Effect of Adding Green Tea Extract, Thyme Oil and/or their Combination to Luncheon Roll Meat during Refrigerate Storage

[*]Ferial M. Abu-Salem, Esmat A. Abou-Arab, Hayam M. Ibrahim and Azza A. Abou-Arab

Department of Food Technology, National Research Centre, Dokki, Cairo, Egypt
[*]ferial_mas@yahoo.com

Abstract: Green tea extract (GTE) and thyme oil extract (TOE) were added individually and/or in combination during the preparation of luncheon roll meat. Some chemical and sensory attributes of the prepared luncheon were investigated during storage at 4 ºC for 4 months. Luncheon samples with (GTE) and (TOE) realized significant reduction towards lowering biogenic amines (BAs) formation, thiobarbituric acid reactive substances (TBARS) levels, volatile basic nitrogen (VBN) and total acidity % relative to control sample. Reduction effect was ranked as: combination of GTE and TOE > GTE > TOE. Phenolic content in green tea extract was significantly higher than in thyme oil extract. Antioxidant activities of (GTE) and (TOE) were evaluated using DPPH radical scavenging assay. Sensory evaluation was acceptable with good scores for luncheon samples. This study indicated that the addition of natural antioxidant extracts (GTE and TOE) during luncheon meat processing could enhance quality and provide safer product.
[Ferial M. Abu-Salem, Esmat A. Abou-Arab, Hayam M. Ibrahim and Azza A. Abou-Arab. Effect of Adding Green Tea Extract, Thyme Oil and/or their Combination to Luncheon Roll Meat during Refrigerate Storage. Journal of American Science 2011; 7(7):538-548]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: Green tea extract-thyme oil extract-antioxidant-luncheon roll meat-chemical and sensory attributes-refrigerated storage.

1. Introduction:

The inhibition of oxidation process is very important in foodstuffs. Oxidative processes lead to the degradation of lipids and proteins and are one of the primary mechanisms of quality deterioration and limiting the shelf life in meat and meat products (Pizzalle *et al*., 2002., Lui *et al*., 2010).Oxidative processes also responsible for the reactive oxygen species (ROS) that cause oxidative changes in carbohydrate, DNA, lipids and protein (Koşar *et al*., 2008).

Antioxidants can delay or inhibit the oxidation propagation of oxidizing chain reactions in the oxidation process (Zheng and Wang, 2001) and considered as important nutraceuticals because of many health benefits (Valko, 2007).

Due to concerns about toxicological safety of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), naturally derived antioxidants are perceived as better and safer than synthetics. Meat products containing natural antioxidants, as opposed to synthetic derivatives, are more desirable from a consumer viewpoint (Mitsumoto, *et al*., 2005; Yen *et al*., 2002). Moreover; natural antioxidants are reported to be more powerful than the synthetic antioxidants, especially, rosemary, sage, and green tea extracts. Therefore, natural antioxidants are very important for human health (Bozkurt, 2006).

The interest in the antioxidant activity of plant extracts has become larger and very important 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 for aging process (Mothana, *et al*., 2008).

The effects of plant extracts or essential oils classified as greatly 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).

Compounds from herbs and spices contain many phytochemicals which are potential sources of natural antioxidants including phenolic diterpenes, flavonoids, tannins and phenolic acids (Dawidowicz *et al*., 2006).Various plant materials containing phenolic compounds have been demonstrated to be effective antioxidants in model systems (Lui *et al*., 2010).

Green tea leaf extracts are becoming increasingly important as a functional food in the diet because of their high poly-phenols contents. (Manzocco *et al*., 1998).These tea compounds promote health by preventing lipid oxidation and providing antibacterial, anti-carcinogenic and antiviral ability (Katiyar and Mukhtar, 1998; Yang *et al*., 2000).The strong free-radical-scavenging ability plus the iron-chelating effects of tea catechins provide a plausible mechanism for the antioxidant effects of added tea catechins in meat system *in vitro* (Tang *et al*., 2002).

Thymbra spicata (a member of *Lamiaceae* family) is used in meat, fish and foodstuffs as a spice.

The major essential oil components derived from *T. spicata* are carvacrol (~86%), thymol (~4%), *E*-3-caren-2-ol (~3%), *C*-terpinene (~1.9%) (Özel. *et al*., 2003). The essential oil has antioxidant, antibacterial, and antifungal activities (Baydar *et al*.,,2004; Dorman, 2003 and Hanci *et al*.; 2003).

Biogenic amines 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 (Önal, 2007).

