ARTICLE

Shelf-life Extension of Beef Patties Using *Gnetum gnemon* Leaf Extract and Modified Atmosphere Packaging

Rio Lawandra[1,*], Albert Kuhon[1], Soottawat Benjakul[2], and Natchapol Bell Buamard[2]

1 Department of Nutrition and Food Technology, Faculty of Life Sciences, Surya University, Tangerang, Banten, Indonesia 15810
2 Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Thailand

E-mail: riolawandra@gmail.com

The effect of *Gnetum gnemon* mature leaf ethanolic extract at various concentrations (0, 200, and 400 ppm) in combination with modified atmosphere packaging (MAP) (30% CO2/ 70% N2) on quality of pre-cooked beef patties during refrigerated storage of 8 days was investigated, in comparison with 200 ppm butylated hydroxytoluene (BHT). The extract possessed DPPH radical scavenging activity (23.394 ± 0.2854 mmol TE/g dry extract), ferric reducing assay power (10.797 ± 0.2273 mmol TE/g dry extract), and metal chelating activity (341.022 ± 0.5660 µmol EDTA equivalent/g dry extract). During the storage, the control had the highest increases in peroxide value (PV), thiobarbituric acid-reactive substances (TBARS) value, total viable count (TVC), and psychrophilic bacterial count (PBC). *Gnetum gnemon* leaf extract (200 ppm) showed the comparable efficacy in retardation of lipid oxidation to BHT at same level used as indicated by similar PV and TBARS value ($p>0.05$). At the same level of extract used, patties packaged under MAP (30% CO2/70% N2) showed lower lipid oxidation and microbial growth than those packaged in air. The increments of PV, TBARS value, TVC, and PBC of patties added with *Gnetum gnemon* leaf extract (400 ppm) and stored under MAP were retarded and the value of all quality indices remained the lowest, compared to other treatments up to 8 days of refrigerated storage ($p<0.05$). Thus, *Gnetum gnemon* mature leaves can be utilized as alternative source of natural antioxidant and antimicrobial agent, especially in conjunction with MAP to lower the deterioration of beef patties during refrigerated storage.

.

Introduction

Over last couple of decades, there has been a general global changing lifestyle and growing interest among consumers towards ready meals due to its convenience and less time spent in preparing foods (Bevis, 2012). The continuing demands in convenient foods which led to an expansion and variation of ready-to-eat or ready-to-cook products.

Burger or patty is one of the most common convenient food products and commonly sold as pre-cooked products which made from grounded meat such as beef, chicken, fish, and pork (Kang, et al., 2013). The favorable sensory characteristics, practicality, and a good source of protein in patty are promoting itself to be a habitually consumed food in many countries (Ramadhan, et al., 2011). Moreover, Frost and Sullivan (2014) reported that Indonesian beef industry, by sales value, was worth 56.2 trillion rupiah in 2013. The beef consumption itself increases each year from 1 kg/capita at 2010 into 2.5 kg/capita at 2014. Lately, there has been increasing consumptions in beef burger so much that a single worldwide retailer could sell more than 100 billion hamburgers annually (Spencer, et al., 2005).

Concerning to such high consumption, consumers also demand acceptable quality of beef patties. However, lipid oxidation remains as a major problem resulting in quality deterioration in beef patty during storage due to its high fat contents. Typically, fat content of a single beef patty is around 10-30% from its total weight, and half of them are composed of unsaturated fats which are very prone to lipid oxidation (Bender, 1992; Johson, et.al., 1994).

Lipid oxidation affects negatively on sensory properties as well as the degradation of nutritional quality (Susan, 2008). The development of secondary lipid oxidation products product produces warmed-over flavors (WOF) which has been described as the “rancid”, “painty” or “card-board like” characteristic off-flavors in pre-cooked meat product. Warmed-over flavors (WOF) are easily detected negatively by consumers at very low threshold values (Vega and Brewer, 1995; Campo, et al., 2006). Thus, mechanisms in controlling lipid oxidations are increasingly important with the rise in popularity of pre-cooked and convenience foods (Das, et. al., 2008).

Modified atmosphere packaging (MAP) has been used as packaging technology to preserve the quality of meat products by inserting different amount and type of gases. Gases such as carbon dioxide (CO2) to inhibit bacterial growth and nitrogen (N2) to inhibit lipid oxidation are

commonly used in MAP to preserve pre-cooked products. Although the application of MAP has increased widely in recent years the optimization of gas composition between CO2 and N2 is essential to preserve the quality of the foods (Zhou, et. al., 2010). Such gas composition is varying in different type of foods.

Besides MAP, antioxidant is successfully known and used to inhibit lipid oxidations and shelf-life extension of meat products without affecting the sensory or nutritional properties (Rojas and Brewer, 2007). Many synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary-butylhydroxyquinone (TBHQ) have been used to retard the oxidation process and avoid the quality deterioration of beef patty (Fasseas, et. al., 2007). However, the uses of synthetic antioxidants is under strict regulation due to their potential health hazard in the promotion of carcinogenesis as well as generals rejection of synthetic food additives by consumers have raised concerns about possible toxicity and food safety (Maqsood et. al., 2013).

