

## ORIGINAL ARTICLES

### Effect of Adding Lemongrass and Lime Peel Extracts on Chicken Patties Quality

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#### ABSTRACT

Lemon grass and lime peel extracts (fresh and oil) were investigated for their antimicrobial and antioxidant activities. The optimum concentrations of the extracts were established and added to chicken patties. Some chemical and microbial characteristics of the prepared patties during storage for 9 days at 4°C were evaluated. Results indicated that the extracts showed high potential antibacterial activity against *Bacillus cereus*, *Salmonella typhimurium* and *Staphylococcus aureus*. The oil extracts exhibited higher antibacterial activity than fresh extracts. Methanolic extract of lemongrass showed significant high antioxidant activity equivalent to gallic acid and to BHA. Water and methanolic extracts of lime peel were of higher inhibition DPPH % radical compared to of lemon grass extracts. During the storage period of prepared chicken patties the optimum concentration of the tested oil extracts used as antioxidants was effective against TBARS formation and inhibited lipid oxidation. pH values of the control patty samples were higher than the other tested samples. Addition of the tested oil extracts reduced the aerobic plate count (APC), mould and yeast counts in the prepared chicken patties and caused decrease in volatile basic nitrogen (VBN) %. It was noticed that addition of optimum concentration of lemongrass and lime peel extracts to chicken patties resulted in a marked reduction in histamine, tyramine and putrescine formation. So, lemongrass and lime peel extracts can play an important role as antioxidant and antibacterial agents in refrigerated chicken patties.

**Key words:** Lemongrass extract – Lime peel extract –Antimicrobial activity – Antioxidant Activity - Chicken Patties.

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#### Introduction

Numerous food products require protection from spoilage during preparation, storage, and distribution to gain them the desired shelf life. The minimally processed, easily prepared and ready-to-eat fresh food products, globalization of food trade and distribution from centralized processing pose major challenges for food safety and quality (Lucera *et al.*, 2012). The quality of food and food products may suffer harmful damaging during their preparation, storage, and distribution by microbiological and/or chemical changes that seem to be a major factor affecting their quality, safety and cost.

Oxidative processes lead to degradation of lipids and proteins and are one of the primary mechanisms of quality deterioration with limiting the shelf life in meat and meat products (Lui *et al.*, 2010). Microbial contamination can cause major public health hazards and economic loss in terms of food poisoning and meat spoilage (Kingchaiyaphum and Rachtanapun 2012). Thus, the application of suitable agents possessing both antioxidant and antimicrobial activities may be useful for maintaining meat quality, extending shelf-life and preventing economic loss (Yin and Cheng 2003). Numerous efforts are conducted to find natural alternatives to prevent bacterial and fungal growth as well to inhibit the oxidation process in foods.

Many researchers have indicated that lipid oxidation and microbial growth in meat products can be controlled or minimized by using either synthetic or natural food additives (Mielnik *et al.*, 2008). Natural agents (e.g. plant and/or herbs) possessing antioxidant have the advantage of being readily accepted by consumers (Kingchaiyaphum and Rachtanapun 2012). Screening of plant extracts revealed that majority of the plant extracts contains phenolic compounds as secondary metabolites. Biological significance of these compounds is immense due to enormous reducing power of free hydroxyl groups (antioxidant property) and protein binding capacity (causes inhibition of microbial growth—antimicrobial property) as reported by Vaithyanathan *et al.*, (2011). It is worthy to note that the demand for medicinal herb (i.e. plants contain phytochemicals) has begun to grow and gain popularity. Chaisawadi *et al.*, (2003) reported that some of the selected medicinal herbs of food ingredients (e.g. coriander, ginger, lemongrass, and sweet basil,...etc) had antimicrobial activity as well the potential use of lime peel as natural antimicrobial have also been confirmed.

Essential oils are organic substances produced by plants which can be obtained from roots, stem, bark, leaves, flowers, fruits and seeds (Tassou *et al.*, 2004). These oils and extracts have recently gained a great

popularity and scientific interest. Moreover, essential oils from many medicinal plants exhibited antimicrobial activity against many pathogenic microbes (Chanthaphon *et al.*, 2008). Essential oils of herbs and their components, (products from the secondary metabolism of plants), have many applications in ethno-medicine, food flavoring and preservation as well as the fragrance and pharmaceutical industries (Fabian *et al.*, 2006).

Essential oils has been regarded as natural alternatives of chemical preservatives and their use in food meets the demands of consumers for mildly processed or natural products, since in modern food industries, mild processes are applied in order to obtain safe products which have a natural or "green" image (Burt 2004). The effects of plant extracts or essential oils classified as general recognized as safe (GRAS) following their addition, have been studied and reported in a variety of meat types including pork, beef and lamb (Nieto *et al.*, 2010). Since the worldwide trend towards the use of natural additives in food; natural medicinal plants/herbs are considered an important target to be investigated in order to provide a new source of natural antioxidants and/or antimicrobial agents from a safety view point. Therefore, the present study is interested to use natural antioxidant and antimicrobial compounds, extracted from lemongrass and lime peels for food preservation.