Luncheon meat is an important industrial meat product, one of the most acceptable food products, widely consumed and used for fast meats. It is usually consist of finely chopped meat and fat with or without some added cereals, cured with salt and nitrite and heat processed (Ranken, 1984).Minced meat undergo oxidative changes and develop rancidity more quickly than intact muscle since grinding exposes more of the muscle surface to air and microbial contamination (Mitsumoto *et al*., 2005). Much attention has been focused on extracts from herbs and spices which have been used traditionally to improve the sensory characteristics and extend the shelf-life of foods (Botsoglou *et al*., 2003). Refrigeration storage is usually the most common preservative method of meat and meat products. In order to extend refrigerated storage time, antimicrobial and antioxidant additives are added to muscle foods (Solomakos *et al*., 2008).So, the use of natural antioxidants to produce luncheon roll meat of high quality and safety is important.

The present study aimed to: i) evaluate the total phenolic compounds, total antioxidant activity of green tea and thyme leaves. ; ii) investigate the effect of using green tea extract and thyme oil extract, applied individually and/or in combination, on biogenic amines, thiobarbituric acid reactive substances (TBARS), volatile basic nitrogen (VBN) and total acidity % as well as sensory attributes of luncheon roll meat during refrigerated storage at 4 ºC for 4 months.

2. Materials and Methods:

Chemicals:

Antioxidant standards of gallic acid (GA), butylated hydroxyanisole (BHA), 1- naphthalene-acetic acid (NAA), 6-benzyladenine (BA),1,1,3,3-tetraethoxypropane (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)**.**

Materials:

Frozen beef meat and back fat were obtained from a local slaughter house. The ingredients used in preparation of the luncheon roll meat were purchased from national food ingredients market. Dried green tea (*Camellia sinensis L*.) leaves and thyme (*Thymbra spicata*, a member of *Lamiaceae* family) leaves were obtained from local market, then powdered and kept in tightly closed amber glass containers.

Preparation of plant extracts:

The green tea extract and thyme oil extract were prepared according to the methods described (Bozkurt , 2006) with a slight modification:

Green tea extract:

About 20 g of air dried and ground green tea leaves were mixed with 500 ml of boiling water and left for 5 min. The extract was obtained by filtration and the soluble solid content of green tea extract was measured using a refractometer (ATAGO, N1E, Japan).

Thyme oil extract:

The separation of essential oil from the air dried thyme leaves was conducted by steam distillation in a Clavenger apparatus for 3 h. The essential oil was dried over anhydrous sodium sulfate and stored in a dark glass bottle until using in formulation of luncheon roll meat.

Luncheon rolls meat preparation:

The luncheon roll meat batter was prepared according to the following formula: 75% minced lean beef meat, 25 % back beef fat, 2.5% salt, 4% soy bean, 1% spices, 1% ground garlic, 2% onion, 4% potato starch, 0.15% $Na\ HP_2\ O_7$, 0.2% STPP, 0.1% sodium nitrite, 0.02% soy protein isolate and 20% ice. The procedure used in preparation of luncheon roll was carried out according to the protocol described by Zhanc *et al*. (2004) with some modifications in a local Egyptian factory pilot plant. Processing of the batter involved blending the frozen minced meat and fat with the other ingredients. The prepared luncheon meat batter was divided into four parts then green tea extract and thyme oil extract

were added: i) Luncheon roll meat sample (1) prepared as control sample without adding any natural extract; ii) Sample (2) was prepared by adding 300ppm green tea extract; iii) Sample (3) was prepared by adding 300ppm of thyme oil extract and iv) Sample (4) was prepared by adding 150ppm green tea extract plus 150ppm of thyme oil extract. Each batter was mixed well to be homogenous and stuffed into round bottom tubes 5-cm diameter and 15-cm long, fibrous casings (about 500g each) and sealed with plastic cable-ties (Maplin electronics, Dublen, Ireland). Luncheon roll meat samples(1-4) were cooked in a thermo statically controlled KERRES smoke-air steam oven (Type CS 350,Raicher-und-Kochanlagen,D-71560 Sulzbach-Murr, Germany) set at 80ºC until the internal temperature reached ~ 67 to 69 ºC, then held under refrigeration (~1-4ºC) for 10 h. After processing, the luncheon roll meat samples were stored for 4 months at 4ºC. The analyses were performed on luncheon samples at different periods of storage.