Therefore, the practice of using natural antioxidants is being increasingly applied as an effective way for the control of lipid oxidation and providing ensured safety to consumers. Many phenolic compounds as the part of plant phenolic extracts have successfully demonstrated the retarding effect on lipid oxidation in different meat products and demonstrated to be the safe additives thus increasing shelf-life and sensory properties of meat products (Fasseas, et. al., 2007; Vaityhiyanathan, et. al., 2011; Yogesh and Ali, 2014).

Gnetum gnemon or often called as “Melinjo”, is an indigenous plant in Southeast Asia especially in Thailand, Malaysia, Vietnam and Indonesia. There are several studies in different parts of the plant that shows possible potential antioxidant activity such as bark and leaf and antimicrobial activity such as seed and peel (Dayana, et. al., 2011; Parhusip and Sitanggang, 2011). The young leaves and seeds are widely used as vegetables and snacks, but the mature leaves remain unused (Lim, 2012). There are no recent studies on its mature leaf to be utilized as a value-added product. Therefore, the utilization of phenolic compounds in Gnetum gnemon mature leaf by conversion into natural antioxidant to inhibit lipid oxidation and antimicrobial agent as safe additives to prolong the shelf-life of beef patties may be an innovative solution.

In this research, different concentrations of ethanol are used as solvent to extract the phenolic compounds of Gnetum gnemon mature leaf. In addition, ultra-sonic assisted extraction is subjected during the extraction using different amplitude and time. The best solvent concentrations and parameters during extraction will be determined based on the highest antioxidant activities among the other treatments. After that, the best solvent concentrations and parameters during extraction are used in Gnetum gnemon mature leaves extraction.

Lastly, different concentrations of Gnetum gnemon mature leaf ethanolic extracts in combination with MAP are tested to determine which treatment gives the highest inhibition of lipid oxidation and microbial growth on pre-cooked beef patty stored in refrigerated storage compared to butylated hydroxytoluene (BHT) synthetic antioxidant as a common antioxidant used in beef patty.

Materials and Methods

Chemicals

All of the chemicals used in research were analytical grade. Sodium Carbonate, Sodium Chloride, Chloroform, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picryl hydrazyl (DPPH), ammonium thiocyanate, and 1,1,3,3-tetramethoxypropane (MDA), Ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gallic acid was purchased from Fluka (St. Louis, MO, USA). Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were obtained from Merck (Darmstadt, Germany).

Preparation of *gnetum gnemon* mature leaf extract

The preparation of Gnetum gnemon mature leaf extract was using method of (Chotphruethipong, et al., 2017) with slight modification in samples used. The fresh Gnetum gnemon mature leaves (1 kg) were harvested from local farm in Hat Yai province in the Southern Thailand during dry season. The leaves were washed with tap water, and dried in an oven (Memmert, Schwabach, Germany) at 50ºC until it reached 10% of moisture content (wet weight basis). The dried leaves were blended and sieved with a sieve size of 80 mesh-sized. After that, the leaves powder were subjected with chloroform using a solid:solvent ratio of 1:20 (w/v) to remove the chlorophyll, and then stirred for 30 minutes. The mixture was filtered using a Whatman filter paper No.1 in a vacuum condition in the fume hood. The treatments with chloroform and filtration were subjected two times. The residues were dried using oven at 105ºC at 1 hour.

The dried leaves powder was dissolved in 60%, ethanol concentrations with a solid:solvent ratio of 1:10 (w/v) at room temperature 25°C using a magnetic stirrer (IKA-Werke, Staufen, Germany) for 5 minutes and subjected for ultrasonic-assisted extraction using sonotrode (Hielscher UIP 1000 hd, France) with different time (10, 20, and 30) minutes and amplitude (50% and 70%). The mixtures were centrifuged (Beckman Coulter, Avanti J-E Centrifuge, Beckman Coulter, Inc., Palo Alto, CA, USA) for 10 min with 5000xg rotation speed at 25ºc then filtered using Whatman filter paper No.1. The filtrate was evaporated at 40ºC using EYELA rotary evaporator (Tokyo Rikakikai, Tokyo, Japan).

The extract was freeze dried using a Scanvac Model Coolsafe 55 freeze dryer (Coolsafe, Lynge, Denmark). The dried extracts were powdered using a mortar and

pestle then were prepared using 60%, ethanol. The powder was kept in an amber bottle and stored in dessicator until prior to analyze. Total phenolic content (TPC) and antioxidant activity (DPPH, FRAP, and metal ions chelating activity) were determined.

