

INVESTIGACIÓN

Antioxidant and antimicrobial effects of some natural plant extracts added to lamb patties during storage

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RESUMEN

Efecto antioxidante y antimicrobiano de algunos extractos de plantas naturales añadidos a pastel de cordero durante el almacenamiento.

Las plantas naturales están consideradas como un importante producto donde buscar y encontrar nuevas fuentes de antioxidantes naturales y/o agentes antimicrobianos. La concentración óptima de algunos extractos de plantas naturales (jojoba, jatropha, ginseng y jengibre) fueron determinados y añadidos a pasteles de cordero. Algunas características químicas y microbiológicas de los pasteles preparados y almacenados durante 9 días a 4°C fueron evaluados. Tanto la adición de estos extractos como el tiempo de almacenamiento tuvieron un efecto significativo en los pasteles en el periodo de almacenamiento. La efectividad de los extractos naturales ensayados puede ser enumerada en el siguiente orden decreciente de valores de substancias reactivas con el ácido tiobarbitúrico (TBARS): ginseng > jatropha > jojoba > jengibre. El recuento de microorganismos decreció significativamente con la adición de los extractos durante el periodo de almacenamiento. Comparado con los pasteles control, la adición de estos extractos naturales fue efectiva como agente antioxidante y antimicrobiano en la mejora de las propiedades de los pasteles de cordero.

PALABRAS-CLAVE: Aminas biogénicas – Antimicrobiano – Antioxidante – Extracto de plantas naturales – Oxidación lipídica – Pastel de cordero – Recuento microbiano.

SUMMARY

Antioxidant and antimicrobial effect of some natural plant extracts added to lamb patties during storage.

Natural plants are considered an important target to investigate in order to provide a new source of natural antioxidants and/or antimicrobial agents. The optimum concentrations of some natural plant (jojoba, jatropha, ginseng and ginger) extracts were determined and added to lamb patties. Some chemical and microbial characteristics of the prepared patties during storage for 9 days at 4°C were evaluated. Both the addition of these extracts and storage time had a significant effect on the patties throughout the storage period. The effectiveness of the tested natural extracts can be listed in the following order of decreasing Thiobarbituric acid reactive substance (TBARS) values: ginseng > jatropha > jojoba > ginger. Aerobic plate count, mould and yeast counts decreased significantly with addition

of the extracts during the storage period. Also, the addition of the extracts was significantly effective in reducing histamine, tyramine and putrescine formation during the storage period. Compared to control patties, the addition of these natural extracts was effective as antioxidant and antimicrobial agents for improving the properties of lamb patties.

KEY-WORDS: Antimicrobial – Antioxidant – Biogenic amines – Lamb patties – Lipid oxidation – Microbial counts – Natural plant extracts.

1. INTRODUCTION

Lipid oxidation and the growth of undesirable microorganisms in food products results in the development of spoilage, off flavor, rancidity, and deterioration, rendering such products unacceptable for human consumption (Bozin *et al.*, 2007) and yielding many compounds that contribute to the pathogenesis of cancer, atherosclerosis, heart and allergic diseases (Mielnik *et al.*, 2008; Tang *et al.*, 2001).

The presence of biogenic amines in food constitutes a potential public health concern due to their physiological and toxicological effects. It is important to monitor biogenic amine levels in fresh and processed food not only due to their toxicity but also because they can be a useful index of spoilage (Önal, 2007).

Lipid oxidation and microbial growth in meat products may be controlled or at least minimized by using either synthetic or natural food additives commonly used in the meat industry (Gray *et al.*, 1996; Lee *et al.*, 1997; Mielnik *et al.*, 2003; Sallam *et al.*, 2004; Estevez and Cava, 2006).

The natural antioxidants found in plants have gained considerable interest for their role in preventing the auto-oxidation of fats, oils and fat containing food products (Reddy *et al.*, 2005). The antioxidant properties of herbs, spices, plant and other food extracts are apparently related to their phenolic content, suggesting that antioxidant action is similar to that of synthetic phenolic antioxidants (Lai *et al.*, 1991).

Since the worldwide trend towards the use of natural additives in food (Yanishlieva *et al.*, 2006); natural plants are considered an important target to investigate in order to provide a new source of natural antioxidants and/or antimicrobial agents from a safety view point. Consequently, there is a practical need for the screening and selection of natural antioxidants as effective alternatives in the prevention of food deterioration (Kikuzaki and Nakatani, 1993). Several plants with very high nutritive values exist and yet remain unexploited for human and animal benefits (Oladele and Oshodi, 2007). Therefore, the search for, and development of other antioxidants and antimicrobials of natural origin are highly desirable.

