and stored at chilling temperatures (5°C). Also to encourage the meat industry to use irradiation processing of fresh meat to produce high quality meat.

## MATERIALS AND METHODS

### Samples preparation

Beef loins (*Longissimus dorsi*) were purchased from local butchery as soon as possible after

slaughter, then rapidly transported to the laboratory in ice box in order to minimize the changes. In the laboratory, the external fascia and visible fat were removed, then meat blocks were ground through a 3mm plate. Marjoram, rosemary and sage extracts were obtained from Research Institute of Horticulture, Aromatic and Medicinal Plants Division, belonging to Ministry of Agriculture and Land Reclamation (Dokki, Giza). Four treatments were prepared: (1) non irradiated control, (2) irradiated control (2 or 4.5 kGy), (3) herbal extracts of marjoram, rosemary or sage (0.04 % of non irradiated meat), (4) irradiated at 2 or 4.5 kGy plus herbal extracts at 0.04 %. Each additive was added to ground beef then mixed for 3 min using Lab blender to ensure uniform distribution of added extract. Ground beef from each treatment was individually packaged in polyethylene bags (50 g of meat in each bag). The heat sealed aerobically packaged samples were stored at 5°C overnight and irradiated.

#### **Irradiation process and storage:**

For irradiation, beef samples were irradiated at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt, where the radiation treatments were applied using the Egypt's Mega Gamma-1 type "J-6500 Co<sup>60</sup>". The dose rate being 8 kGy per hour. The samples in chilled state, received radiation dose in ice box to keep the temperature around 5°C during irradiation. The applied irradiation doses were 2 or 4.5 kGy, as monitored by radiochromic film

(McLaughlin et al., 1985). After irradiation, the irradiated and nonirradiated meat samples were immediately stored at 5°C. Samples were withdrawn periodically for analysis.

A schematic diagram of all steps of preparation and treatments of minced beef are summarized in (Fig. 1).

#### **Chemical analysis**

Both irradiated and non-irradiated beef samples were analyzed for thiobarbituric acid-reactive substances (TBARS) according to the method of Du and Ahn, (2002) as follow: Five grams of meat were weight and homogenized with 15 mL of deionized distilled water. One milliliter of the meat homogenate was transferred to a test tube and 50 µL of butylated hydroxytoluene (7.2 %) and 2 mL of thiobarbituric acid (TBA)-trichloroacetic acid (TCA) (15 mM TBA-15% TCA) were added. The mixture was vortexed and then incubated in a boiling water bath for 15 min to develop color. Then sample was cooled in cold water for 10 min, vortexed again, and centrifuged for 15 min at 2500 x g. The absorbance of the resulting supernatant solution was determined at 531 nm against a blank containing 1 mL of deionized water and 2 mL of TBA-TCA solution. The amounts of TBARS were expressed as milligrams of malonaldehyde per kilogram of meat.

#### **Sensory evaluation:**

The irradiation flavor, color and overall accepta-

bility of minced beef (treated and untreated) were evaluated by at least 3 persons every time of analysis using 7 hedonic scale described by (Nam et al. 2002). Panelists were trained to be familiarized with the irradiation off-flavor. Panelists were instructed to smell the samples in random order and record the intensity of irradiation off-odor based on the hedonic scale with 1 (not detectable) and 7 (extremely intense). Panelists were asked to assign a numerical value between 1 (dislike extremely) and 7 (like extremely) for color of treated and untreated minced beef. They also asked to assign the same values depends on the overall acceptability of odor and color of the minced beef.

### Bacteriological examination

Ten grams of meat from each bag were separately homogenized in sterile stomacher bag with 90 ml of Ringer's solution (Merck) for one min using stomacher (Lab-blender 400, Seward, UAC house Friars Road, London SE 19 UG-model No. 6021) to provide dilution of  $10^{-1}$ . From the original homogenate ten fold serial dilutions were prepared (APHA, 1992). Psychrotrophic bacterial count was estimated by inoculation onto standard plate count agar and incubation at  $7^{\circ}\text{C}$  for 7 days (Cousin et al., 1992).