Chemical analyses:

Total phenolic compounds content:

Total phenolic compounds were estimated according to the method described by Meda *et al.* (2005). A known quantity of green tea or thyme leaves was taken in 100 ml conical flask and 25 ml 0.3 N HCl in methanol was added, and then kept on a shaker at 150rpm for an hour. After shaking, crude extract was filtered through filter paper (Whatman No.1). The filtrate obtained was evaporated to dryness in a boiling water bath. Hot water was added to the residue and final volume was adjusted to 100 ml with distilled water, then 1 ml aliquot was taken in a test tube and 1 ml each of Folin-Ciocalteu reagent (dilute 1:2) and 35% sodium carbonate were added and mixed well. After 10 min, 2 ml of distilled water was added and intensity of the color was recorded at 620nm in the UV spectrophotometer (model T80 x UVNIS Spectrometer PG Instruments Ltd) against the reagent blank. The content of total phenolic compounds was determined using a standard curve prepared with gallic acid.

Total antioxidant activities:

Fine ground powder (150mg) of green tea leaves or thyme leaves was taken in 250 ml conical flasks and 50 ml water was added then kept on a shaker at 150rpm for an hour. The flask contents were filtered using filter paper (Whatman No.1). Similarly, methanol extracts were also prepared. The filtrate was used directly for 1.1-dipheny-2-picrythydrazyl (DPPH) assay without storage.

DPPH radical scavenging activity:

The method was adopted as described Sharma and Bhat, (2009). Methanolic and water extracts of green tea leaves and thyme leaves 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 (200 mM) in methanol was added to the sample tube and mixed vigorously for 15s. The sample tube was then kept in a water bath at 37ºC 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. DPPH radical scavenging effect was calculated as "inhibition of percentage" according to the following formula:

Inhibition of percentage (%)=$[A_{c\,(0)}-A_{a\,(t)}/A_{c\,(0)}]$x100

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.

Total acidity:

The total acidity was obtained by direct titration with (0.1M) NaOH and phenol- phthalein as indicator (Egan *et al.*; 1987).Ten grams of each sample were magnetically stirred in a total volume of 100 ml distilled water for 30 min, and filtered. 10 ml filtrate was titrated with (0.1M) NaOH using three drops of phenolphthalein as indicator. The total acidity was calculated as 1.0 ml of (0.1M) NaOH = 0.0090 g lactic acid.

Total volatile basic nitrogen (VBN):

A sample (10 g) was minced with 100 ml distilled water and washed into a distillation flask with 100 ml distilled water; then 2g 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 luncheon meat as described Pearson (1976).

Thiobarbituric acid reactive substances (TBARS):

The TBARS values were determined spectrophotometricaly according to the method described by Byun *et al.* (2001). Homogenized luncheon samples (2g) were taken and TBARS were extracted twice with 10 ml of 0.4 M perchloric acid. Extracts were collected and made up to 25 ml with 0.4 M perchloric acid and then centrifuged for 5 min at 1790*g*. After centrifugation, 1ml of the extract was poured into a glass test-tube with a stopper. TBARS

reagent (5ml) was added and the extract was heated in a boiling water bath for 35 min. After cooling in tap-water, the absorbance of the sample was read against the appropriate blank at 538 nm. A standard curve was prepared using 1, 1, 3, 3-tetraethoxypropane (TEP).

Biogenic amines:

Histamine, tyramine, putrescine and tryptamine were extracted as follows: five grams of the sample were blended with 25 ml 5% trichloroacetic acid. Filtration was achieved using filter paper whatman No.1. Five ml of the extract were transferred into a suitable culture tube with 4 g NaCl and 1ml of 50% NaOH then shacked for 2 min. Centrifugation were carried out for 5 min at 5000xg and the upper layer was transferred to 50 ml separating funnel. To the upper layer extract, 15 ml of *n*-heptane were added and extracted 3 times with 1ml portions of 0.2 N HCl. The extracts were collected in a glass stoppard tube and evaporated to dryness using water bath at 95ºC with the aid of a gentle current of air. This was followed by the formation of Dansylamines as described by (Mijalla and Eerola, 1993).

Biogenic amines concentrations were determined Deabes, (2000) using HPLC. The HPLC system (Waters 600) equipped with delivery system. HPLC column: Reverse phase C18 Nucleosil column 250x4 mm, 10 µm packing, (Macherey-Naggl). The detection was performed using U.V detector (waters 486) at 254 nm wavelength, using linear program of 25 min period and 1 ml/min constant solvent flow rate. Data were integrated and recorded using a Millennium Chromatography; Manger software 2010, (Waters, Milford MA 01757).