Jatropha curcas is a nut belonging to the *Euphobiaceae* family. Recently, the tree of this plant has been successfully cultivated in Upper Egypt (Hawash *et al.*, 2009) and all parts of it have specific uses as determined by Gubitz *et al.*, (1999) and Makkar *et al.*, (1997). El Diwani *et al.*, (2009) reported that the residue of the methanolic extract of Egyptian *Jatropha curcas* contains bioactive substances such phenolic compounds, which were successful as natural antioxidants against oxidative deterioration.

The flavonoid profile of the fruits of the Jojoba plant, *Simmondsia chinensis* may place this family among other families which are rich in flavonol methyl ethers and flavonoid content which make the pericarp a valuable source for antioxidant and hepato-protective compounds (El-Halwany, 2002). This plant extract has been reported to be useful as a dietary supplement for use in a weight control regiment in humans, a component of functional food, a food additive, a medical food, or as a therapeutic agent (Teague *et al.*, 2005).

Ginseng is an herbaceous perennial belonging to the *Aralia* family. It is used early for medicinal purposes and used widely in herbal, health food and cosmetic applications (Rangahau, 2001). Various formulations prepared from the *Panax* ginseng root have been marketed as dietary supplements, especially in China where it is frequently used as a food additive and as raw materials of healthy food rather than therapeutic agents (Gillis, 1997; Shen *et al.*, 2003). Bioactive compounds from medicinal plants including ginseng are known to protect against oxidative stress from reactive oxygen species and prevent lipid per-oxidation (Sievenpiper *et al.*, 2003; Fuzzati, 2004).

The rhizome of the popular ginger species, *Zinger officinale*, is widely used as a spice and food seasoning due to its sweet aroma and pungent taste. It is well known to have antioxidant activity (Jitoe *et al.*, 1992; Zia-ur-Rehman *et al.*, 2003,) and effective antimicrobial agents. A ginger rhizome extract exhibited the highest antioxidant activity (Mansour and Khalil, 2000) due to the effect of its total phenols (Stoilova *et al.*, 2007).

The interest in the antioxidant activity of plant extracts has become greater and very important (Joyeux *et al.*, 1995; Azaizeh *et al.*, 2005; Alma

et al., 2003) due to the fact that free radicals e.g. reactive oxygen species (ROS) can be responsible for various diseases, e.g. heart diseases, stroke, arteriosclerosis and cancer, as well as being involved in the aging process (Willcox *et al.*, 2004). The effects of plant extracts or essential oils classified as greatly recognized as safe (GRAS) following their addition, have been studied extensively and reported in a variety of meat types, including pork (Nissen *et al.*, 2004); beef (Solomakos *et al.*, 2008); lamb (Camo *et al.*, 2008).

Lamb meat contains higher levels of ω -3 polyunsaturated fatty acids (PUFAs) compared to beef or pork, which is beneficial to human health (Wood *et al.*, 1999); however, PUFAs increase the susceptibility of meat to oxidative processes such as lipid oxidation ultimately leading to off odors and warmed over flavor (Jeremiah, 2001).

The objectives of the present study were: i) To establish the optimum concentrations of some natural plant extracts: jojoba (Jo), *Jatropha* (Ja), ginseng (Gg) and ginger (Gr) as sources of natural antioxidants and/or antimicrobial agents to be added to lamb patties in order to diminish oxidative and microbiological deterioration. ii) To evaluate the effects of the natural extracts at the optimum concentrations on the evolution of quality parameters (thiobarbituric acid reactive substances (TBARS), biogenic amines (BA), microbiological count) in the prepared lamb patties stored at 4°C for 9 days.

2. MATERIALS AND METHODS

2.1. Materials

Four natural plants (Jojoba *Simmondsia chinensis*, *Jatropha curcas*, *Panax* ginseng and ginger, *Zinger officinale*) were used as sources of antioxidants and antimicrobial agents. Jojoba (pericarp) was purchased from the Egyptian Natural Oils Company, Cairo, Egypt. *Jatropha curcas* (leaves and roots) were obtained from the Ministry of Agriculture and Reclamation Land., Egypt. Jojoba and *Jatropha* were air-dried, powdered and kept in tightly closed amber glass containers. Ginger rhizomes (*Zingiber officinale*) were purchased from the local market. a ginseng extract was obtained from the Korean Society of ginseng, Seoul, Korea as a gift. Thiobarbituric acid (TBA), 1,1,3,3-tetraethoxypropan (TEP) and other chemicals used were of analytical grade and obtained from Sigma Chemical Co (St. Louis, MO).