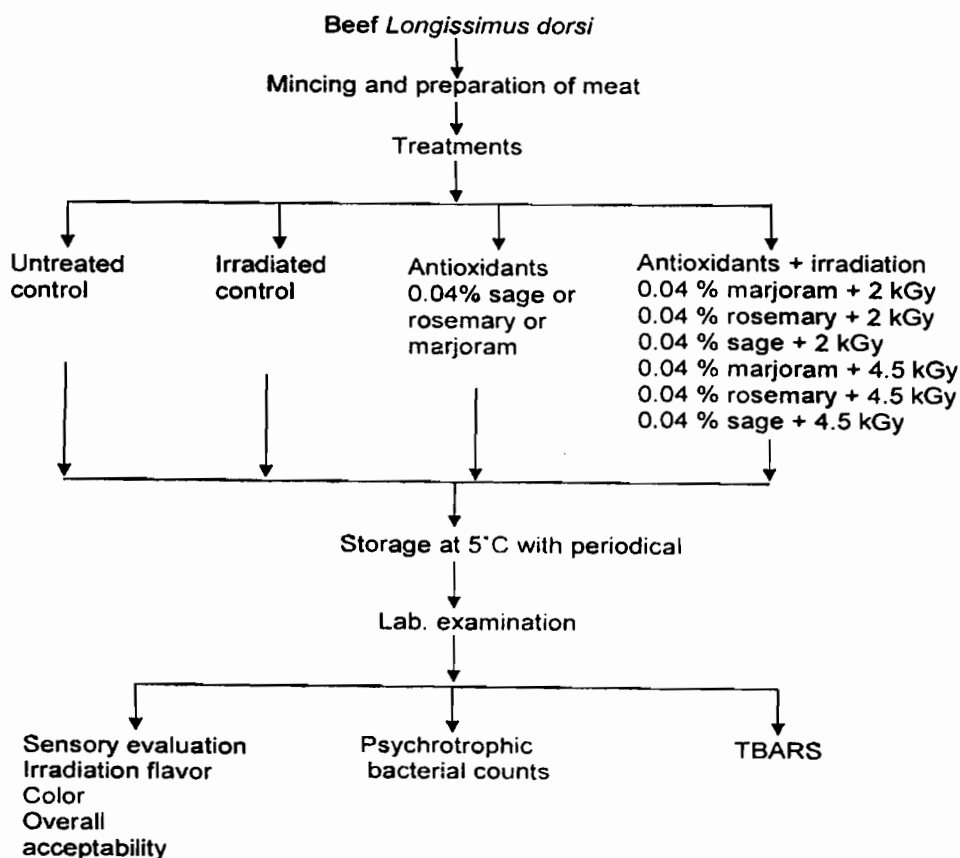


Fig.1: Experimental design

### **Statistical analysis:**

Statistical data analysis was carried out using Minitab Statistical Program (Minitab Inc., State College, Pa.). One-way analysis of variance was performed to compare the effect of the treatments. Multiple comparisons of means were done using Tukey's at family error rate 0.05.

## **RESULTS AND DISCUSSION**

### **Lipid oxidation**

The data illustrated in Fig 2A, B and C showed the effect of irradiation processing up to 4.5 kGy, herbal extracts and herbal extracts plus irradiation up to 4.5 kGy on TBARS of minced beef after treatments and during refrigerated storage. Irradiation alone resulted in dose dependent increase of TBARS post-irradiation and during storage at 5°C. This observation is in agreement with Lefebvre et al. (1994), Hampson et al. (1996), Luchsinger et al. (1996), Lee et al. (1999), Ahn et al. (2000), Formanek et al. (2003) and Kanatt et al. (2005). The increase of TBARS owing to irradiation may be explained by the autoxidation of fat

which is accelerated by presence of oxygen (Nawar, 1985).