Sensory evaluation:

The sensorial criteria (taste, flavor, texture and color) of the four luncheon samples under investigation were evaluated by twenty five untrained panelists. Luncheon samples were cut into 2mm thick slices and served in numerically-coded glass petri dishes. Each panelist received four coded samples (one from each tested samples) then independently evaluated the luncheon meat for texture, flavor, color and taste using a 5-point hedonic scale (1= extremely poor, 2 = poor, 3= acceptable, 4= good, 5= excellent), according to the described method Lavrova and Krilov, (1975).

Statistical analysis:

The conventional statistical methods were used to calculate means and standard deviations. All the measurements were replicated three times and the data are presented as mean ± SD. The effects of natural antioxidant extracts addition and storage period 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 (PC-STAT, 1985).

3. Results and Discussion

Total phenolic compounds:

Total phenolic compounds contents of green tea and thyme leaves are recorded in Table1. The total phenolic compounds content (on dry weight basis) in green tea leaves (34.32mg/g) was significantly higher ($p< 0.05$) than the corresponding in thyme leaves (24.30 mg/g). Green tea leaf extracts had high polyphenols content (Manzocco *et al*., 1998). It has been mentioned Wanasundara and Shahidi, (1998) that polyphenols content in green tea can increase up to 36% (dry basis) due to climate, season or variety. Catechins is a predominant group of polyphenols present in green tea leaves composed of four compounds epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate (Zhong *et al*., 2009). The phenolic monoterpenes in thyme, thymol and carvacrol, are the primary compounds that contribute to the characteristic aroma of its essential oil and also known to inhibit lipid peroxidation (Yanishlleva *et al*., 2006). Phenolic compounds are very important plant constituents because of their scavenging ability due to the presence of their hydroxyl groups (Oktay *et al*., 2003).

Table (1): Total phenolic compounds content of green tea and thyme leaves

Total phenolic content of green tea and thyme leaves (mg/g dry weight basis) (Mean ±SD)	
Green tea leaves	Thyme leaves
34.32 ± 0.59^{a}	24.30 ± 0.37^{b}

All values are mean of triplicate determinations ± standard deviation (SD)
Means within row with different letters are significantly different ($P<0.05$)

Antioxidant activity:

The antioxidant activity of water and methanolic extracts of green tea and thyme leaves equivalent to gallic acid (GA) and butylated hydroxyanisole (BHA) were determined. The results in (Table 2) demonstrated the absorption at 593nm against (GA) and (BHA) as standards in water and methanolic extracts of green tea and thyme leaves. Gallic acid was found to be the strongest antioxidant in both water and methanolic extracts, whereas BHA proved to be a weak antioxidant. Methanolic extract of green tea showed significant ($p<0.05$) high antioxidant activity equivalent to gallic acid (11.55mg) and to BHA (34.71mg). Meanwhile, water extracts of green tea and thyme leaves did not show 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 green tea and thyme. On expressing the antioxidant activity in terms of gallic acid as a standard the methanolic extracts of green tea and thyme leaves showed slightly higher values; whereas on expressing in terms of BHA the methanolic leaves extract showed higher value compared to the water extract. It was reported that green tea leaves (*Camellia sinensis L.*) contain 10-30% (dry leaf weight) of polyphenols, including catechins, flavonols, flavanones, phenolic acids, glycosides and the aglycones of plant pigments (Farhoosh *et al*., 2007). The author added that tea polyphenols have a stronger anti-oxidative activity than butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and DL-α-tocopherol; and the toxicity of tea polyphenols is lower than that of BHA, BHT and DL-α-tocopherol.

Table (2): Antioxidant activity and inhibition DPPH radical of green tea and thyme leaves

Antioxidant activity of water and methanolic extracts of green tea and thyme leaves equivalent to (GA)* and (BHA) (Mean±SD)**

	Antioxidant activity(mg equivalent /g on dry weight basis)			
	Green tea leaves extract		Thyme leaves extract	
Item	**water**	**Methanolic**	**water**	**Methanolic**
GA	9.69 ± 0.16^{b2}	11.55 ± 0.16^{b1}	8.62 ± 0.11^{b3}	9.76 ± 0.05^{b2}
BHA	19.74 ± 012^{a2}	34.71 ± 0.16^{a1}	18.75 ± 0.13^{a3}	17.90 ± 0.09^{a4}
The inhibition (%) of DPPH radical with Different extracts of green tea and thyme leaves				
Inhibition %	35.58 ± 0.20^{b3}	31.78 ± 15^{b4}	53.39 ± 0.33^{b2}	54.37 ± 0.30^{b1}
IC_{50} µg of sample	622.15 ± 5.18^{a2}	682.82 ± 1.06^{a1}	462.09 ± 0.79^{a3}	452.21 ± 1.67^{a4}

Sample 1= control without any extract, Sample 2=with green tea extract, Sample 3=with thyme oil extract, Sample 4= with combination of green tea extract + thyme oil extract.