Lemongrass (*Cymbopogon citratus*), a tall perennial grass comprising of about 55 species, is native to warm region and grows in almost all tropical and subtropical countries (Cheel *et al.*, 2005). It is an aromatic herb, known in the North and West tropical Africa, in Arabian Peninsula and in Egypt (Khadri *et al.*, 2010). The biologically active constituent of lemon grass is citral constituting more than 75% (w/w) of its essential oil (Huynh *et al.*, 2008). It is widely used as an herb in Asian cuisine, has a subtle citrus flavor and can be dried and powdered, or used fresh. It is commonly used in teas, soups, and curries, also is suitable for poultry, fish, beef, and seafood. Moreover, lemongrass is used as a preservative (Shadab *et al.*, 1992). Also, lemongrass essential oil is applied for its medicinal value to cure acne, oily skin, flatulence, headaches, and blood circulation problems (Pearson 2010).

Lemongrass is a rich source of citral, which is used in perfumery, pharmaceutical industries, and bioactive compounds (flavonoids and vitamin C). The natural flavonoids are also attracting more and more attention not only due to their antioxidant properties, but also as anti-carcinogenic and anti-inflammatory agents because of their lipid anti-peroxidation effects (Martin *et al.*, 2002). Flavonoids extracted from lemongrass are of considerable interest as natural plant components with antioxidant and antifungal activity. Moreover, of the flavonoids present in lemongrass, licochalcone A and licochalcone B which have equal antioxidant activity of vitamin E and glabrene which is 3 times as active when compared with vitamin E (Abd-El Fattah *et al.*, 2010).

Several studies have shown that the lemongrass has antibacterial and antifungal properties and many other studies proved the antimicrobial effect of lemongrass using reference strains of variety of bacteria and fungi as reported by Singh *et al.*, (2011). Wannissorn *et al.*, (2009) cleared in their preliminary analysis of antimicrobial activities of 28 essential oils against food poisoning and food spoilage bacteria that the essential oils obtained from lemongrass was capable of inhibiting 5-7 types of tested bacteria. Meanwhile, Abd-El Fattah *et al.*, (2010) reported that lemongrass had medical functions and safe as well antifungal activity, make its oil may be potential multi-functional food additives.

Peels are the primary by-products during citrus juice processing and if unused, they turn out to be waste and represent a source of environmental pollution. However, studies conducted on several fruits (citrus, apples, grapes and berries) showed that peels are the major source of natural antioxidants (de Moraes Barros *et al.*, 2012). Phenolic compounds in peels and fruit by-products can be used in food products as active ingredients or as substitutes for synthetic preservatives (Ignat *et al.*, 2011).

Chaisawadi *et al.*, (2005) reported that lime and its derivatives including lime juice, lime peel and lime oil provides a whole range of medicinal properties. Especially, d-limonin from lime oil is suggested to have cancer-chemo preventive and anti-carcinogenic properties making it a potential candidate for industrial production for use as a medicinal herb. Also, the same authors cleared that the waste utilization products are important ingredients for traditional medicine and nutritional health products. In addition, lime peel processing products are used as ingredients in health foods and medicine. Literature exiting on the antimicrobial activities of lime oil states its potent antibacterial and antifungal effects. In-addition to antimicrobial activities, lime essential oil was found to have several medicinal properties and potential health benefits which make it a good candidate as a natural antimicrobial preservative in food products. Also, the essential oil of *C. acida* var. sour lime peels were found to possess antimicrobial and antioxidative potential which might be utilized in food and chemical industry (Mahmud *et al.*, 2009).

The use of natural antioxidant as well antibacterial compounds, such as extracts of spices and herbs, essential oils, organic acids, salts, and bacteriocins has been reported to improve the shelf life of meat (Jamilah *et al.*, 2008; Jąłosińska and Wilczak 2009) and to prevent bacterial and fungal growth.

Chicken patties are considered as one of the popular foodstuffs among food products and widely spread all over the world. However, during storage, quality attributes of the product deteriorate due to lipid oxidation, microbial growth, and also may be due to formation of biogenic amines which are basic nitrogenous compounds found in a wide variety of foods such as meat and meat products (Vinci and Antonelli 2002). The presence of biogenic amines in food constitutes a potential public health concern due to their physiological and toxicological

effects. Biogenic amines can be produced during storage or processing of the products by thermal or bacterial enzymatic decarboxylation of free amino acids, or exposed to microbial contamination during processing or storage. Their production depends on the quality of raw materials and hygienic conditions during processing and storage (Onal 2007).

Accordingly, the objectives of this study were intended to: i) investigate the antimicrobial and the antioxidant activities of lemon grass and lime peels extracts (fresh and oil); ii) set up the optimum concentrations of lemon grass and lime peel extracts as sources of natural antioxidants and/or antimicrobial agents to be added to chicken patties in order to reduce oxidative and microbiological deterioration and iii) evaluate the effects of adding the natural extracts at the optimum concentrations on some quality parameters e.g. pH values, thiobarbituric acid reactive substances (TBARS), biogenic amines (BA), volatile basic nitrogen (VBN) and microbiological counts of the prepared chicken patties stored at 4°C for 9 days.

## Materials and Methods

### Materials:

#### Chemicals

Antioxidant standards of gallic acid (GA), butylated hydroxyanisole (BHA), 1-naphthalene-acetic acid (NAA), 6-benzyladenine (BA), 1,1,3,3-tetraethoxypropan (TEP), 2-thiobarbituric acid, histamine dihydrochloride, putrescine, tyramine hydrochloride and tryptamine hydrochloride, Sodium carbonate and methanol were obtained from Sigma Chemical Co (St. Louis, MO). Dimethyl sulphoxide (DMSO) was purchased from Merck Co (Darmstadt, Germany). Other chemicals used were of analytical grade and obtained from Sigma Chemical Co (St. Louis, MO).