Total phenolic content

Total phenolic content were determined by colorimetric method of Folin-Ciocalteu reagent (Slinkard and Singleton, 1997). Phenolic compounds in plant extracts react with Folin-Ciocalteu reagent to form blue complex from yellow under alkaline condition that can be quantified by visible-light spectrophotometry. The melinjo extract from different ethanol concentration were diluted 10-folded. An aliquot of 100 µL was mixed with 0.75 mL of Folin-Ciocalteau reagent, which was pre-diluted 10-fold with distilled water. After 3 minute, the mixture was added with 0.75 mL of 6% (v/v) sodium carbonate then mixed vigorously using a vortex mixer and allowed to stand for 1 h at room temperature. The absorbance at 760 nm was read using UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). Standard solutions of gallic acid (0-600 ppm) were used for standard curve preparation. The phenolic content was expressed as mg gallic acid equivalents per gram dry weight of melinjo leaves extract (Rodrigues and Pinto, 2007).

DPPH radical scavenging activity

DPPH radical scavenging activity was determined as method described by (Binsan, et al., 2008) with a slight modification. DPPH (1,1-diphenyl-2-picrylhydrazyl) was a stable free radical compound with violet color due to the unpaired nitrogen electron. After reaction with oxygen atom of a radical scavenger, the reduced DPPH formed yellow in color. An aliquot of 0.5 mL of DPPH in 95% ethanol was added with 0.5 mL of pre-diluted melinjo leave extract in 100-fold times with distilled water. The solution was then mixed vigorously using a vortex mixer and allowed to stand at room temperature in the dark for 30 minutes.

The color change can be monitored from absorbance of the resulting solution at 517 nm using a spectrophotometer. Sample blank was prepared in the same manner except that ethanol was used instead of DPPH solution. The standard curve of Trolox (60–600 µM) was prepared in the same manner. The activity was expressed as micromole Trolox equivalent (TE) per gram of dry melinjo leaves extract.

Ferric reducing assay power (FRAP)

Ferric reducing antioxidant power (FRAP) was determined as described by (Benzie and Strain, 1996) with a slight modification. It is a method to measure the reducing ability of the Fe3+ to Fe2+ transformation. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity in secondary mechanism of antioxidant. The color changing from reduction of a ferric tripyridyltriazine (TPTZ) complex to ferrous-(2,4,6-trpyridyl-s-triazine)2 from brown to violet can be observed by measuring absorbance at 593 nm.

An aliquot of 2.85 ml of freshly prepared FRAP solution 2.85 ml (2.5 ml of a 10 mM TPTZ solution in 40 mM HCl, 2.5 mL of 20 mM FeCl3.6H2O solution, and 25 mL of 300 mM acetate buffer, pH 3.6) was incubated at 37 °C for 30 min before mixing with 150 µL of sample.

The mixture was allowed to react in the dark at room temperature with melinjo leaves extract prior pre-diluted 100-folded times by distilled water. Absorbance at 593 nm was measured after 30 minutes of reaction. Sample blank was prepared by substituting FeCl3 with distilled water from the FRAP solution. The standard curve of Trolox (60–600 µM) was prepared in the same manner. FRAP was expressed as micromole TE per gram of dry extract.

Metal ions chelating activity

The chelating activity on Fe2+ of sample was determined using the method of Decker and Welch (1990). Ferrous iron (Fe2+) can initiate lipid peroxidation by the fenton reaction as well as accelerating peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals.

Ferrozine can form complexes with ferrous ions. In the presence of chelating agents, the complex formation in magenta is interrupted and as a result, the red color of the complex is decreased. Thus, the chelating effect of the coexisting chelator can be determined by measuring the rate of color reduction (Nagulendran, et.al., 2007). One millilitre of sample solution was mixed with 3.7 ml of distilled water. The mixture was then reacted with 0.1 ml of 2 mM FeCl2 and 0.2 ml of 5 mM ferrozine for 20 minutes at room temperature.

Preparation of beef patties using Gnetum gnemon mature leaf extract and MAP

Beef briskets were purchased fresh from a local market (Hat Yai, Thailand). The beef meats were cut in small cubes and ground separately using a meat grinder (National Model MK-5080M, Selangor, Malaysia) for 2 minutes. Bastos, et al., (2014) formulation of beef patties was followed with slight modification. The beef patties were prepared by mixing 88% of lean meat of beef, 10.5% cold mineral water, 1.5% iodized salt, and 0.1% antioxidant. After mixing, the meat was formed using a beef patties former (Italmans, Italy) and placed on oven trays prior to be cooked.

The 50 g of beef patties were divided into control beef patty (without additive mixture using only ethanol 60% as extract solvent), beef patty with added 200 ppm and 400 ppm of melinjo mature leaf extract, and 200 ppm of butylated hydroxytoluene (BHT). The beef patties were pre-cooked at 130ºC by heat in an oven cooker (FM 2011 E3, Forno Misto, Italy).