2.2. Preparation of the plant extracts

Jojoba extract

Air-dried powdered pericarp (one kg) of jojoba was exhaustively defatted using hexane and then extracted with ethanol 70% by percolation. The ethanolic extract was combined and evaporated under reduced pressure to yield 50gm of dry residue.

The residue was suspended in water (250ml) and partitioned successively with chloroform ($5 \times 50\text{ml}$) followed by ethyl acetate ($5 \times 50\text{ml}$) and *n*-butanol ($7 \times 50\text{ml}$). The solvents were evaporated under reduced pressure to give chloroform fraction (11gm), ethyl acetate fraction (4gm) and butanol fraction (10gm) (El-Halwany, 2002).

Jatropha extract

Ten grams of the air-dried powder of the leaves and roots of jatropha were extracted successively under shaking with chloroform (CHCl_3) three to five times at room temperature, with 90% methanol (CH_3OH) in a water-bath at 50°C three to five times and finely with water in a water-bath at 70°C . The obtained extract was filtered and evaporated using a vacuum evaporator to give the crude dried extract (Mothana and Likdequist, 2005).

Ginger extract

Ginger rhizomes were ground and passed through a 60 mesh screen. One hundred grams of ground ginger were defatted by shaking three times with four volumes of petroleum ether in a rotary shaker for 1 h. The residue obtained after filtration was dried overnight under a hood until all traces of petroleum ether were removed. The dried residue was extracted three times with four volumes of 90% ethanol by shaking for 1 h. and filtered. The combined filtrate was concentrated in a rotavapor and placed under a hood to remove the residual ethanol. The obtained aqueous extract was frozen overnight and freeze-dried at -60°C (Dura-Dry, USA). The freeze-dried extract was stored in airtight containers at 5°C until use (Mansour and Khalil, 2000).

Ginseng extract

Korean red ginseng extract was obtained from the Korean Society of ginseng, Seoul, Korea by Prof Dr. Mosaad A. Abdel-Wahhab, Food Toxicology and Contamination Dept., National Research Center, Cairo, Egypt; who supplied it for us as a gift

2.3. Determination of optimum concentration of the natural extracts

Jojoba (Jo), jatropha (Ja), ginseng (Gg) and ginger (Gr) extracts were screened at levels ranging from 0 to 1% to determine their optimum working concentrations. Fresh lamb meat was obtained from a local slaughter house and kept overnight at 0°C . The lamb meat was minced using a meat mincer. The four natural plant extracts were mixed with the minced lamb meat and formed into patties using a patty former. The potential antioxidants of the test extracts were determined through thiobarbituric acid reactive substances (TBARS), which were assessed in the lamb patties.

The optimum concentrations for the individual test extracts were identified during the screening trials and assessed simultaneously.

2.4. Preparation of Lamb Patties

Minced lamb meat was subdivided into five equal parts. Lamb patties were prepared to provide five treatment samples. A control treatment was formulated without plant extracts. The other treatments were prepared by adding the optimum concentrations determined of the tested extracts to lamb meat as follows: 0.1% jojoba (Jo) extract (sample patties with Jo), 0.1% jatropha (Ja) extract (sample patties with Ja), 0.25% ginseng (Gg) extract (sample patties with Gg) and 0.25% ginger (Gr) extract (sample patties with Gg); then mixed well and formed into patties (100g) using a meat former. Lamb patties were placed on plastic foam meat trays, wrapped with polyethylene film and kept in a refrigerator at 4°C for 9 days. The effect of the optimum concentration of the test extracts on thiobarbituric acid reactive substances (TBARS), pH, biogenic amines (BAs), mould and yeast counts & aerobic plate count (APC) were determined in lamb patties for 0, 3, 6, and 9 days of storage time at 4°C . Hence, the patties quality and safety could be assessed.

2.5. Chemical analyses

pH determination

A lamb patty sample (10 g) was homogenized in 100 ml distilled water for 1 min in a blender and the pH was measured using a digital pH-meter (HAANA, HI902 meter, Germany). Two readings were taken from each of the three lamb patty samples.