#### Strains of microorganisms:

The microorganisms used were as follows: *Bacillus cereus* (ATCC 10907), *Salmonella typhimurium* (ATCC 14028) and *Staphylococcus aureus* (ATCC 29213) bacteria strains were obtained from Naval Medical Research Unit 3 (NAMRU-3) Cairo, Egypt.

#### Culture media:

Peptone wastes (Oxoid CM 9, UK), nutrient agar (Lab M, UK), (Baird parker Medium, Oxoid CM 275, UK), Brain Heart Infusion Broth (BHI-Oxoid CM 225, UK), violet red (Scharlau Chemie, EU), Perfringens agar (Lab M, UK) *Salmonella-shigella* agar (Difco, France) CSM for *B. cereus* (lab M, UK) and potato dextrose agar. Plant extracts check.

#### Chicken meat:

The chickens were obtained from local poultry market. The birds were slaughtered, dressed and de-boned manually in a commercial poultry processing unit and packed in polyethylene bags and stored overnight at  $4 \pm 1^\circ\text{C}$  in a refrigerator. Chicken meat was cut into small pieces and minced twice in a meat mincer (Model TC 22; RIO INOX, Sirman, Italy).

#### Plant material:

Lemongrass and lime peel were used in this study as sources of antioxidant and antimicrobial agents. Fresh lemongrass and lime fruit were purchased from a local market.

#### Preparation of plant samples:

Lemongrass leaves were separated from stems, washed in clean water, and dried at room temperature nearly  $25^\circ\text{C}$  (Gupta *et al.*, 1993). The plants were milled to a fine powder.

Peels of limes were taken off from the fruits. For preparation of lime peel, the fruits were cut into half and squeezed by a hand-pressed juice extractor (Chinapongtitiwat *et al.*, 2013). The peels were removed and dried at room temperature ( $25 \pm 2^\circ\text{C}$ ). The dried peels were powdered in a heavy duty grinder. Lemongrass and lime peel powders were kept in closed containers in a refrigerator at  $4^\circ\text{C}$  until required.

*Extraction of the plant:*

Each sample of lemongrass and lime peel was prepared into fresh and oil extracts. Twenty grams of each sample were prepared for each extract. Fresh extracts were prepared by using a manual extractor and squeezing. The oil extracts of the tested plants were extracted with methanol according to Khadri *et al.*, (2010). Methanol extracts were obtained by Soxhlet extraction of 20 g of each sample for 6 h in about 250 ml methanol then concentrated to dryness under reduced pressure using rotatory evaporator and the residues were stored at 4 °C.

*Antimicrobial activity screening of lemon grass and lime peels extracts:*

The first screening step, in this study, was carried out to prop the antimicrobial activity of fresh and oil extracts of lemongrass and lime peel against the tested bacteria strains: *Bacillus cereus*, *Salmonella typhimurium* and *Staphylococcus auras*. The antibacterial activity of the tested extracts was measured by the inhibition zones produced. All experiments were duplicated. The diameter in mm of the clear zone indicated the inhibition activity (the second screening step).

Antibacterial activity of fresh and oil extracts of lemon grass and lime peels was determined according to Sahin *et al.*, (2004) using disk diffusion assay as follows: The tested bacteria strains were suspended in 5ml 0.1% peptone water and 100µl of suspension were swabbed on entire surface of Plate Count Agar (PCA) for bacteria. Sterile 6-mm filter paper discs (Whatman, Kent, UK) immersed with fresh and oil extracts of lemongrass and lime peels individually were aseptically placed on the center of the inoculated plates. The diameters of inhibition zones were measured in mm after incubation at 37 °C for 24 hr. All the testes were performed in duplicate. A disk with methanol was used as control

*Antioxidant activity (DPPH radical scavenging activity):*

The method was adopted as described by Sharma and Bhat (2009). Methanolic and water extracts of lemongrass and lime peel were evaluated in terms of their hydrogen donating or radical scavenging ability using DPPH radical. For assay, 200µl filtrate was taken in test tubes and volume made up to 1 ml with methanol. Three milliliters of the freshly prepared solution of DPPH (200mM) in methanol was added to the sample tube and mixed vigorously for 15s. The sample tube was then kept in a water bath at 37 °C for 20 min. The absorbance of the sample was measured at 517nm using the UV Spectrophotometer (model T80 x UVNIS Spectrometer PG Instruments Ltd). Gallic acid and BHA were used as standard references. The DPPH radical scavenging effect was calculated as “inhibition of percentage” According to the following formula:

$$\text{Inhibition of percentage (\%)} = [A_{c(0)} - A_{a(t)} / A_{c(0)}] \times 100$$

Where:  $A_{c(0)}$  is an absorbance of control DPPH solution at 0 min and  $A_{a(t)}$  is absorbance of test sample after 20 min.

*Determination of optimum concentration of the natural oil extracts:*

The oil extracts of lemon grass and lime peel were screened at levels ranging from 0 to 1% to determine their optimum working concentrations. The two extracts were mixed with the minced chicken 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 chicken patties. The optimum concentrations for the individual test extracts were identified during the screening trials and assessed simultaneously.

*Preparation of chicken patties:*

The minced chicken meat was subdivided into three equal parts. Chicken patties were prepared to provide three 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 the chicken meat as follows: 0.25% lemongrass extract and 0.25% lime peel extract then mixed well and formed into patties (100g) using a meat former. Chicken 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 chicken patties during the storage time at 4 °C.