Addition of herbal extracts caused significant reduction ( $P \leq 0.05$ ) of TBARS in both irradiated and non-irradiated minced beef. Similarly, Formanek et al. (2001) observed that antioxidant supplementation increased the oxidative stability of non-irradiated minced beef. Moreover, Nam and Ahn (2003) reported that antioxidant combinations decreased TBARS in both non-irradiated and irradiated pork patties under aerobic conditions. Chen et al. (1999) also reported that phenolic antioxidants were effective in reducing lipid oxidation in aerobically packaged irradiated pork patties. TBARS of samples treated with herbal extracts plus irradiation at 2 kGy were about to be the same as that of non-irradiated one (control). As a function of stored period, the combination effect between herbal extract and radiation treatment decreased the level of TBARS more than irradiation treatment do alone; these results are in good agreement with Nam et al. (2002) and Kanatt et al. (2005).

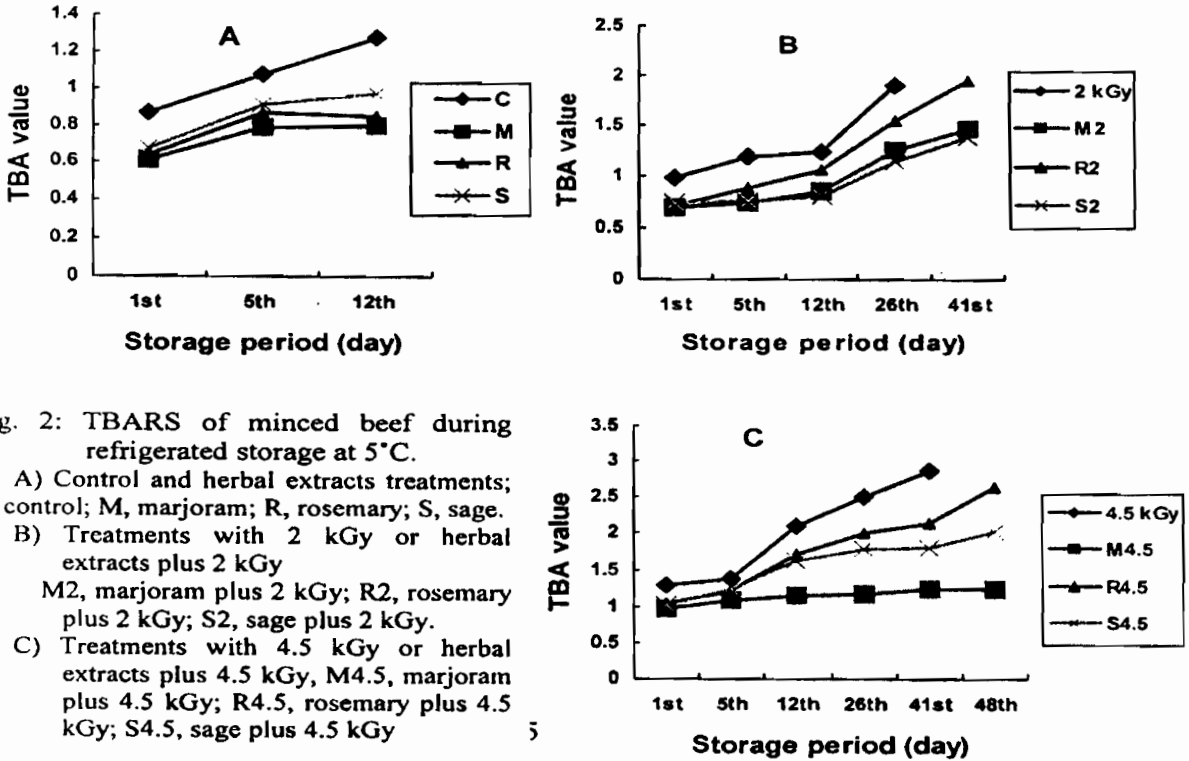


Fig. 2: TBARS of minced beef during refrigerated storage at 5°C.  
 A) Control and herbal extracts treatments; C, control; M, marjoram; R, rosemary; S, sage.  
 B) Treatments with 2 kGy or herbal extracts plus 2 kGy M2, marjoram plus 2 kGy; R2, rosemary plus 2 kGy; S2, sage plus 2 kGy.  
 C) Treatments with 4.5 kGy or herbal extracts plus 4.5 kGy, M4.5, marjoram plus 4.5 kGy; R4.5, rosemary plus 4.5 kGy; S4.5, sage plus 4.5 kGy