Thiobarbituric acid reactive substances (TBARS) value

The TBARS values were determined spectrophotometrically according to Byun *et al.*, (2001). Patty samples were analyzed for the optimum concentration of each extract. Homogenized patty samples (2g) were taken and TBARS were extracted twice with 10 ml of 0.4M perchloric acid. Extracts were collected and made up to 25ml with 0.4M perchloric acid and then centrifuged for 5 min at 1790g. After centrifugation, 1ml of extract was poured into a glass stoppered test-tube. A TBARS reagent (5ml) was added and the extract was heated in a boiling water bath for 35 min. After cooling under tap-water, the absorbance of the sample was read against the appropriate blank at 538nm. A standard curve was prepared using 1,1,3,3 - tetraethoxypropane (TEP).

Biogenic amines

Histamine, tyramine and putrescine were extracted as follows: five grams of the sample were blended

with 25ml 5% trichloroacetic acid. Filtration was achieved using whatman filter paper No.1. Five ml. of the extract were transferred into a suitable culture tube with 4g NaCl and 1ml of 50%Na OH and then shaken for 2 min. Centrifugation were carried out for 5 min at 5000 xg and the upper layer was transferred to a 50ml separating funnel. 15ml of *n*-heptane were added to the upper layer extract and extracted 3 times with 1ml portions of 0.2 N HCl. The extracts were collected in a glass stoppered tube and evaporated to dryness using a water bath at 95°C with the aid of a gentle current of air. This was followed by the formation of Dansylamines as described by Majjala and Eerola, (1993). Biogenic amine concentrations were determined according to Deabes, (2000) using the HPLC. The HPLC system was equipped with a (Waters 600) delivery system. The HPLC column was a reverse phase C18 Nucleosil column 250 × 4mm, 10µm packing, (Macherey-Naggl). The detection was performed using a U.V detector (waters 486) at 254nm wavelength, using a linear program of 25 min. periods and 1ml/min constant solvent flow rate. Data were integrated and recorded using Millennium Chromatography; Manger software 2010, (Waters, Milford MA 01757)

2.6. Microbial determinations

Aerobic plate count (APC)

The aerobic plate count was determined on nutrient agar medium as recommended by the American Public Health Association for food stuff examination (APHA, 1992). Plates seeded with serial dilutions of the samples were incubated at 37°C for 24-48 h.

Mould and yeast counts

Mould and yeast counts were estimated on Potato Agar according to APHA, (1992). The medium was acidified to pH 3.5 by adding a sterile 10% lactic acid solution; incubation was carried out at 25-28°C for 72 h.

2.7. Statistical analysis

The conventional statistical methods were used to calculate means and standard deviations. All the

measurements were performed in triplicate and the data are presented as mean ± SD. The effects of the addition of natural antioxidant extracts and storage time were analyzed and the obtained data were subjected to analysis of variance (ANOVA) according to PC-STAT, Version I A Copyright 1985, the University of Georgia.

3. RESULTS AND DISCUSSION

3.1. Optimum concentration of natural plant extracts

Based on preliminary trials, the optimum concentrations of the four tested natural plant (Jo, Ja, Gg, Gr) extracts were incorporated into lamb patties. Potential antioxidant properties of each of the tested natural plant extracts was determined through TBARS analysis. The concentration range employed for each test extract screened was from 0 to 1.0%. Doubling of the natural extracts addition rates was used in patty processing i.e. 0, 0.01, 0.05, 0.10, 0.25, 0.50, and 1.0% in order to have a greater effect on the assessment of the extracts' performance. Owing to the huge amount of data generated during screening; only the optimum concentrations of each of the four tested natural plant extracts are presented in Table 1. Thus, the optimum test extract addition rates based on the identified levels of antioxidant activity were determined as: jojoba (0.1%), Jatropha (0.1%), ginseng (0.25%) and ginger (0.25%).

3.2. pH changes

The effect of the optimum concentrations of natural plant extracts under investigation on the pH values of lamb patties stored at 4°C for 9 days is shown in Table 2. At zero time the pH of the control and all tested samples had the same value (5.92). Control samples, generally, had higher pH values than the other samples throughout the storage time. The pH values of the control and tested lamb patties containing natural antioxidant extracts were significantly ($p < 0.05$) increased gradually throughout the storage period. During storage time (3-9days) it was noticed that the pH value of the control samples was higher (6.29) than the other tested samples. At the 9th day lamb

Table 1
Optimum concentration of natural plant extracts determined in Lamb patties.