*Analyses of the chicken patty samples:**pH determination:*

A chicken 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). Three readings were taken from each of the three chicken patty samples.

*Thiobarbituric acid reactive substances (TBARS) value:*

The TBARS values were determined spectro-photometrically according to Byun *et al.*, (2001). Patty samples were analyzed for the optimum concentration of each extract. Homogenized patty samples (2 g) 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 1790xg. After centrifugation, 1ml of extract was poured into a glass stoppered test-tube. TBARS reagent (5 ml) 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% NaOH and then shaken for 2 min. Centrifugation were carried out for 5 min at 5000xg and the upper layer was transferred to a 50ml separating funnel. Fifteen ml 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 Maijala and Eerola (1993). Biogenic amine concentrations were determined according to Eerola *et al.*, (1993) 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 x3.4 mm, 10 µm 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)

*Volatile basic nitrogen:*

A sample (10 g) was minced with 100 ml distilled water and washed into a distillation flask with 100 ml distilled water; then 2 g of magnesium oxide and an antifoaming agent were added. The mixture was distilled using the micro Kjeldahl distillation apparatus. Distillate was collected for 25 min into 25 ml 4% boric acid and five drops of Tashero indicator. The solution was titrated using (0.1 M) HCl to calculate the total volatile basic nitrogen in the sample in terms of mg VBN/100g chicken patty sample (Pearson 1976).

*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.

*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, USA.

## Results and Discussion

### Antibacterial activity:

The Antibacterial activity of lemongrass and lime peels extracts (fresh and oil extracts) against *Bacillus cereus* (Gram+), *Staphylococcus aureus* (Gram+) and *Salmonella typhimurium* (Gram-) was shown in Tables 1 and 2.

In the first preliminary screening step, lemongrass showed antibacterial activity with clear zone (+) against *Bacillus cereus* and *Staphylococcus aureus* in oil extracts only; meanwhile, for fresh extracts these two bacteria strains showed activity with no clear zone (-). Antibacterial activity with no clear zone was noticed against *Salmonella typhimurium* in both fresh and oil extracts. Regarding, lime peels extracts, antibacterial activity was displayed with clear zone against *Bacillus cereus* in both fresh and oil extracts. Activity with clear zone (+) was noticed against *Salmonella typhimurium* and *Staphylococcus aureus* in oil extracts; but in fresh extract these strains showed antibacterial activity with no clear zone (Table 1).

The second screening step for antibacterial activity using the Disk Diffusion Test on Agar Diffusion Method. The inhibitory activity was measured by zone diameter (mm) of inhibition. Data predicted in Table 2 showed that in lime peels extracts, the largest zones of inhibition were observed against *Staphylococcus aureus* followed by *Bacillus cereus* in both fresh and oil extracts; while the inhibition zones against *Salmonella typhimurium* were equal either in fresh or oil extracts. Worthy to note that lemongrass extracts (fresh and oil) had the same pattern of antibacterial activity but with slight decrease in zone diameter (mm) of inhibition. It was also demonstrated that the tested oil extracts exhibited higher antibacterial activity than fresh extracts. In a previous study it has been found by Behboud *et al.*, (2012) that methanol extract of lemongrass plant prevents bacterial growth of *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli*; with increasing concentration, their antibacterial effect also increased. These authors reported that most plant extracts have inhibition effect on Gram positive and little effect on Gram negative bacteria. This inhibition effect can be related to the biological active components. Also, essential oil of kaffir lime (*Citrus hystrix* DC) peel exhibited antimicrobial activity against Gram positive bacteria namely *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, Gram negative bacteria namely *Escherichia coli*, *Salmonella Typhimurium*, lactic acid bacteria and mold. (Kingchaiyaphum and Rachtanapun, 2012). Thus, lemongrass and lime peel extracts showed high potential antibacterial activity against *Bacillus cereus*, *Salmonella typhimurium* and *Staphylococcus aureus* and this indicated the possibility of using these medicinal plants as natural antibacterial agents.

**Table 1:** Antimicrobial activity of lemongrass and lime peel extracts on the first screening step

Medicinal herbs	<i>Bacillus cereus</i>		<i>Salmonella typhimurium</i>		<i>Staphylococcus aureus</i>	
	Oil Extract	Fresh Extract	Oil Extract	Fresh Extract	Oil Extract	Fresh Extract
Lemongrass	+	-	-	-	+	-
Lime peels	+	+	+	-	+	-

(+) = with clear zone, (-) = no clear zone

**Table 2:** Antimicrobial activity against *Bacillus cereus*, *Salmonella typhimurium* and *Staphylococcus aureus* of lemongrass and lime peel extracts on second step

Medicinal herbs	<i>Bacillus cereus</i>				<i>Salmonella typhimurium</i>				<i>Staphylococcus aureus</i>			
	Fresh extracts		Oil extracts		Fresh extracts		Oil extracts		Fresh extracts		Oil extracts	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
	Dia. of clear Zone (mm)	Dia. of clear Zone (mm)	Dia. of clear Zone (mm)	Dia. of clear Zone (mm)	Dia. of clear Zone (mm)	Dia. of clear Zone (mm)	Dia. of clear Zone (mm)	Dia. of clear Zone (mm)	Dia. of clear Zone (mm)	Dia. of clear Zone (mm)	Dia. of clear Zone (mm)	Dia. of clear Zone (mm)
Lemongrass	8	8	13	13	8	8	9	9	10	13	15	16
Lime peel	10	10	14	14	9	9	9	9	10	12	16	17