**Sensory evaluation**

It is well established that irradiation of raw meat develop a characteristic repulsive odor (irradiation flavor) due to oxidation of polyunsaturated fatty acids, destruction of antioxidants in muscle by free radicals and production of volatile compounds resulted from irradiation treatment (Thayer et al., 1993; Lakritz et al., 1995; Giroux and Lacroix, 1998). Results in Fig 3A and B revealed that irradiation flavor scores increased as a function of irradiation dose. The addition of herbal extracts resulted in lowering of irradiation flavor scores post irradiation and during refrigerated storage. Marjoram and sage resulted in significant reduction ( $P \leq 0.05$ ) in flavor scores in both

samples treated with 2 or 4.5 kGy, however, rosemary resulted in significant reduction of scores with samples treated at dose level of 4.5 kGy only. The panelists could not detect any irradiation flavor in samples treated with marjoram plus 2 or 4.5 kGy at the 8<sup>th</sup> day of storage period, however, they could not detect the flavor in samples treated with sage plus 2 kGy at the 5<sup>th</sup> day of storage period, same was samples treated with sage plus 4.5 kGy at the 12<sup>nd</sup> day of storage period. The Figure 3A and B also revealed a lowering of irradiation flavor scores during storage which may be attributed to volatilization of sulfur-containing compounds as mercaptomethane and dimethyldisulfide that are responsible for most of

irradiation off-odor (Ahn et al., 2001). The reduction of irradiation flavor scores that achieved by addition of studied natural herbal extracts may be attributed to the antioxidant activity of these extracts. This antioxidant activity act as scavengers for free radicals so can interrupt free radical chain reactions and chelate catalytic metals therefore, retard oxidative deterioration and minimize off-flavor production in meat (Lee et al., 1999; Ahn et al., 1997). The antioxidant activity of rose-

mary and sage was reported by many researchers (Barbut et al., 1985; Buckley et al., 1995). Chen et al. (1999) reported that sesamol reduced off-odor in irradiated pork patties. Du and Ahn (2002) found that antioxidants had a little effect on the off-odor of irradiated turkey sausages, they attributed that to the processing and cooking method which produced more radiolytic changes that interrupt the function of antioxidants as scavengers for free radicals.

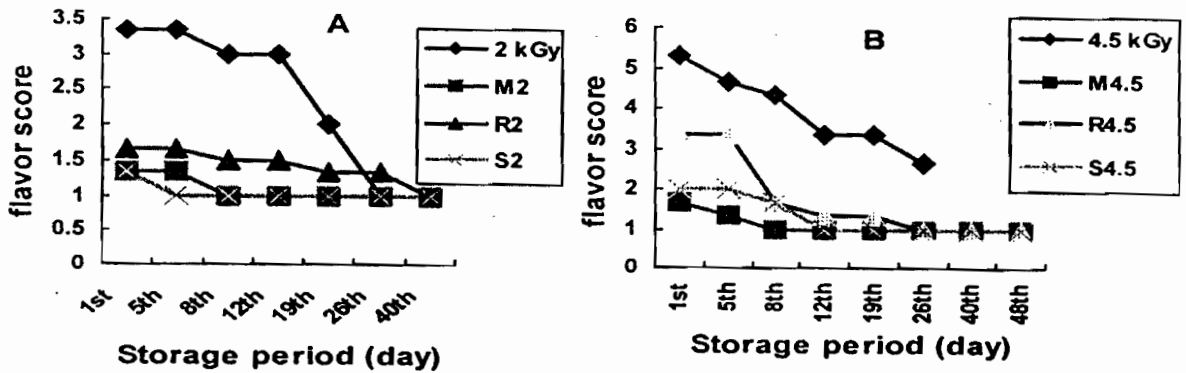


Fig. 3: Irradiation flavor scores of minced beef during refrigerated storage.