Natural plant extract	Concentration range screened %	Optimum working concentration range %	Optimum concentration determined %
Jojoba (Jo)	0 – 1	0.1 – 0.50	0.1
Jatropha (Ja)	0 – 1	0.1 – 0.25	0.1
Ginseng (Gg)	0 – 1	0.25 – 1.00	0.25
Ginger (Gr)	0 – 1	0.25 – 1.00	0.25

Table 2
Effect of the optimum concentration of natural plant extracts on pH changes and TBARS values of lamb patties stored at 4°C for 9 days.

Lamb patties sample*	Day 0 M ± SD	Day 3 M ± SD	Day 6 M ± SD	Day 9 M ± SD
<i>pH values</i>				
Control	5.92 ± 0.00 ^z	5.99 ± 0.00a ^y	6.16 ± 0.00a ^x	6.29 ± 0.00a ^w
Jo	5.92 ± 0.00 ^z	5.94 ± 0.00d ^y	5.99 ± 0.00e ^x	6.16 ± 0.00c ^w
Ja	5.92 ± 0.00 ^z	5.95 ± 0.00c ^y	6.01 ± 0.00c ^x	6.14 ± 0.00d ^w
Gg	5.92 ± 0.00 ^z	5.95 ± 0.00c ^y	6.00 ± 0.00d ^x	6.13 ± 0.00e ^w
Gr	5.92 ± 0.00 ^z	5.97 ± 0.00b ^y	6.12 ± 0.00b ^x	6.19 ± 0.00b ^w
<i>TBARS values (malonaldehyde mg/ kg meat)</i>				
Control	0.158 ± 0.006 ^z	0.264 ± 0.009a ^y	0.494 ± 0.024a ^x	0.626 ± 0.006a ^w
Jo	0.161 ± 0.002 ^z	0.174 ± 0.004cd ^y	0.326 ± 0.004b ^x	0.405 ± 0.004c ^w
Ja	0.161 ± 0.002 ^z	0.174 ± 0.004cd ^y	0.324 ± 0.004c ^x	0.404 ± 0.001c ^w
Gg	0.160 ± 0.003 ^z	0.165 ± 0.005d ^y	0.304 ± 0.004c ^x	0.393 ± 0.003d ^w
Gr	0.161 ± 0.003 ^z	0.185 ± 0.009b ^y	0.345 ± 0.004b ^x	0.436 ± 0.004b ^w

*Jo = patties with jojoba extract. Ja = patties with jatropha extract. Gg = patties with ginseng extract. Gg = patties with ginger extract. Control = patties without any extract. M ± SD = Mean ± Standard Deviation.

*Letters a-e to show significant differences ($P < 0.05$) between the same column. Letters w- z to show significant differences ($P < 0.05$) between rows.

patties containing ginger extract (sample Gr) had the highest pH value and patties containing ginseng extract (sample Gg) had of the lowest value. Similar findings in pork patties and in ground buffalo meat containing BHA/BHT antioxidants during refrigerated and frozen storage, respectively have been reported by (McCarthy *et al.*, 2001; Sahoo, 1995). The increase in pH may be due to the accumulation of metabolites by bacterial action in meat and deaminations of proteins (Jay, 1996). Bacteria, upon exhaustion of stored glucose, utilize amino acids released during protein breakdown and, as a product of amino acid degradation, ammonia accumulates and pH rises (Gill, 1983).

3.3. Thiobarbituric acid reactive substances (TBARS)

The data presented in Table 2 show the changes of TBARS values in the lamb patties containing optimum concentrations of the tested natural extracts stored at 4°C for 9 days. The screened natural extracts were effective as antioxidants and had lower TBARS values than the control samples throughout the storage period. The effectiveness of the added natural plant extracts as antioxidants inhibiting lipid oxidation throughout storage time could be listed in the following order of decreasing TBARS values: Ginseng > jatropha > jojoba > ginger. Results also show that the ginseng extract was the most effective antioxidant and ginger had the lowest effect. This can indicate that the optimum concentration of the natural plants used as antioxidants was effective against TBARS

formation in a different way when incorporated into lamb patties. Estevez *et al.*, (2004); Formanek *et al.*, (2001); McCarthy *et al.*, (2001); Chen *et al.*, (1999), reported that dried herbs and their essential oils were successfully used to reduce lipid oxidation in meat products. Polyphenolic extracts are excellent electron and proton donors, and their intermediate radicals are quite stable due to electron delocalization phenomena and owing to the lack of positions attackable by O₂ (Djenane *et al.*, 2005). In the present study, since the natural extracts used in preparing lamb patties contain bioactive substances e.g. phenolic compounds in Jatropha and ginger (El-Diwani *et al.*, 2009; Stoilova *et al.*, 2007), flavonoids in jojoba (El-Halawany, 2002) and triterpenes saponins in ginseng (Fuzzati, 2004); these substances could cause an inhibition of the chain reactions during lipid oxidation.