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

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

IC_{50}: Concentration required for 50 % inhibition

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

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 517nm. The inhibition % of DPPH radical with different extracts of green tea and thyme leaves are recorded in Table 2.Water extracts of green tea and thyme leaves inhibitions % were 35.58 and 53.39 %; whereas they reached 31.78 and 54.37%s for methanolic extract respectively. Also, the results in the same Table indicated that IC_{50} was found to be 622.15 and 462.09µg for green tea and thyme samples for water extract and 682.82 and 452.21µg for methanolic extracts, respectively. Water and methanolic extracts of thyme were of higher inhibition percent than DPPH radical compared to water and methanolic extract of green tea leaves. Tea extracts are powerful antioxidants, mainly owing to the presence of catechin compounds which are effective free radical-scavengers (Salah et *al*., 1995). Also the anti-oxidative effect of thyme ethanol extract is associated with the high content of carvacrol and thymol (Nguyen *et al*., 2000).

The present work was continued to evaluate the effects of adding green tea and thyme extracts on some chemical characters: Thiobarbituric acid reactive substances (TBARS), Biogenic Amines (BAs), volatile basic nitrogen (VBN) and total acidity of the prepared luncheon meat samples during refrigerated storage at 4°C in order to shed more light on their safety and quality.

Thiobarbituric acid reactive substances (TBARS):

TBARS values were affected significantly ($p< 0.05$) by the storage time and addition of green tea extract, thyme oil extract and their combination to luncheon rolls meat (Table 3). TBARS values of the luncheon meat samples (1-4) showed no differences between all prepared samples with 0.197 mg malonaldehyde/kg luncheon meat at zero time. These values increased for all the investigated samples gradually during storage period. Addition of the natural antioxidant extracts (green tea and thyme extracts) reduced TBARS values of luncheon meat samples compared to control sample. For instance, the highest TBARS values at the end of storage were found in control sample; while the lowest were noticed in sample 4 (containing combination of green tea plus thyme oil extracts).Thus, the best antioxidative effect was obtained by the combination of green tea and thyme oil extracts, as noticed in sample 4 containing the combination of both extracts which had significant lower TBARS concentrations than those containing the individual extracts. Green tea extract decreased the formation of TBARS and added tea catechins significantly reduced the TBARS values of beef, duck, ostrich, pork and chicken during 10 days refrigerated storage as reported (Bozkurt, 2006., Tang *et al*., 2001 and Choi *et al*., 2003).The phenolic mono-terpenes in thyme, thymol and carvacrol are known to inhibit lipid per-oxidation, *p*-cumene-2, 3-diol isolated from thyme was reported as a strong antioxidant (Yanishlleva *et al*., 2006). So, green tea extract and thyme oil extract containing high level of total phenolic compounds content can contribute in retardation of lipid per-oxidation and hence for gaining low TBARS concentration during luncheon roll meat processing.

Table (3): Thiobarbituric acid (TBARS) values (mg malonaldehyde/kg) of the luncheon rolls meat samples during storage at 4°

Thiobarbituric acid values (mg / kg) of Luncheon meat samples (Mean ± SD)				
Storage period (month)	***Sample 1**	***Sample2**	***Sample 3**	***Sample 4**
0	0.197 ± 0.003^{e}	0.197 ± 0.003^{e}	0.197 ± 0.003^{e}	0.197 ± 0.003^{e}
1	0.272 ± 0.004^{d1}	0.231 ± 0.004^{d2}	0.238 ± 0.004^{d2}	0.216 ± 0.004^{d3}
2	0.389 ± 0.004^{c1}	0.286 ± 0.004^{c3}	0.295 ± 0.004^{c2}	0.242 ± 0.003^{c4}
3	0.459 ± 0.006^{b1}	0.314 ± 0.005^{b2}	0.322 ± 0.004^{b2}	0.284 ± 0.004^{b3}
4	0.508 ± 0.008^{a1}	0.370 ± 0.002^{a2}	$0.378\pm0.oo3^{a2}$	0.313 ± 0.005^{a3}

*Sample 1= control without any extract, Sample 2=with green tea extract, Sample 3=with thyme oil extract, Sample 4= with combination of green tea extract + thyme oil extract.
All values are mean of triplicate determinations ± standard deviation (SD).
Means within column and row with different letters and numbers are significantly different ($P<0.05$)

Biogenic amines (BAs):

The effect of adding green tea and thyme oil extracts and their combination to the prepared luncheon meat samples stored for four months at 4°C on formation of biogenic amines is presented in Table 4. Tyramine, histamine, putrescine and tryptamine were not detected in luncheon meat samples at zero time of storage period. It was generally observed that storage time and addition of natural antioxidant extracts caused a significant effect ($p<0.05$) on the formation of all the estimated biogenic amines.