The patties were cooled in a room temperature (25ºC) for 10 minutes. A patty was placed on a polystyrene tray (15 x 25 cm) and the trays were sealed with nylon bags with low gas permeabiality (O2 transmission rate = 3-6 cm3 m-2 day-1 at 4.4 ºC, 1 atm pressure and 0% relative humidity) using modified atmosphere packaging machine Henkovac type 1000 (Tecnovac, Italy). The trays were filled with either 30% CO2+70% N2 or air with nylon bag/ gas ratio of 1:2 (v/v). The samples were stored at 4ºC incubator and removed for chemical and microbiological analysis every 2 days up to 8 days.

Thiobarbituric acid reactive substances (TBARS)

The control was prepared in the same manner except that distilled water was used instead of the sample. A standard curve was prepared using EDTA. The activity was expressed as µmol EDTA equivalent (EDTA)/g extract. All samples were analyzed in three replications. Determination of TBARS was determined as described by (Benjakul, et. al., 2009) with modification. The sample (0.5 g) was homogenized with 2.5 ml of solution containing 1.875% thiobarbituric acid (w/v), 25% trichloroacetic acid (w/v) and 0.124 M HCl. The mixture was heated in a boiling water bath (95-100oC) for 10 min to develop a pink color then cooled with running water. Centrifugation at 5000 RCF at 25ºC for 5 min was conducted to the mixture. The absorbance of supernatant was measured at 532 nm. A standard curve was made using 1,1,3,3-tetramethoxypropane at the concentrations ranging from 0 to 6 ppm. TBARS was expressed as mg malondialdehyde/kg sample.

Peroxide value (PV)

Peroxide value was determined as per the method of (Richards and Hultin, 2002) with a slight modification. Ground sample (1 g) was homogenized at a speed of 13,500 rpm for 2 min in 11 ml of chloroform/methanol (2:1 v/v). Homogenate was filtered using Whatman No.1 filter paper. Two mililiter of 0.5% NaCl was then added to 7 ml of the filtrate. The mixture was vortexed at a moderate speed for 30 s and then centrifuged at 3,000 RCF for 3 min to separate the sample into two phases. Two mililiter of cold chloroform/methanol (2:1) were added to 3 ml of the lower phase. Twenty five microliter of ammonium thiocyanate and 25 microliter of iron (II) chloride were added to the mixture (Shantha and Decker, 1994). Reaction mixture was allowed to stand for 20 minutes at room temperature prior to reading the absorbance at 500 nm. A standard curve was prepared using cumene hydroperoxide at a concentration range of 0.5 - 2 ppm.

Microbiological analysis

Beef patties (25 g) were collected aseptically in a stomacher bag and 10 volumes of sterile saline solution (0.85 g/100 ml) were added. After homogenizing in a stomacher blender M400 (Seward, UK) for 1 min, a series of 10-fold dilutions was made using normal saline solution (0,85%) for microbiological analysis. Mesophilic or total viable count (TVC) and psychrophilic bacterial counts (PBC) were determined by plate count agar (PCA) with the incubation at 35oC for 2 days (Hasegawa, 1987) and 7o C for 7 days (Cousin et. al., 1992), respectively. Microbial counts were expressed as log cfu/g.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) and mean comparison of means was carried out by Duncan's multiple range test (Steel and Torrie, 1980). Analysis was performed using the SPSS 20.0 package (SPSS for windows, SPSS Inc., Chicago, IL, USA). The p-value of the data < 0.05 were considered having statistically significant differences.

Results and Discussion

Total phenolic content

The total phenolic content among all samples subjected with different time and amplitude during ultrasonic-assisted extraction were determined by Folin-Ciocalteau reagent thus the total phenolic compounds in extract could be estimated. Phenolic compounds such as flavonoid, tannins, and saponin had hydroxyl functional group which act as free radical terminators (Maqsood, et al., 2013). The total phenolic contents which responsible for antioxidant effect in Gnetum gnemon mature leaf extracts were presented in Table 7 below.

Table 1. Total phenolic content of 60% ethanol *Gnetum gnemon* mature leaf extract in ultrasonic-assisted extraction

Sample	Total Phenolic Content (mg GAE/g dry sample)
Control	984.4976 ± 7.05609^{a}
10 Min, 50% Amp	$1062.2964 \pm 21.31087^{c}$
10 Min, 70% Amp	$1074.8150 \pm 7.93030^{c}$
20 Min, 50% Amp	$1079.4224 \pm 19.91044^{c}$
20 Min, 70% Amp	$1116.1877 \pm 24.67681^{d}$
30 Min, 50% Amp	$1016.7957 \pm 10.65871^{b}$
30 Min, 70% Amp	$1048.8159 \pm 13.73808^{c}$

Table 2. DPPH radical scavenging activity of 60% ethanol *Gnetum gnemon* mature leaf extract in ultrasonic-assisted extraction