Dia. = Diameter (mm)

### Antioxidant activity and inhibition DPPH radical:

#### Antioxidant activity:

The antioxidant activity of water and methanolic extracts of lemongrass and lime peels equivalent to gallic acid (GA) and butylated hydroxyanisole (BHA) were determined. Results in Table 3 demonstrated the



absorption at 593nm against (GA) and (BHA) as standards in water and methanolic extracts of lemongrass and lime peels. Gallic acid was found to be the strongest antioxidant in both extracts; whereas BHA proved to be a weak antioxidant. Methanolic extract of lemongrass showed significant ( $p<0.05$ ) antioxidant activity equivalent to gallic acid (11.45 mg) and to BHA (33.56 mg). Meanwhile, water extracts of lemongrass and lime peels did not show a significant difference in antioxidant activity when expressed in terms of BHA and were significantly ( $p<0.05$ ) of high activity compared to the methanolic extracts of lemongrass and lime peels.

On expressing the antioxidant activity in terms of gallic acid as a standard, the methanolic extracts of lemongrass and lime peels showed slightly higher values; whereas on expressing in terms of BHA, the methanolic extract showed higher value compared to the water extract. Many studies have pointed to the effect of the poly-phenols on lipid oxidation in meat products (Gobert *et al.*, 2010; Sayago-Ayerdi *et al.*, 2009; Viuda-Martos *et al.*, 2010) and the antioxidant effect of the some plant essential oils on meat products was generally accepted (Mielnik *et al.*, 2008; Sayago-Ayerdi *et al.*, 2009). This antioxidant activity is related to the capacity of polyphenols to act as metal-chelators, free radical scavengers, hydrogen donors and inhibitors of the enzymatic systems responsible for initiating oxidation reaction.

#### DPPH radical scavenging assay;

DPPH radicals are widely used to investigate the scavenging activities of several natural compounds. The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability or radical scavenging activity. When DPPH radical is scavenged, the color of the reaction mixture changed from purple to yellow with decreasing of absorbance at wave length 517 nm, Djenane *et al.*, (2005) cleared that 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  $O_2$ .

**Table 3:** Antioxidant activity and inhibition DPPH radical of lemongrass and lime peel extracts

Antioxidant activity of water and methanolic extracts of lemongrass and lime peel equivalent to (GA) and (BHA)**				
Antioxidant activity(mg equivalent /g on dry weight basis)				
Item	Lemongrass extract		Lime peel extract	
	water	Methanolic	water	Methanolic
	Mean±SD	Mean±SD	Mean±SD	Mean±SD
GA	9.54±0.011 <sup>Cb</sup>	11.45±0.020 <sup>Ab</sup>	8.42±0.020 <sup>Db</sup>	9.69±0.025 <sup>Bb</sup>
BHA	18.74±0.016 <sup>Ba</sup>	33.56±0.025 <sup>Aa</sup>	18.01±0.031 <sup>Ca</sup>	17.83±0.021 <sup>Da</sup>
The inhibition (%) of DPPH radical with different extracts of lemongrass and lime peel				
Inhibition %	35.02±0.020 <sup>C</sup>	31.23±0.030 <sup>D</sup>	52.01±0.030 <sup>B</sup>	53.38±0.031 <sup>A</sup>
IC <sub>50</sub> µg of sample	620.22±0.791 <sup>D</sup>	679.05±0.000 <sup>A</sup>	459.06±0.952 <sup>C</sup>	45.21±0.810 <sup>D</sup>

All values are mean of triplicate determinations ± standard deviation (SD).

\* GA= Gallic acid. \*\* BHA= butylated hydroxyl anisole

IC<sub>50</sub>: Concentration required for 50 % inhibition

Means within column and row superscript with different letters are significantly different ( $P<0.05$ )

The inhibition % of DPPH radical with different extracts of lemon grass and lime peel are recorded in Table 3. Water extracts of lemon grass and lime peel inhibitions %s were 35.02 and 52.01; whereas they reached 31.23 and 53.38 % for methanolic extract respectively. Also, the data in Table 3 indicated that IC<sub>50</sub> was found to be 620.22 and 459.06µg for lemon grass and lime peel samples for water extract and 679.05 and 450.21µg for methanolic extracts, respectively. Water and methanolic extracts of lime peel were of higher inhibition DPPH radical percents compared to extracts of lemon grass. Since IC<sub>50</sub> means the concentration of the extract that is able to scavenge half of the DPPH free radical present in test solution, the lower this value the higher is the antioxidant activity of the extract (Khadri *et al.*, 2010).

#### Optimum concentration of lemon grass and Lime peel extracts:

The optimum concentrations of the lemongrass and lime peel oil extracts were added to chicken patties according to the results of preliminary trials. Through TBARS analysis, potential antioxidant properties of each of the two tested oil extracts were determined. For each screened test extract the concentration range used was from 0 to 1.0%. In order to obtain a greater effect on the assessment of the extracts' performance, doubling of lemongrass and lime peel extracts addition rates was used in patty processing i.e. 0, 0.01, 0.05, 0.10, 0.25, 0.50, and 1.0%. During screening, huge amount of data were collected, only the optimum concentrations of each of the two tested extracts are presented in Table 4. Thus, the optimum test extract addition rates based on the identified levels of antioxidant activity were determined and used as (0.25%) for both lemongrass and lime peel oil extracts.