Tyramine concentration increased gradually in all luncheon meat samples during storage period. Tyramine concentration in red meat and in sausages increased during 36 days and during 7 days storage period, respectively (Vinci and Antonelli, 2002 and Eerola *et al*., 1997). Sharp increase was observed in tyramine concentrations of control sample and reached 220 mg/kg at the end of storage time. This concentration was found in the safe range and within the permitted level. The permitted level of tyramine in foods is 100-800 mg/kg; while 1080 mg/kg is toxic (Shalaby, 1996).The tyramine contents in the studied luncheon meat samples containing green tea extract, thyme oil extract and their combination were found to be less than that of control. During the storage period the highest tyramine level was observed in control sample (1) and the lowest tyramine formation was in sample 4 (containing combination of green tea extract plus thyme oil extract). Thus, addition of this natural antioxidant extracts decreased tyramine formation in the investigated luncheon meat samples in the following order: green tea extract + thyme oil extract > green tea extract > thyme oil extract. The reduction in tyramine formation by 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 (Ruiz-Capillas and Jimenez-Colmenero, 2004).

Histamine concentrations increased significantly during storage period in all the investigated luncheon samples (Table 4). It was observed that histamine level was the highest in control sample 1; whereas its level varied from 21mg/kg at 1st month to 243 mg/kg at the end of storage period at 4°C. Significant decrease in histamine levels was noticed in luncheon meat samples (2-4) compared to the control. The order of decline in histamine level can be arranged as follows: green tea extract + thyme oil extract > green tea extract > thyme oil extract.

Mixing of the tested plant antioxidant extracts to the luncheon meat samples stored at 4°C affected significantly ($p<0.05$) putrescine formation (Table 4). Its concentration was noticed to increase gradually during the refrigerated storage period. At the end of storage period, the highest putrescine concentration was observed in control sample, while the lowest was for sample 4 containing combined green tea and thyme oil extracts. Apparently, this reduction in putrescine level could be due to the antimicrobial activities of the natural extracts. Ranking in decreasing order of the effectiveness of the used extracts on putrescine concentration in the luncheon samples was: combination of green tea and thyme oil extracts > green tea extract >thyme oil extract. The decrease in putresine formation can also, achieved by reduction of total aerobic viable count. Antimicrobial activity of green tea extract and T. *spicata* has been previously reported (Baydar *et al*., 2004.; Higdon and Frei, 2003.; Manzocco *et al*., 1998 and Tang.; et *al*., 2001).

Spices (including thyme), in general, are well known for their antimicrobial ability mainly due to their content of phenolic compounds. The possible mechanisms for antimicrobial effect of phenolic compounds include altering microbial cell permeability; interfering with membrane function including electron transport, nutrient uptake, protein and nucleic acid synthesis, and enzyme activity; interacting with membrane proteins causing deformation in structure and functionality; and substituting alkyls into phenol nucleus (Zhang *et al*., 2010).

Reduction of tyrptamine in luncheon samples (2-4) containing the natural extracts under investigation relative to control sample (Table 4) was about 10.27% for sample 2 (containing green tea extract), 6.10% for sample 3 (mixed with thyme oil extract) and 22.91% for sample 4 (mixed with combination of green tea and thyme oil extracts) at the end of storage time. Storage time had a significant effect ($p<0.05$) on tryptamine formation, its concentrations in the tested samples increased significantly during storage period and reached at the end of 4th month to 10.60, 22.02, 29.01and 37.01mg/kg in the three luncheon samples(2-4), respectively. So, combination of green tea and thyme oil extracts realized the lowest tryptamine contents over storage time in the studied luncheon roll meat samples.

Therefore, it can be stated that the addition of natural plant extracts (green tea, thyme oil and their combination) realized significant reduction and showed marked effect towards histamine, tyramine, putrescine and tryptamine formation in the refrigerated stored luncheon roll meat samples.