Sample	DPPH Radical Scavenging Activity (µmol TE/g dry sample)
Control	$4693.5689 \pm 56.46279^{a}$
10 Min, 50% Amp	$16935.6115 \pm 385.40748^{b}$
10 Min, 70% Amp	$1928.0363 \pm 652.60883^{d}$
20 Min, 50% Amp	$20646.9346 \pm 246.32644^{e}$
20 Min, 70% Amp	$23393.9437 \pm 285.39200^{f}$
30 Min, 50% Amp	$18195.2933 \pm 219.55415^{c}$
30 Min, 70% Amp	$21225.2068 \pm 78.43314^{e}$

Table 3. Ferric reducing assay power of 60% ethanol *Gnetum gnemon* mature leaf extract in ultrasonic-assisted extraction

Sample	FRAP (µmol TE/g dry sample)
Control	8012.4986 ± 125.68158[a]
10 Min, 50% Amp	8982.5254 ± 360.02923[b]
10 Min, 70% Amp	9934.8048 ± 345.95566[c]
20 Min, 50% Amp	10404.3083 ± 408.36528[c-d]
20 Min, 70% Amp	10796.5598 ± 227.25629[d]
30 Min, 50% Amp	8120.5343 ± 431.84464[a]
30 Min, 70% Amp	9135.8209 ± 1070.07181[b]

Table 4. Metal ions chelating activity of 60% ethanol *Gnetum gnemon* mature leaf extract in ultrasonic-assisted extraction

Sample	Chelating Activity (µmol EDTA equivalent/g dry extract)
Control	287.1359 ± 2.54276[a]
10 Min, 50% Amp	284.9443 ± 16.38763[a]
10 Min, 70% Amp	289.2938 ± 4.90113[a]
20 Min, 50% Amp	322.5719 ± 4.23461[b]
20 Min, 70% Amp	341.0218 ± 0.56600[c]
30 Min, 50% Amp	322.2598 ± 3.33414[b]
30 Min, 70% Amp	329.9856 ± 6.48136[c]

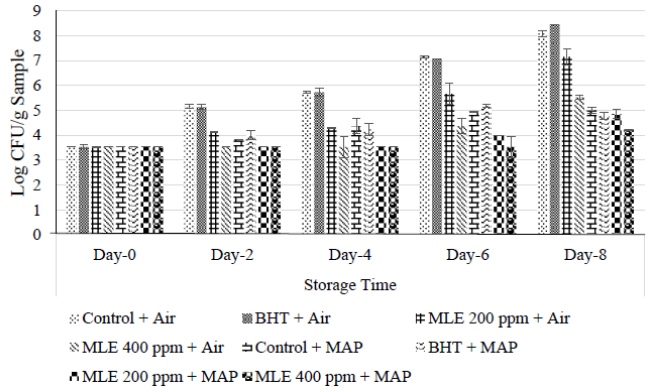

Fig. **1.** Graph of PBC of pre-cooked beef patties

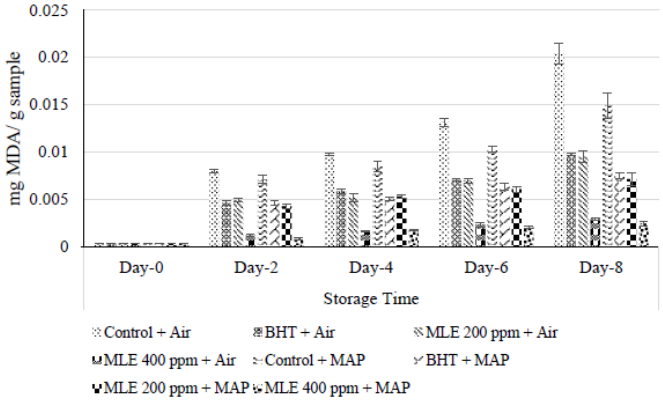

Fig. **2**. Graph of TBARS value of pre-cooked beef patties

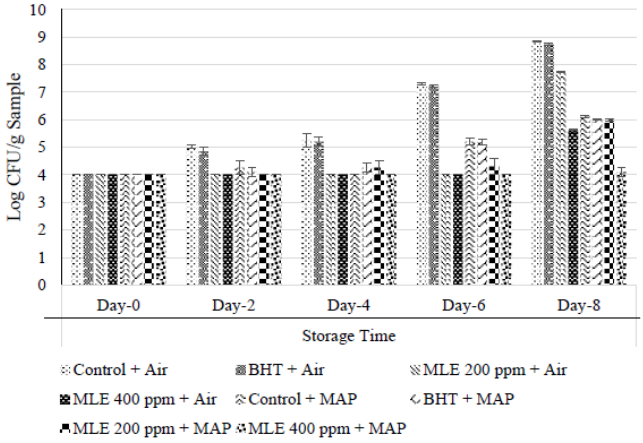

Fig. **3.** Graph of TVC of pre-cooked beef patties

The highest total phenolic content of melinjo leaves extract was showed by ultra-sound assisted extraction within 20 minutes and 70% amplitude. Chotphruethipong, Benjakul, and Kijroongrojan (2017) studied the effect of ultra-sonic assisted extraction of young cashew leaves and resulted in same parameter of time (20 minutes) and amplitude (70%) by response surface methodology. There were statistical differences between samples with the same level of time elapsed during extraction but higher amplitude, yielded in higher total phenolic contents ($p < 0.05$).