**Table 4:** Optimum concentration of lemongrass and lime peel extracts determined in chicken patties

Medicinal herbs extracts	Concentration range screened %	Optimum working concentration range %	Optimum concentration determined %
Lemongrass	0 – 1	0.25 – 1.00	0.25
Lime peel	0-1	0.25 – 1.00	0.25

*Thiobarbituric acid reactive substances (TBARS):*

The data presented in Table 5 show the changes of TBARS values in the chicken patties containing optimum concentrations of oil extracts of lemongrass and lime peel stored at 4°C for 9 days. During the storage period, the screened tested extracts were effective as antioxidants and had lower TBARS values than the control samples. Lemongrass and lime peel oil extracts inhibited lipid oxidation throughout storage time. Generally, storage time significantly ( $P<0.05$ ) affected TBARS values, whereas treatment showed only little effect on TBARS values on the 3<sup>rd</sup> day compared to the increase of TBARS values which were noticed on the 6<sup>th</sup> and 9<sup>th</sup> days. This indicated that the optimum concentration of these two oil extracts used as antioxidants was effective against TBARS formation when incorporated into chicken patties. Lemongrass and lime peels extracts are rich sources of bioactive compounds e.g. flavonoides and vitamin C and hence their addition to chicken patties in the present study could cause an inhibition of the chain reactions during lipid oxidation and may exert higher health-promoting effects. de Moraes Barros *et al.*, (2012) reported that the antioxidant capacity of citrus was correlated both to vitamin C and phenolics. Also the citrus peels are good sources of bioactive compounds and minerals, and can be explored for their health promoting values in food products. They can be applied as a source of functional compounds, or as natural preservatives, improving the lipid oxidation of meals and fat products. Formanek *et al.*, (2001) and McCarthy *et al.*, (2001) reported that dried herbs and their essential oils were successfully used to reduce lipid oxidation in meat products.

*pH determination:*

The effect of the optimum concentrations of lemongrass and lime peel oil extracts on the pH values of chicken patties stored at 4°C for 9 days is shown in Table 5. At zero time, the pH of all the tested chicken patty samples including control had the same pH value (5.85). Control samples, generally, had higher pH values than the other samples throughout the storage time. The pH values of the control and tested chicken patties containing the tested extracts were

**Table 5:** Effect of the optimum concentration of lemongrass and lime peel extracts on pH changes and TBARS\* values of chicken patties stored at 4°C for 9 days

Chicken patties sample	Day 0 M ± SD	Day 3 M ± SD	Day 6 M ± SD	Day 9 M ± SD
pH values				
Control	5.85±0.010 <sup>D</sup>	5.95±0.007 <sup>C a</sup>	6.15±0.010 <sup>B a</sup>	6.25±0.050 <sup>A a</sup>
Lemongrass	5.85±0.010 <sup>D</sup>	5.92±0.002 <sup>C b</sup>	5.98±0.010 <sup>B b</sup>	6.12±0.004 <sup>A b</sup>
Lime peel	5.85±0.010 <sup>D</sup>	5.91±0.002 <sup>C b</sup>	6.03±0.067 <sup>B b</sup>	6.11±0.008 <sup>A b</sup>
TBARS values (malonaldehyde mg/ kg sample)				
Control	0.15±0.001 <sup>D</sup>	0.26±0.002 <sup>C a</sup>	0.33±0.009 <sup>B a</sup>	0.44±0.004 <sup>A a</sup>
Lemongrass	0.15±0.001 <sup>D</sup>	0.18±0.003 <sup>C b</sup>	0.30±0.003 <sup>B b</sup>	0.39±0.003 <sup>A b</sup>
Lime peel	0.15±0.001 <sup>D</sup>	0.19±0.002 <sup>C b</sup>	0.31±0.001 <sup>B b</sup>	0.39±0.002 <sup>A b</sup>

TBARS\* = Thiobarbituric acid reactive substances.

All values are mean of triplicate determinations ± standard deviation (SD).

Means within column and row superscript with different letter are significantly different ( $P<0.05$ ) significantly ( $p<0.05$ )

increased gradually throughout storage period. At the end of storage time, it was noticed that the pH value of the control samples was higher (6.25) than the two other tested samples. At the 9th day of storage, pH values of both chicken patty samples containing lemon grass and lime peel extracts were nearly similar (6.12 and 6.11 respectively). The increase in pH may be due to the accumulation of metabolites by bacterial action in meat and deamination of proteins (Jay, 1996). The changes in pH of chicken patty samples were significant ( $p<0.05$ ) due to storage and addition of lemon grass and lime peel extracts containing phenolic content.

In this concern, Vaithyanathan *et al.* (2011) and Sylvestre *et al.*, (2001) reported that the treatment due to pomegranate fruit juice phenolics resulted in increased ( $P<0.05$ ) pH values of meat through the storage period. Moreover, the storage of meat at refrigeration temperature (4 °C) causes many chemical reactions including enzymatic reactions.



*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 (Nevás *et al.*, 2004).