3.4. Microbial changes

Meat is prone to both microbial and oxidative spoilage and therefore it is important to use a preservative with both antioxidant and antimicrobial properties (Kanatt *et al.*, 2008). The growing concern about the safety of foods has led to the development of natural antimicrobials to control food-borne pathogen (Nevas *et al.*, 2004).

Aerobic plate count (APC)

Table 3 shows the effect of adding optimum concentrations of the natural plant extracts to the prepared lamb patties stored at 4°C for 9 days on

Table 3
Changes in Aerobic plate count, mould and yeast counts of lamb patties stored at 4°C for 9 days.

Lamb patties sample*	Day 0	Day 3	Day 6	Day 9
	M ± SD	M ± SD	M ± SD	M ± SD
<i>Aerobic plate count (Log CFU/ g)</i>				
Control	4.06 ± 0.019 ^z	4.99 ± 0.012a ^y	6.23 ± 0.010a ^x	7.57 ± 0.027a ^w
Jo	4.06 ± 0.019 ^z	4.17 ± 0.050c ^y	4.30 ± 0.019c ^x	4.94 ± 0.040b ^w
Ja	4.06 ± 0.019 ^z	4.18 ± 0.064c ^y	4.30 ± 0.031c ^x	4.96 ± 0.021b ^w
Gg	4.06 ± 0.019 ^y	4.10 ± 0.009d ^y	4.18 ± 0.019d ^x	4.82 ± 0.035b ^w
Gr	4.06 ± 0.019 ^y	4.26 ± 0.009b ^x	4.45 ± 0.035b ^x	4.84 ± 0.312b ^w
<i>Mould and yeast counts (Log CFU /g)</i>				
Control	2.32 ± 0.019 ^z	2.88 ± 0.015a ^y	3.22 ± 0.015a ^x	3.66 ± 0.015a ^w
Jo	2.32 ± 0.019 ^z	2.43 ± 0.047c ^y	2.52 ± 0.025 ^x	2.59 ± 0.009c ^w
Ja	2.32 ± 0.019 ^y	2.42 ± 0.058c ^x	2.53 ± 0.015c ^w	2.59 ± 0.021c ^w
Gg	2.32 ± 0.019 ^y	2.35 ± 0.045d ^y	2.43 ± 0.019 ^x	2.51 ± 0.020d ^w
Gr	2.32 ± 0.019 ^z	2.50 ± 0.010b ^y	2.60 ± 0.021 ^x	2.79 ± 0.029b ^w

*Jo = patties with jojoba extract. Ja = patties with jatropha extract. Gg = patties with ginseng extract. Gg = patties with ginger extract. Control = patties without any extract. M ± SD = Mean ± Standard Deviation. *Letters a-e to show significant differences ($P < 0.05$) between column. Letters w- z to show significant differences ($P < 0.05$) between rows.

aerobic plate count (APC). A remarkable increase was noticed in APC throughout storage, especially in the control sample at days 6 and 9 (from 6.23 to 7.57 Log CFU/g) respectively. It has been reported by Insausti *et al.*, (2001) that meat spoilage cannot be said to occur until Total Variable Count (TVC) counts reach 10^6 - 10^8 CFU g^{-1} (limit of microbiological acceptability). In general, a significant decrease was noticed for all tested patty samples in their aerobic plate count during the storage period (3-9 days). Worth noting is the fact that sample Gg (containing ginseng extract) was of the lowest APC and the patties with ginger extract (sample Gr) had the highest compared to the other samples. Thus, the results show that the aerobic plate count (APC) decreased significantly with the addition of the natural extracts during storage at 4°C for 9 days. Igbinosa *et al.*, (2009) concluded that *Jatropha curcas* stem bark could be a potential source of active antimicrobial agents. Also, Jitoe *et al.*, (1992); Zia-ur-Rehman *et al.*, (2003) found that ginger has antioxidant activity and effective antimicrobial agents.