Table 5. Peroxide value (PV) of pre-cooked beef patties with different treatments during 8 days refrigerated storage

Treatments	PV during Storage time (day)				
	0	2	4	6	8
Control+ Air	0.001128± 0.0000849[a,A]	0.006533± 0.0003921[e,B]	0.007700± 0.0005434[e, B]	0.009410± 0.0006063[e, C]	0.011211 ±0.00048 47[f,D]
BHT + Air	0.001128± 0.0000849[a,A]	0.005225± 0.0002029[d,B]	0.006746± 0.0004963[d, B]	0.008907± 0.0006960[d, C]	0.008576 ±0.00064 38[d,C]
MLE 200 ppm + Air	0.001128± 0.0000849[a,A]	0.004904± 0.0002805[c-d,B]	0.006488± 0.0001642[d, C]	0.008280± 0.0003274[d, D]	0.007464 ±0.00064 38[d,D]
MLE 400 ppm + Air	0.001128± 0.0000849[a,A]	0.001740± 0.0001280[b,B]	0.002498± 0.0000960[b, C]	0.002918± 0.0002401[b, C]	0.004207 ±0.00040 33[b,D]
Control + MAP	0.001128± 0.0000849[a,A]	0.005365± 0.0002327[d,B]	0.006639± 0.0005708[d, C]	0.008021± 0.0000436[c, D]	0.008694 ±0.00045 38[e,D]
BHT + MAP	0.001128± 0.0000849[a,A]	0.004934±0. 0001976[c,B]	0.005534± 0.0001262[c, B]	0.006721± 0.0003342[b, C]	0.006799 ±0.00055 69[c,D]
MLE 200 ppm + MAP	0.001128± 0.0000849[a,A]	0.002549±0. 0000968[b,B]	0.003712± 0.0001756[b, C]	0.006035± 0.0002114[b, D]	0.006155 ±0.00018 63[c,E]
MLE 400 ppm + MAP	0.001128± 0.0000849[a,A]	0.001908±0. 0001628[a,B]	0.003001± 0.0001756[a, C]	0.003897± 0.0000752[a, D]	0.004126 ±0.00022 52[a,E]

a-f Values within each column with different superscripts are significantly different ($p<0.05$); A-E Values within each row with different superscripts are significantly different ($p<0.05$)

Table 6. Thiobarbituric acid reactive (TBARS) value of pre-cooked beef patties with different treatments during 8 days refrigerated storage

Treatments	Storage time (day)				
	0	2	4	6	8
Control + Air	0.0003602 ±0.00003040^{a,A}	0.007987 ±0.00018219^{e,B}	0.009724 ±0.00010890^{e,B}	0.013169 ±0.00043188^{e,C}	0.020389 ±0.00113044^{f,D}
BHT + Air	0.0003602 ±0.00003040^{a,A}	0.004597 ±0.00021335^{d,B}	0.005855 ±0.00021207^{d,B}	0.007057 ±0.00016698^{d,C}	0.009741 ±0.00015186^{d,C}
MLE 200 ppm + Air	0.0003602 ±0.00003040^{a,A}	0.004904 ±0.00021479$^{c-d,B}$	0.005170 ±0.00048186^{d,C}	0.006981 ±0.00026617^{d,D}	0.009496 ±0.00065553^{d,D}
MLE 400 ppm + Air	0.0003602 ±0.00003040^{a,A}	0.001187 ±0.00008763^{b,B}	0.001637 ±0.00005537^{b,C}	0.002330 ±0.00021796^{b,C}	0.002878 ±0.00014247^{b,D}
Control + MAP	0.0003602 ±0.00003040^{a,A}	0.007117 ±0.00044788^{d,B}	0.008511 ±0.00054206^{d,C}	0.010256 ±0.00029054^{c,D}	0.014891 ±0.00138729^{e,D}
BHT + MAP	0.0003602 ±0.00003040^{a,A}	0.004594 ±0.00024011^{c,B}	0.005066 ±0.00018451^{c,B}	0.006332 ±0.00033520^{b,C}	0.007442 ±0.00030954^{c,D}
MLE 200 ppm + MAP	0.0003602 ±0.00003040^{a,A}	0.004253 ±0.00026845^{b,B}	0.005293 ±0.00014360^{b,C}	0.006062 ±0.00028821^{b,D}	0.007134 ±0.00068115^{c,E}
MLE 400 ppm + MAP	0.0003602 ±0.00003040^{a,A}	0.000832 ±0.00007072^{a,B}	0.001705 ±0.00006574^{a,C}	0.002046 ±0.00011169^{a,D}	0.002426 ±0.00022216^{a,E}

a-f Values within each column with different superscripts are significantly different (p<0.05); A-E Values within each row with different superscripts are significantly different (p<0.05)