*Aerobic plate count (APC):*

Data in Table 6 show the effect of adding optimum concentrations of the lemongrass and lime peel oil extracts to the prepared chicken patties stored at 4°C for 9 days on aerobic plate count (APC). A remarkable increase was noticed in APC throughout storage, especially in the control sample at the 6<sup>th</sup> and 9<sup>th</sup> days (6.13, 7.33 Log CFU/g) respectively. It has been reported by Insausti *et al.*, (2001) that meat spoilage cannot be supposed to occur until Total Viable Count (TVC) counts reach 10<sup>6</sup>-10<sup>8</sup> CFU/g (limit of microbiological acceptability). In general, a significant decrease was noticed for all tested chicken patty samples in their aerobic plate count during the storage period (3-9 days) as compared to the control sample. Worth noting that the chicken patty samples containing the two extracts at 3 and 6 days were of lower APC than the 9<sup>th</sup> day of storage period. Thus, the results realized that the aerobic plate count (APC) decreased significantly with the addition of the tested extracts to chicken patties during storage at 4°C for 9 days.

*Mould and yeast counts:*

Mould and yeast counts of the prepared chicken patties containing the optimum concentrations of the two tested extracts during storage for 9 days at 4°C are given in Table 6. 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 tested extracts increased gradually in their mould and yeast counts till the end of storage period. A reduction in mould and yeast counts was observed at the 9<sup>th</sup> day of storage in the patties containing lemongrass (2.59 Log CFU/g) during the storage time compared to sample containing lime peel extract (2.79 Log CFU/g). Thus, addition of both tested natural extracts reduced the mould and yeast counts in the prepared chicken patties. In this concern, Al Bulushi *et al.*, (2010) found that as a result of the inhibition of spoilage microorganisms, levels of chemical compounds, for example, TMA, total volatile nitrogen, hypoxanthine and biogenic amines, which are chemical indicators of microbial spoilage of food, are also reduced.

**Table 6:** Changes in Aerobic plate count, mould and yeast counts of chicken patties stored at 4°C for 9 days

Chicken patties sample	Day 0 M ± SD	Day 3 M ± SD	Day 6 M ± SD	Day 9 M ± SD
Aerobic plate count (Log CFU/ g)				
Control	4.01±0.021 <sup>D</sup>	4.97±0.001 <sup>C a</sup>	6.13±0.035 <sup>B a</sup>	7.33±0.021 <sup>A a</sup>
Lemongrass	4.01±0.017 <sup>D</sup>	4.10±0.015 <sup>C a</sup>	4.18±0.015 <sup>B b</sup>	4.82±0.015 <sup>A b</sup>
Lime peel	4.01±0.001 <sup>D</sup>	4.12±0.010 <sup>C b</sup>	4.19±0.020 <sup>B b</sup>	4.85±0.006 <sup>A b</sup>
Mould and yeast counts (Log CFU /g)				
Control	2.12±0.010 <sup>D</sup>	2.81±0.010 <sup>C a</sup>	3.19±0.001 <sup>B a</sup>	3.61±0.026 <sup>A a</sup>
Lemongrass	2.12±0.010 <sup>D</sup>	2.35±0.020 <sup>C c</sup>		2.59±0.001 <sup>A c</sup>
Lime peel	2.12±0.010 <sup>D</sup>	2.40±0.030 <sup>C b</sup>	2.53±0.015 <sup>B b</sup>	2.79±0.002 <sup>A b</sup>

All values are mean of triplicate determinations ± standard deviation (SD).

Means within column and row superscript with different letters are significantly different ( $P < 0.05$ ).

*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 and decarboxylase activities which interact with each other (Latorre-Moratalla *et al.*, 2008; Suzzi and Gardini, 2003).

The effect of adding optimum concentrations of lemongrass and lime peel extracts to the prepared chicken patties stored at 4°C for 9 days on the formation of biogenic amines (histamine, tyramine and putrescine) were not detected in chicken patties at zero time of storage (Table 7). It was observed that storage time had a significant effect ( $p < 0.05$ ) for up to 9 days at 4°C on the formation of all the estimated biogenic amines.

Histamine concentrations varied from 4.15 to 10.85 mg/kg in the control sample during storage at 4°C for 9 days. Data in Table 7 showed that on 3, 6 and 9 days histamine concentrations of all chicken patty samples were significantly ( $p < 0.05$ ) increased gradually. The two tested extracts used in the present study were effective in

producing lower histamine concentrations than control samples over the storage period of chicken patties. Results showed that lemongrass extract was the more effective against histamine formation. Masniyom (2011) reported that biogenic amines, including agmatine, cadaverine, dopamine, histamine, noradrenaline, putrescine, serotonin, spermidine, spermine, tryptamine and tyramine are products of bacterial spoilage and their contents are often used as an index to assess the keeping quality and shelf-life of fishery products. Thus, an increase in biogenic amines content reflects the decomposition of amino acids during storage of seafood varied with differences in species, concentrations of substrate in tissue, handling procedures and conditions of storage.

Tyramine concentrations, in the present study, were found in the safe range and lower than the permitted level. The permitted level of tyramine in foods is 100- 800mg/kg, while 1080mg/kg is toxic (Shalaby 1996). They varied in the control patty sample from 5.29 to 12.93 mg/kg during the 3-9 days of storage at 4°C (Table 7). The tyramine contents in the chicken patties containing the tested extracts were less than the control level all over the storage period. Using of the tested extracts was found to significantly reduce ( $P<0.05$ ) tyramine formation. The lemongrass extract showed the lowest tyramine content (7.43 mg/kg) at 9<sup>th</sup> day of storage; 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 it has been previously reported by Ruiz-Capillas and Jimenez-Colmenero (2004).