A) Treatments with 2 kGy or herbal extracts plus 2 kGy

M2, marjoram plus 2 kGy; R2, rosemary plus 2 kGy; S2, sage plus 2 kGy.

B) Treatments with 4.5 kGy or herbal extracts plus 4.5 kGy

M4.5, marjoram plus 4.5 kGy; R4.5, rosemary plus 4.5 kGy; S4.5, sage plus 4.5 kGy.

The data illustrated in Fig 4A, B and C clearly represented that irradiation produce dose dependant reduction in color scores after irradiation and during storage. The changes in processed meat color scores may be explained upon the fact that the free binding sites of myoglobin react with the free radicals generated upon irradiation to form the brown colored metmyoglobin (Giroux et al., 2001). Treatments of minced beef with natural herbal extracts alone did not

result in significant change in color scores whereas, the addition of these extracts resulted in an increase in the color scores of irradiated minced beef after irradiation and during storage specially with samples treated with 4.5 kGy. This may be explained that the applied natural herbal extracts have an effective antioxidant activity that protect the free binding sites of myoglobin to be reacted with the radiolytic products formed during irradiation treatment.

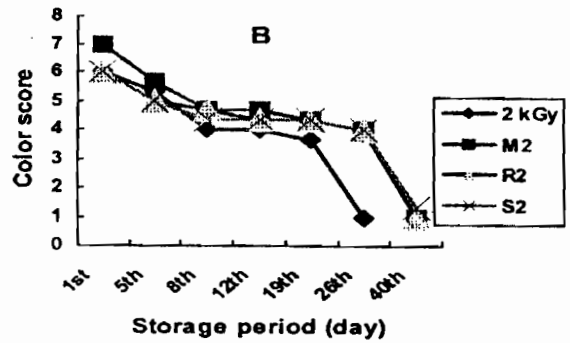
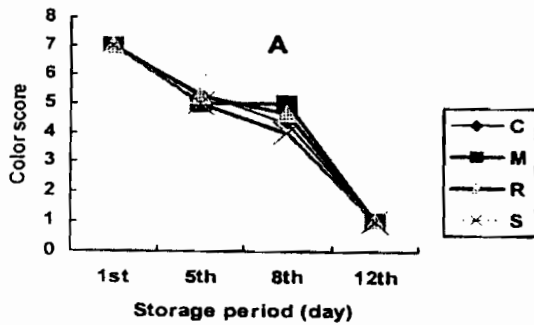
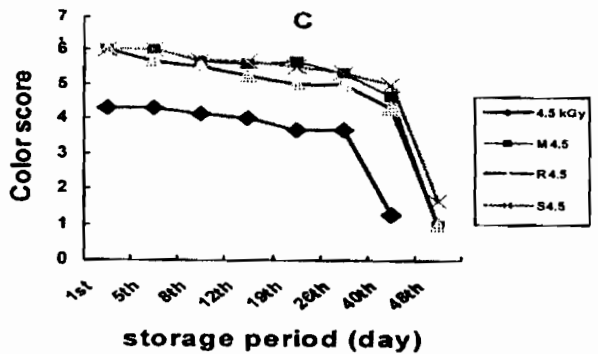


Fig. 4: Color scores of minced beef during refrigerated storage at 5°C.

- A) Control and herbal extracts treatments; C, control; M, marjoram; R, rosemary; S, sage.
- B) Treatments with 2 kGy or herbal extracts plus 2 kGy  
M2, marjoram plus 2 kGy; R2, rosemary plus 2 kGy; S2, sage plus 2 kGy.
- C) Treatments with 4.5 kGy or herbal extracts then 4.5 kGy, M4.5, marjoram plus 4.5 kGy; R4.5, rosemary plus 4.5 kGy; S4.5, sage plus 4.5 kGy



Regarding the overall acceptability of minced beef supplemented with natural herbal extracts alone there was no significant change ( $P \geq 0.05$ ) in the acceptability scores of beef (Fig 5A). Treatments of samples with radiation alone resulted in significant reduction ( $P \leq 0.05$ ) of acceptability scores of meat. However, the addition of herbal extracts before irradiation resulted in significant ( $P \leq 0.05$ ) increase in the acceptability scores, of irradiated samples post irradiation and

during storage (Fig 5B and C). The marjoram was superior to rosemary and sage in increasing the acceptability scores of irradiated minced beef after treatments and during storage. Depending on the above sensory analyses, the improvement in acceptability of irradiated beef by addition of natural herbal extracts may be attributed to oxidative stability, reduction of irradiation off-odor and increasing of color scores of irradiated minced beef.