Mould and yeast counts

Mould and yeast counts of the prepared lamb patties containing the optimum concentrations of the tested natural extracts during storage for 9 days at 4°C are given in Table 3. It was observed that both the addition of the natural extracts and the storage time had a significant effect on the mould and yeast counts. The control samples had the highest mould and yeast counts throughout the storage period. In general, the patty samples containing the natural extracts Jo, Ja, Gg and Gr increased in their mould and yeast counts at the end of storage period.

Lamb patties containing ginger extract (sample Gr) were noticed to be of high mould and yeast counts. A higher reduction in mould and yeast counts was observed in the patties containing ginseng extract (sample Gg) than in samples Jo, Ja and Gr during the storage time. Meanwhile, samples containing jojoba and *Jatropha* (Jo and Ja) were of nearly the same mould and yeast counts. Thus, addition of the tested natural extracts reduced the mould and yeast counts in the prepared lamb patties. Spices and herbs used in food stuffs for enhancing flavor or color attributes, generally have antimicrobial as well as antioxidant activities (Baydar *et al.*, 2004; Sağdic and Özcan 2003; Yanishlieva and Marinova 2001). The addition of ginseng extract was found to be more effective in reducing APC and mould & yeast counts in the tested lamb patties.

3.5. Biogenic Amines (BAs)

The production of biogenic amines during the storage or processing of food products is an extremely complex phenomenon depending on several variables, such as the growth of microorganisms, several extrinsic and intrinsic factors during the manufacturing process such as formulation, some physico-chemical parameters and proteolytic & decarboxylase activities which interact with each other (Latorre-Moratalla *et al.*, 2008; Suzzi and Gardini, 2003).

Table 4 shows the effect of adding optimum concentrations of natural extracts to the prepared lamb patties stored at 4°C for 9 days on the formation of biogenic amines. Histamine, tyramine and putrescine (mg/kg) were not detected in lamb patties at zero time of storage. It was observed that storage time had a significant effect ($p < 0.05$)

Table 4
Effect of the optimum concentration of natural plant extracts on concentration of biogenicamines in lamb patties stored at 4°C for 9 days.

Lamb patties samples*	Day 0	Day 3	Day 6	Day 9
	M ± SD	M ± SD	M ± SD	M ± SD
<i>Histamine concentration (mg/kg)</i>				
Control	ND	4.26 ± 0.187a ^y	6.84 ± 0.127a ^x	11.04 ± 0.147a ^w
Jo	ND	2.89 ± 0.100bc ^y	4.03 ± 0.057c ^x	6.36 ± 0.138 c ^w
Ja	ND	2.90 ± 0.089bc ^y	3.98 ± 0.068cd ^x	6.36 ± 0.100c ^w
Gg	ND	2.67 ± 0.318c ^y	3.74 ± 0.25d ^x	6.10 ± 0.163d ^w
Gr	ND	3.14 ± 0.136 b ^y	4.35 ± 0.125 b ^x	7.04 ± 0.064b ^w
<i>Tyramine concentration (mg/kg)</i>				
Control	ND	5.38 ± 0.170a ^y	7.84 ± 0.140a ^x	13.34 ± 0.555a ^w
Jo	ND	3.94 ± 0.055cd ^y	5.89 ± 0.061cd ^x	8.04 ± 0.074c ^w
Ja	ND	4.02 ± 0.031c ^y	5.95 ± 0.045bc ^x	8.10 ± 0.119c ^w
Gg	ND	3.72 ± 0.076d ^y	5.68 ± 0.252 d ^x	7.43 ± 0.130d ^w
Gr	ND	4.46 ± 0.201b ^y	6.15 ± 0.130b ^x	8.85 ± 0.127b ^w
<i>Putrescine concentration (mg/kg)</i>				
Control	ND	6.14 ± 0.085 a ^y	8.22 ± 0.157a ^x	12.58 ± 0.407a ^w
Jo	ND	4.16 ± 0.115c ^y	5.53 ± 0.231c ^x	7.90 ± 0.096c ^w
Ja	ND	4.18 ± 0.095c ^y	5.54 ± 0.216c ^x	8.06 ± 0.081c ^w
Gg	ND	3.42 ± 0.126d ^y	4.81 ± 0.165d ^x	6.40 ± 0.180d ^w
Gr	ND	4.45 ± 0.065b ^y	6.25 ± 0.122b ^x	8.84 ± 0.140b ^w

*Jo = patties with jojoba extract. Ja = patties with jatropha extract. Gg = patties with ginseng extract. Gg = patties with ginger extract. Control = patties without any extract. ND = none detected. M ± SD = Mean ± Standard Deviation. Letters a-e to show significant differences ($P < 0.05$) between column. Letters w- z to show significant differences ($P < 0.05$) between rows.

for up to 9 days at 4°C on the formation of all the estimated biogenic amines.