Volatile basic nitrogen (VBN):

Effects of addition green tea and thyme oil extracts individually or combined to the studied luncheon roll meat on the volatile basic nitrogen % were determined and_compared (Table 5). At zero time of the refrigerated storage period, the VBN of luncheon meat samples showed no significant differences between the control sample and the studied samples (2-4) containing green tea extract, thyme oil extract and their combination. During refrigeration at 4°C and storage for 4 months of the luncheon meat samples the VBN % tended to increase gradually. The control sample had higher VBN % than samples 2, 3 and 4. The luncheon sample 4 mixed with combination of the two extracts was significantly of lower VBN% than sample 2 (with green tea extract) or sample 3 (with thyme oil extract). Addition of green tea or thyme oil extracts either individual or combined caused decrease in VBN% and thus improvement of the luncheon meat characters can occur. In pork sausages, green tea powder could partly substitute nitrite, and resulted in lower TBARS value and decreased volatile basic nitrogen contents compared to samples prepared with nitrite alone (Choi *et al*., 2003).

Total acidity:

Total acidity (% lactic acid) of all the tested luncheon meat samples stored for 4 months and refrigerated at 4ºC were of the same acidity % at zero time of storage (Table5). Total acidity percentage was observed to increase throughout the storage period. The initial amount of total acidity in luncheon increased with increasing storage time was reported (Al Bachir, 2005). Higher acidity of meat may be a positive indicator of storability due to the inhibition of microbial growth by acid and can improve the storability of the luncheon meat treated with natural extracts (AL-Bachir and Mehio, 2001).

Table (4): Changes of Biogenic amine concentrations of the luncheon roll meat sample during storage at 4ºC.

Storage period (month)		Biogenic amine concentrations (mg / kg) of luncheon meat samples (Mean ± SD) Tyramine	Histamine	Putrescine	Tryptamine
*Sample 1	0	ND	ND	ND	ND
	1	25.01±0.110^{d}	21.00±0.261^{d}	42.00±0.264^{d}	20.03±0.101^{d}
	2	41.08±0.149^{c}	83.05±0.474^{c}	79.09±0.159^{c}	32.02±0.371^{c}
	3	98.00±0.198^{b}	135.07±0.203^{b}	122.00±0.238^{b}	39.02±0.263^{b}
	4	220.01±0.265^{a}	243.04±0.043^{a}	162.00±0.140^{a}	48.01±0.259^{a}
Sample 2	0	ND	ND	ND	ND
	1	19.12±0.093^{d}	17.07±0.218^{d}	30.08±0.150^{d}	16.13±0.080^{d}
	2	28.00±0.049^{c}	55.08±0.281^{c}	49.03±0.161^{c}	24.01±0.362^{c}
	3	41.08±0.162^{b}	79.01±0.263^{b}	75.07±0.165^{b}	33.01±0.165^{b}
	4	69.02±0.237^{a}	86.09±0.083^{a}	89.01±0.207^{a}	43.08±0.216^{a}
Sample 3	0	ND	ND	ND	ND
	1	22.08±0.167^{d}	19.08±0.158^{d}	33.00±0.173^{d}	18.02±0.170^{d}
	2	34.01±0.274^{c}	59.09±0.153^{c}	55.00±0.133^{c}	26.01±0.278^{b}
	3	43.03±0.223^{b}	82.00±0.104^{b}	78.06±0.193^{b}	36.01±0.424^{c}
	4	73.013±0.263^{a}	93.01±0.133^{a}	92.01±0.180^{a}	45.08±0.168^{a}
Sample 4	0	ND	ND	ND	ND
	1	13.03±0.274^{d}	15.03±0.191^{d}	20.02±0.184^{d}	10.60±0.122^{d}
	2	21.01±0.263^{c}	51.06±0.195^{c}	41.01±0.274^{c}	22.02±0.090^{c}
	3	35.02±0.250^{b}	73.34±0.4239^{b}	63.02±0.108^{b}	29.01±0.261^{b}
	4	49.01±0.372^{a}	79.08±0.070^{a}	79.02±0.238^{a}	37.01±0.358^{a}

*Sample 1= control without any extract, Sample 2=with green tea extract,
Sample 3=with thyme oil extract, Sample 4= with combination of green tea extract + thyme oil extract. All values determinations ± standard deviation (SD) are mean of triplicate. ND = not detected.
Means within column with different letters are significantly different (P<0.05).