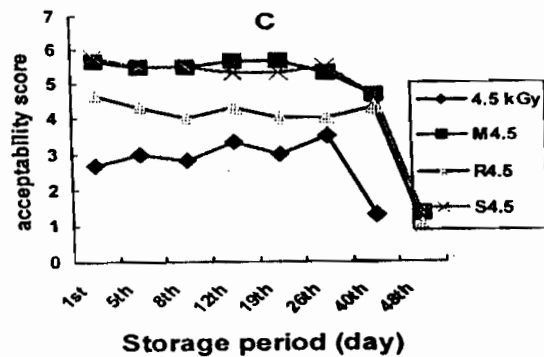
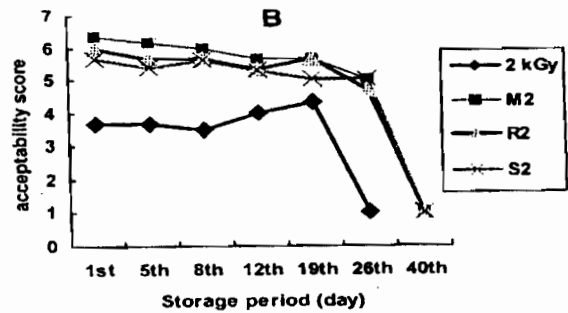
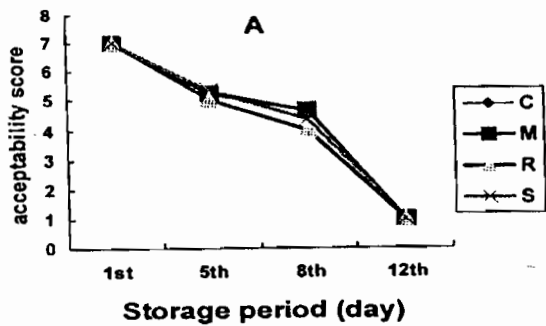


Fig. 5: Overall acceptability scores of minced beef during refrigerated storage

- A) Control and herbal extracts treatments; C, control; M, marjoram; R, rosemary; S, sage.  
 B) Treatments with 2 kGy or herbal extracts plus 2 kGy  
 M2, marjoram plus 2 kGy; R2, rosemary plus 2 kGy; S2, sage plus 2 kGy.  
 C) Treatments with 4.5 kGy or herbal extracts then 4.5 kGy  
 M4.5, marjoram plus 4.5 kGy; R4.5, rosemary plus 4.5 kGy; S4.5, sage plus 4.5 kGy

### Bacteriological quality

The log CFU g<sup>-1</sup> of Psychrotrophic bacterial counts of untreated and treated samples are illustrated in Fig. 6A, B and C. From the figure, it is clear that treatment with natural herbal extracts alone did not affect the psychrotrophic counts of minced beef and the counts reached 10<sup>8</sup> CFU g<sup>-1</sup> in control and samples treated with extracts at the 12<sup>th</sup> day of storage with the appearance of signs of spoilage in the form of change in color, odor and texture.