The addition of the tested plant extracts in the preparation of the chicken patties stored at 4°C for 9 days significantly affected ( $p<0.05$ ) the formation of putrescine. Its concentration increased up to 12.31, 6.41 and 7.81 gm/kg in the control and patty samples containing lemongrass and lime peel extracts respectively at the end of the storage period (Table 7). The highest putrescine concentration was observed in the control sample; while the lowest was for chicken samples containing lemongrass extract. Thus, the addition of natural plant extracts was found to be effective in reducing the formation of putrescine. This reduction could also be indirectly due to the antimicrobial activities of the used 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 lemon grass and lime peel extracts to chicken patties resulted in a marked reduction in histamine, tyramine and putrescine formation.

**Table 7:** Effect of the optimum concentration of lemongrass and lime peel extracts on concentration of biogenic amines in chicken patties stored at 4°C for 9 days.

Chicken patties sample	Day 0 M ± SD	Day 3 M ± SD	Day 6 M ± SD	Day 9 M ± SD
Histamine concentration (mg/kg)				
Control	ND	4.15±0.025 <sup>C a</sup>	6.54±0.030 <sup>B a</sup>	10.85±0.040 <sup>B a</sup>
Lemon grass	ND	2.95±0.035 <sup>C c</sup>	3.85±0.025 <sup>B c</sup>	6.73±0.014 <sup>A c</sup>
Lime peel	ND	3.01±0.015 <sup>C b</sup>	3.95±0.010 <sup>B b</sup>	6.92±0.015 <sup>A b</sup>
Tyramine concentration (mg/kg)				
Control	ND	5.29±0.030 <sup>C a</sup>	7.89±0.010 <sup>B a</sup>	12.93±0.020 <sup>A a</sup>
Lemon grass	ND	3.85±0.020 <sup>C b</sup>	5.94±0.040 <sup>B b</sup>	7.43±0.015 <sup>A c</sup>
Lime peel	ND	3.74±0.010 <sup>C c</sup>	5.90±0.059 <sup>B b</sup>	7.92±0.020 <sup>A b</sup>
Putrescine concentration (mg/kg)				
Control	ND	6.09±0.020 <sup>C a</sup>	8.03±0.025 <sup>B b</sup>	12.31±0.020 <sup>A a</sup>
Lemon grass	ND	3.42±0.025 <sup>C c</sup>	4.85±0.030 <sup>B c</sup>	6.41±0.030 <sup>A c</sup>
Lime peel	ND	4.05±0.030 <sup>C b</sup>	5.21±0.010 <sup>B b</sup>	7.81±0.030 <sup>A b</sup>

All values are mean of triplicate determinations ± standard deviation (SD).

Means within column and row superscript with different letters are significantly different ( $P<0.05$ ).

#### Volatile basic nitrogen (VBN):

Effects of adding lemongrass and lime peel oil extracts to the chicken patties on the volatile basic nitrogen % were determined and compared (Table 8). At zero time of the refrigerated storage period, the VBN of the tested patty samples showed no significant differences between the control and the other studied samples containing lemongrass and lime peel extracts. During storage period of the tested patty samples for 9 days at 4°C the VBN % tended to gradual slight increase. The control sample had higher VBN % than the extract-treated samples. This increase of VBN in patties sample during cold storage might be attributed to the breakdown protein as a result of activity of microbial strains and proteolysis enzymes. Addition of lemongrass and lime peel oil extracts caused decrease in VBN%. Sacchetti *et al.*, (2005) found that the treatment with natural plants (5% thyme and marjoram) was effective in delaying the formation rate of TVB-N during subsequent cold storage. This is due to the role of such spices on microbial population and the growth of bacteria as antimicrobial agent.

**Table 8:** Volatile basic nitrogen (%) of chicken patties samples during storage at 4°C for 9 days.

Chicken patties sample	Day 0	Day 3	Day 6	Day 9
	M ± SD	M ± SD	M ± SD	M ± SD
Control	0.034±0.001 <sup>D</sup>	0.042±0.001 <sup>C</sup>	0.046±0.003 <sup>B</sup>	0.057±0.001 <sup>Aa</sup>
Lemongrass	0.034±0.001 <sup>D</sup>	0.040±0.002 <sup>C</sup>	0.045±0.002 <sup>B</sup>	0.049±0.001 <sup>Ab</sup>
Lime peel	0.034±0.001 <sup>D</sup>	0.041±0.002 <sup>C</sup>	0.046±0.001 <sup>B</sup>	0.051±0.001 <sup>Ab</sup>

All values are mean of triplicate determinations ± standard deviation (SD).

Means within column and row superscript with different letters are significantly different (P<0.05).

### Conclusion:

Lemongrass and lime peel extracts are promising sources of bioactive compounds which can be used for their health properties in food products. Also, the extracts can be used as natural meat preservatives with both antioxidants and antimicrobial activities against food borne pathogens and spoilage organisms. Comparison of control and treated chicken patty samples with lemongrass and lime peel extracts during storage at 4°C for 9 days showed that the addition of these extracts was effective as antioxidant and anti microbial agents for improving the properties of the prepared chicken patties from a quality and safety view point. It was concluded that the optimum concentration of the investigated natural extracts were effective in reducing TBARS, the aerobic plate count (APC), mould and yeast counts as well as against the formation of biogenic amines (histamine, tyramine and putrescine) when incorporated into chicken patties.

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