Table (5): Volatile basic nitrogen (%) and total acidity (% lactic acid) of the luncheon rolls meat samples during storage at 4 °C

Storage period (month)	Volatile basic nitrogen (%) of Luncheon meat samples (Mean ± SD) *Sample 1	*Sample2	*Sample 3	*Sample 4
0	0.037±0.002^{e}	0.037±0.002^{e}	0.037±0.002^{e}	0.037±0.002^{e}
1	0.043±0.003^{d}	0.041±0.001^{d}	0.043±0.0001^{d}	0.040±0.0006^{d}
2	0.049±0.003^{c}	0.046±0.002^{c}	0.047±0.002^{c}	0.045±0.002^{c}
3	0.057±0.003b^{1}	0.053±0.003^{b2,3}	0.054±0.003^{b1,2}	0.049±0.0006^{b3}
4	0.064±0.002a^{1}	0.060±0.001^{a2,3}	0.063±0.002^{a1,2}	0.059±0.002^{a3}
	Total acidity (% lactic acid) of Luncheon meat samples			
0	0.36±0.015^{e}	0.36±0.015^{e}	0.36±0.015^{e}	0.36±0.015^{e}
1	0.42±0.025^{d}	0.39±0.010^{d}	0.40±0.010^{d}	0.39±0.006^{d}
2	0.49±0.019^{c1}	0.43±0.015^{c2}	0.44±0.019^{c2}	0.42±0.009^{c2}
3	0.54±0.015^{b}	0.51±0.019^{b}	0.52±0.031^{c}	0.49±0.019^{c}
4	0.59±0.020^{a1}	0.57±0.015^{a1,2}	0.58±0.099^{a1}	0.54±0.010^{a2}

*Sample 1= control without any extract, Sample 2=with green tea extract,
Sample 3=with thyme oil extract, Sample 4= with combination of green tea extract + thyme oil extract.
All values determinations ± standard deviation (SD) are mean of triplicate. ND = not detected.
Means within column and row with different letters and numbers are significantly different (P<0.05).

Sensory Evaluation:

The sensorial criteria (texture, flavor, color and taste) of the tested luncheon meat samples were evaluated at the end of storage and presented in Table 6. The results of sensory tasting showed that the taste was acceptable with good score for all treated luncheon meat samples. The order of taste scores of the tested samples was found as: combination of green tea extract and Thyme oil extract > green tea extract> thyme oil extract > control sample. With regard to texture, sample 4 (mixed with combination of green tea extract and thyme oil extract) was found to be of highest texture score; while sample 3 containing thyme oil extract was of lowest score. Flavor score was found to be the highest in luncheon sample 3 compared to other studied luncheon samples; while control sample was of worst score. The phenolic monoterpenes in thyme, thymol and carvacrol, are the primary compounds that contribute to the characteristic aroma of its essential oil (Yanishlieva *et al.*; 2006). The highest color score was for sample 4, followed by sample 2, meanwhile the control sample 1 showed a relative low score. However, all the investigated samples (including the control) realized good color scores.

Table (6): Sensory evaluation of the luncheon roll meat samples during storage at 4ºC (Mean ± SD).

Luncheon meat samples	Texture	Flavor	Color	Taste
Sample 1	3.72±0.010^{c}	3.69±0.009^{d}	4.06±0.010^{d}	3.81±0.009^{d}
*Sample 2	3.87±0.010^{b}	3.93±0.010^{c}	4.29±0.009^{b}	3.97±0.009^{b}
*Sample 3	3.65±0.009^{d}	4.18±0.009^{a}	4.18±0.009^{c}	3.90±0.00^{c}
*Sample 4	3.94±0.010^{a}	4.02±0.001^{b}	4.40±0.010^{a}	4.06±0.010^{a}

*Sample 1= control without any extract, Sample 2=with green tea extract, Sample 3=with thyme oil extract, Sample 4= with combination of green tea extract + thyme oil extract.
All values are mean of triplicate determinations ± standard deviation (SD).
Means within column with different letters are significantly different (P<0.05).

Conclusion:

The obtained results indicated that green tea and thyme leaves extracts contained high level of total phenolic compounds content. The addition of green tea extract, thyme oil extract either individually or combination (as natural antioxidant extracts) to luncheon roll meat was found to be effective towards reducing BAs formation, TBARS levels, VBN% and acidity %; hence improvement of the stored and refrigerated luncheon meat samples characters can occur. The best anti-oxidative effect was obtained by the combination of green tea and thyme oil extracts. Also, the sensorial criteria of the prepared luncheon roll samples gained acceptable and good scores. Thus, the use of natural antioxidants is important to preserve the quality of meat products and prevent their oxidative deterioration in order to produce luncheon roll meat of high quality and safety.

Corresponding author
Ferial M. Abu-Salem
Department of Food Technology, National Research Centre, Dokki, Cairo, Egypt
ferial_mas@yahoo.com

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6/28/2011