Treatment of minced beef through gamma radiations caused dose dependent reduction in psychrotrophic counts. The 2 kGy irradiation caused 3

log CFU g<sup>-1</sup> reductions in bacterial count while in samples treated with 4.5 kGy, the psychrotrophic bacterial counts were under the detectable level immediately after irradiation. Irradiation also resulted in extension of shelf life of treated minced beef. The count reached 10<sup>8</sup> CFU g<sup>-1</sup> in samples treated with 2 kGy at the 26<sup>th</sup> day of storage while the samples treated with 4.5 kGy reached 10<sup>7</sup> CFUg<sup>-1</sup> at the 40<sup>th</sup> day of storage. The decrease in total bacterial populations due to irradiation was in agreement with other studies (Naik et al., 1994; Luchsinger et al., 1996; Kanatt et al., 1997 and Kanatt et al., 2005). It has been reported that a dose of 1 kGy significantly decreased the total aerobic plate counts in compari-

son to untreated samples (Lacroix and Ouattara, 2000). Murano et al. (1998) and Lee et al. (1999) also found that the shelf life of ground beef patties was extended to 55 days when they were stored at 4°C after irradiation.

Addition of herbal extracts to minced beef before irradiation resulted in non-significant reduction ( $P \geq 0.05$ ) in Psychrotrophic counts. Addition of these extracts at the level used (0.04%) proved to help in extending the shelf life of samples treated with 2 kGy and 4.5 kGy for 2 weeks and one week respectively more than samples irradiated alone. Several previous reports on the combination of gamma irradiation and other treatments suggested that microorganisms which can survive

radiation treatment will probably be more sensitive to environmental conditions (temperature, pH, nutrients, inhibitors, etc.) than untreated cells (Farkas, 1990; Lacroix and Ouattara, 2000). Lee et al. (1999) and Giroux et al. (2000) found that incorporation of ascorbyl palmitate (200 ppm) in ground beef prior to irradiation at 1.5 kGy resulted in an additional 3 log units reduction in total aerobic and lactic acid bacteria counts.

In conclusion, although, the radiation processing can extend the shelf life of raw beef, it induce changes in its quality in the form of changes in color, production of off-odor and lipid oxidation which subsequently decrease the acceptability of irradiated chilled aerobically packaged minced