Table 7. Log value of total viable counts (TVC) of pre-cooked beef patties with different treatments during 8 days refrigerated storage

Treatments	Storage time (day)				
	0	2	4	6	8
Control+ Air	4.000±0.0000^{a,A}	5.011±0.0632^{e,B}	5.259 ±0.2413^{e,B}	7.298 ±0.0258^{e,C}	8.839± 0.0289^{f,D}
BHT + Air	4.000±0.0000^{a,A}	4.852±0.1351^{d,B}	5.201 ±0.1738^{d,B}	7.225 ±0.0274^{d,C}	8.736± 0.0300^{d,C}
MLE 200 ppm + Air	4.000±0.0000^{a,A}	4.000±0.0000$^{c-d,B}$	4.000 ±0.0000^{d,C}	4.000 ±0.0000^{d,D}	7.737± 0.0201^{d,D}
MLE 400 ppm + Air	4.000±0.0000^{a,A}	4.000±0.0000^{b,B}	4.000 ±0.0000^{b,C}	4.000± 0.0000^{b,}	5.630± 0.0258^{b,D}
Control + MAP	4.000±0.0000^{a,A}	4.259±0.2413^{d,B}	4.000 ±0.0000^{d,C}	5.209 ±0.1317^{c,D}	6.113± 0.0368^{c,D}
BHT + MAP	4.000±0.0000^{a,A}	4.100±0.1738^{c,B}	4.259 ±0.1512^{c,B}	5.180 ±0.0906^{b,C}	6.004± 0.0156^{c,D}
MLE 200 ppm + MAP	4.000±0.0000^{a,A}	4.000±0.000^{b,B}	4.259 ±0.2413$^{b,C C}$	4.301 ±0.3010$^{b,D ,D}$	5.997± 0.0453^{c,E}
MLE 400 ppm + MAP	4.000±0.0000^{a,A}	4.000±0.000^{a,B}	4.000 ±0.0000^{a,,C}	4.000 ±0.0000^{a,D}	4.100± 0.1738^{a,E}

Values within each column with different superscripts are significantly different (p<0.05); A-E Values within each row with different superscripts are significantly different (p<0.05)

The main reason why the ultrasonic-assisted extract was higher than conventional extraction was because ultrasound waves created during ultrasonic-assisted extraction disrupted the plant cell walls, creating cavitations in the plant cells so the solvent penetration towards the cells were improved and enhanced the mass transfer of phenolic compounds across cell membrane (Hossain, et al., 2012). Additionally, the results also showed that there were no statistical differences between samples extracted within 10 minutes and 30 minutes at same level of amplitude used (p > 0.05). This was mainly caused by the longer time in extraction yielded in feed-back results. Such results were happened due to the destruction because of heat generated during extraction for longer exposure of time and vibrations thus disrupted the labile phenolic compounds inside the extract (Ammar, et. al., 2016).

DPPH radical scavenging activity

The DPPH radical scavenging activity among all samples subjected with different time and amplitude during ultrasonic-assisted extraction were determined. The results of DPPH radical scavenging activity from all samples were showed in Table 8 below.

Table 8. Log Value of Psychrophilic Bacterial Count (PBC) of Pre-cooked Beef Patties with Different Treatments during 8 Days Refrigerated Storage

Treatments	Storage time (day)				
	0	2	4	6	8
Control+ Air	4.000±0.0000^{a,A}	5.171 ±0.0808^{e,B}	5.794 ±0.0277^{e,B}	7.155 ±0.0440^{e,C}	8.073± 0.1292^{f,D}
BHT + Air	4.000±0.0000^{a,A}	5.117 ±0.1000^{d,B}	5.799 ±0.0246^{d,B}	7.079 ±0.0096^{d,C}	8.444± 0.0087^{d,C}
MLE 200 ppm + Air	4.000±0.0000^{a,A}	4.100 ±0.1738$^{c-d,B}$	5.768 ±0.0173^{d,C}	5.657 ±0.0874^{d,D}	7.148± 0.0417^{d,D}
MLE 400 ppm + Air	4.000±0.0000^{a,A}	4.000 ±0.0000^{b,B}	4.254 ±0.04404^{b,C}	4.301 ±0.3010^{c,D}	5.5090± 0.0847^{b,D}
Control + MAP	4.000±0.0000^{a,A}	4.000 ±0.0000^{d,B}	4.360 ±0.3177^{d,C}	4.937 ±0.0295^{b,C}	4.989± 0.1257^{c,D}
BHT + MAP	4.000±0.0000^{a,A}	4.100 ±0.1738^{c,B}	4.259 ±0.2413^{,B}	5.172 ±0.0741^{b,D}	4.761± 0.1512^{c,D}
MLE 200 ppm + MAP	4.000±0.0000^{a,A}	4.000 ±0.0000^{b,B}	4.000 ±0.0000^{b,C}	4.000 ±0.0000^{a,D}	4.845± 0.1738^{c,E}
MLE 400 ppm + MAP	4.000±0.0000^{a,A}	4.000 ±0.0000^{a,B}	4.000 ±0.0000^{b,C}	3.523 ±0.4134^{a,D}	4.222± 0.0138^{a,E}