Histamine concentrations varied from 4.26 to 11.04mg/kg in the control sample during storage at 4°C for 9 days. Data in Table 4 show that on 3, 6 and 9 days histamine concentrations of all lamb patty samples were significantly ($p < 0.05$) increased gradually. All natural plant extracts were effective in producing lower histamine concentrations than control samples over the storage period. Results show that ginseng extract was the most effective, while ginger was the least effective. Meanwhile, jatropha and jojoba extracts were nearly equal in their effectiveness against histamine formation.

The permitted level of tyramine in foods is 100-800mg/kg, while 1080mg/kg is toxic (Shalaby, 1996). Tyramine concentrations, in the present study, were found in the safe range and lower than the permitted level. They varied in the control patty sample from 3.72 to 13.34mg/kg during the 3-9 days of storage at 4°C (Table 4). The tyramine contents in the patties containing the tested extracts were still less than the control level at day 9. Using the tested natural antioxidants was found to significantly reduce ($P < 0.05$) tyramine formation. The reduction of tyramine in lamb patty samples with Jo, Ja, Gg and Gr relative to the control sample was about 39.8% for jojoba, 39.4% for jatropha,

54.4% for ginseng and 33.7% for ginger extracts at the end of the storage period. Storage time had a significant effect ($p < 0.05$) on tyramine formation, its concentrations in the tested patties samples increased significantly during the storage period and at day 9 reached 8.04, 8.10, 7.43 and 8.85mg/kg in the four lamb patty samples Jo, Ja, Gg and Gr respectively. The ginseng extract showed the lowest tyramine content 7.43mg/kg thus presenting a marked effect on this BA formation. Eerola *et al.*, (1997) observed that tyramine concentration in sausages increased during 7 days of storage at 4°C. The reduction in tyramine formation through natural antioxidant extracts is important with respect to human health because tyramine causes migraine headaches, increased blood pressure and an increase in noradrenalin as has been previously reported by Ruiz-Capillas and Jimenez-Colmenero, (2004).

The addition of the tested plant extracts in the preparation of the lamb patties stored at 4°C for 9 days significantly affected ($p < 0.05$) the formation of putrescine. Its concentration increased up to 12.58, 7.90, 8.06, 6.40 and 8.84 gm/kg in the control and patty samples containing Jo, Ja, Gg and Gr respectively at the end of the storage period (Table 4). The highest putrescine concentration was observed in the control sample; while the

lowest was for sample Gg containing the ginseng extract. Thus, the addition of natural plant extracts was found to be effective in reducing the formation of putrescine. The ranking in decreasing order of effectiveness of the applied extracts on putrescine concentration in the lamb patty samples is: ginseng > jojoba > jatrophia > ginger. This reduction could also be due to the antimicrobial activities of the natural extracts. Putrescine formation depends on the total aerobic count where a high total aerobic count results in high putrescine formation (Ruiz-Capillas and Jimenez-Colmenero, 2004).

Therefore, it can be stated that the addition of optimum concentration of the used natural plant extracts to lamb patties resulted in a marked significant reduction in histamine, tyramine and putrescine formation.

4. CONCLUSION

Comparison of control and treated lamb patty samples during storage at 4°C for 9 days showed that the addition of the investigated natural plant extracts was effective as antioxidant and antimicrobial agents for improving the properties of the prepared lamb patties from a quality and safety view point. The results show that, in general, the ginseng extract was the most effective while the ginger extract was the least. Also, It was noticed that the optimum concentration of the investigated natural extracts were effective in reducing the aerobic plate count (APC), mould & yeast counts and against the formation of biogenic amines (histamine, tyramine and putrescine) as well as reducing TBARS; although their performance differed when incorporated into lamb patties. The effectiveness of the tested natural antioxidant extracts could be listed in the following order of decreasing TBARS value: Ginseng > jatrophia > jojoba > ginger.

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Recibido: 7/6/10
Aceptado: 24/8/10