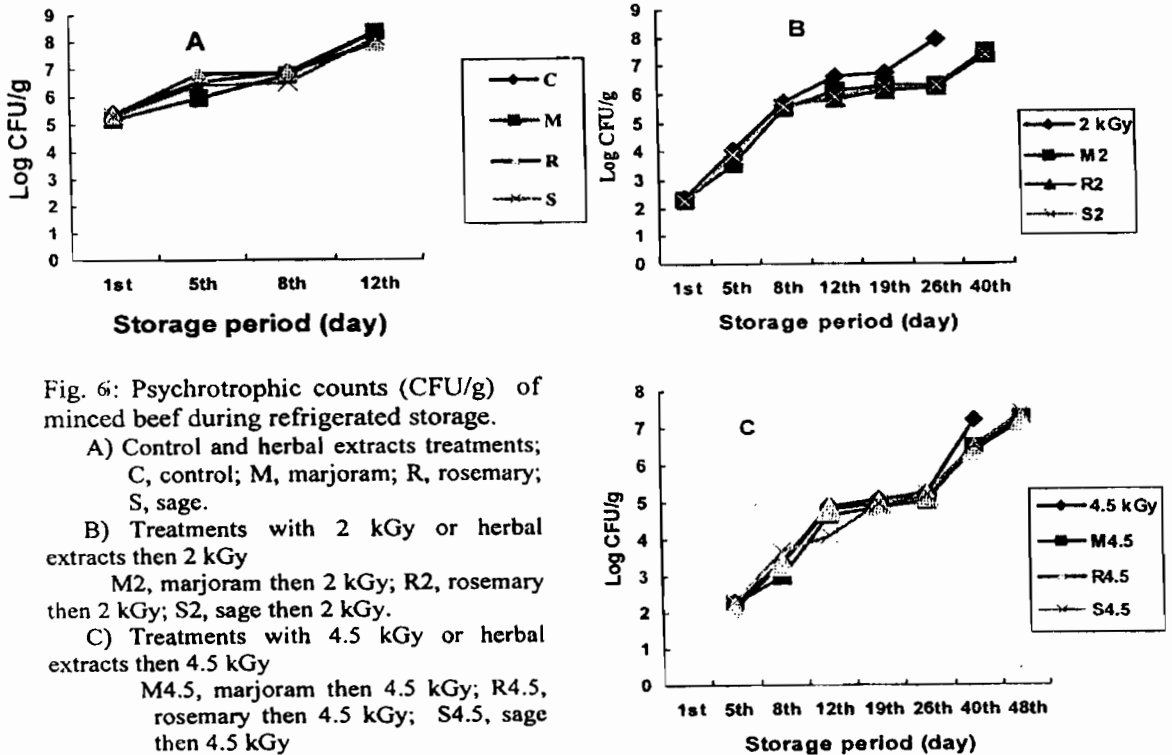


Fig. 6: Psychrotrophic counts (CFU/g) of minced beef during refrigerated storage.

- A) Control and herbal extracts treatments; C, control; M, marjoram; R, rosemary; S, sage.  
 B) Treatments with 2 kGy or herbal extracts then 2 kGy  
 M2, marjoram then 2 kGy; R2, rosemary then 2 kGy; S2, sage then 2 kGy.  
 C) Treatments with 4.5 kGy or herbal extracts then 4.5 kGy  
 M4.5, marjoram then 4.5 kGy; R4.5, rosemary then 4.5 kGy; S4.5, sage then 4.5 kGy

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## تحسين ثبات الليبيدات والخواص الحسية للحم البقرى المفروم والمعالج إشعاعياً باستخدام المستخلصات الطبيعية للأعشاب

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تهدف هذه الدراسة لتقليل الأكسدة الدهنية وتحسين الخواص الحسية للحم البقرى المعامل بالإشعاع حيث تمت معاملة اللحم البقرى المفروم بعد وضعه فى أكياس من البولى إيثيلين (٥٠ جم لكل كيس) بإحدى المعاملات الآتية: ١- بدون تشعيع ٢- تشعيع عند ٢ أو ٤.٥ كيلو جراى ٣- بدون تشعيع وإضافة ٠.٠٤٪ من إحدى مستخلصات البردقوش، حصا ألبان أو المريمية ٤- تشعيع عند ٢ أو ٤.٥ كيلو جراى مع إضافة إحدى المستخلصات العشبية السابقة. تم أخذ العينات على فترات وتحليلها من حيث قيمة حامض الثيوباربتوريك، الخواص الحسية وعد الميكروبات المحبة للبرودة. أدت إضافة مستخلص كل من البردقوش وحصا البان إلى حدوث نقص ملحوظ لقيمة حامض الثيوباربتوريك فى كل من العينات المشعة والغيرمشعة. فى حين أن مستخلص المريمية أدى إلى حدوث نقص ملحوظ فى قيمة حامض الثيوباربتوريك وذلك بالنسبة للعينات التى تمت معاملتها بـ ٢ كيلو جراى. إضافة إلى ذلك فقد أدت إضافة مستخلص كل من المريمية والبردقوش إلى النقص الملحوظ فى درجة النكهة فى كل من العينات المعاملة ، إما بـ ٢ كيلو جراى أو ٤.٥ جراى فقط ، فى حين أن مستخلص حصا البان أدى إلى نقص ملحوظ فى درجة النكهة فى العينات المعاملة بـ ٤.٥ ك جراى فقط، كما أن إضافة هذه المستخلصات العشبية قبل عملية التشعيع أدت إلى زيادة ملحوظة فى درجة القابلية بعد التشعيع وخلال فترة التخزين. فضلاً عن أن إضافة هذه المستخلصات العشبية للحم البقرى قبل التشعيع أدى إلى خفض بسيط فى العد البكتيرى. كما أن التأثير الأحدى للمستخلصات العشبية مع التشعيع أدى إلى زيادة فترة الصلاحية للعينات المعاملة بـ ٢ كيلو جراى و ٤.٥ كيلو جراى لمدة أسبوع وإسبوعين على التوالى وذلك أكثر من العينات المعاملة بالتشعيع فقط . وبذلك فإن إضافة المستخلصات العشبية من الممكن أن تقلل من الأكسدة الدهنية، تحسن اللون وتقلل من الروائح الكريهة.