Values within each column with different superscripts are significantly different (p<0.05); A-E Values within each row with different superscripts are significantly different (p<0.05)

The highest radical scavenging activity of melinjo leaf extract was showed by sonication within 20 minutes with 70% amplitude and proved to be responsible to the increments of antioxidant activity of plants compared to the conventional extraction. The same trend of the results was showed with total phenolic contents and both of them had the similar correation. The results were in agreement with Ammar, et. al. (2016) that the highest total phenolic contents and DPPH free-radical scavenging rate was showed at the same level of amplitude which was 70% amplitude.

Increasing amplitudes and times affected the higher temperature might be produced during extraction due to an increase of molecular movements. At this stage, there would be some chance that accelerated the extraction of phenolic compounds from plant cells. However, there was also a possibility that elevated temperature would lead to the denaturation of thermo-sensitive antioxidants, thus optimum parameters of ultra-sonic assisted extraction must be determined. In addition, higher amplitudes also might cause the collapse of bubbles in the samples, thus the time was not sufficient enough for cavitation bubbles extracting all the target compounds (Chang, et al., 2015).

Ferric reducing assay power (FRAP)

The reducing antioxidant activity of *Gnetum gnemon* mature leaf extracts from different parameter used during ultrasonic-assisted extraction was shown in Table 3 below.

FRAP assay is a method used to determine the antioxidant activity of sample based on secondary antioxidant mechanisms which is on electron transfer reaction (Huang, et. al., 2005). The other previous studies also reported that the reducing power of antioxidant in natural source might serve as a significant indicator of potential antioxidant activity because the antioxidant activity had been proposed to be related to reducing power. Halvorsen, et. al. (2006) showed that most of the secondary metabolites in various food product samples were redox-active compounds that detected from FRAP assay.

The reducing properties was based on electron donation that responsible in the reduction of Fe^{3+} into Fe^{2+} called reductones. They exerted secondary antioxidant mechanisms by breaking the free-radical chain. Statistical analysis was conducted and showed that treatment within 20 minute extraction using either 50% or 70% amplitude were remained the highest ferric reducing assay power among all the others.

The extraction yields of FRAP by higher extraction time and amplitude were remained to be the lowest among all samples. Longer times accounted for more energy created from the vibration and heat during ultrasonic-assisted extraction from the phenolic compounds extracted and might disrupted the extracted compounds. The optimum extraction time to achieve the highest FRAP was at 20 minute, and decreased after.

Metal ions chelating activity

The metal ions chelating activity of *Gnetum gnemon* mature leaf extract with ethanol 60% as solvent during ultrasonic-assisted extractions with different time and amplitude were showed in Table 4 below. The metal ions chelating activity was expressed by µmol EDTA equivalent/g dry extract because EDTA served as standard chelating agent in this assay.

In the presence of chelating agents or antioxidants from *Gnetum gnemon*, the complex formation of ferrozine with Fe^{2+} was disrupted and resulting the decreases of purple color complexes. The highest metal ions chelating activity of melinjo leaf extract was showed by sonication within 20 minutes with 70% amplitude.

As seen from the Table 4, increasing time and amplitude yielded in higher metal ions chelating activity and no feed-back effect were happened on the highest time (30 minutes) and amplitude used (70% amplitude). These results were quite different in trends showed compared to the results of total phenolic contents, DPPH, and FRAP values. This was mainly caused by the relatively thermo-stable compounds were being extracted, compared to the compounds having role in scavenging and reducing were more labile.

Peroxide value (PV)

The result of peroxide value (PV) determination of refrigerated beef patties with different treatments in addition of extracts and packaging were represented in Table 5 and Fig. 1 below.

There were 8 different treatments in total applied in refrigerated pre-cooked beef patties which were: 1) control/no additives and stored in air; 2) control/no additives and stored in MAP; 3) BHT 200 ppm and stored in air; 4) BHT 200 ppm and stored in MAP; 5) Melinjo leaf extract (MLE) 200 ppm and stored in air; 6) Melinjo leaf extract (MLE) 200 ppm and stored in MAP; 7) Melinjo leaf extract (MLE) 400 ppm and stored in air; 8) Melinjo leaf extract (MLE) 400 ppm and stored in MAP.

The levels of primary oxidation products in the lipid fraction of the beef patties during the 8-day storage period represented by the peroxide value. The peroxide value of same sample during each days are significantly different ($p < 0.05$) due to oxidation process were occurring. This happened due to internal oxygen absence inside the meat system.

During the same day of the storage, on the starting day there was no significant difference among the samples ($p > 0.05$) due the absence of oxidation stability of beef patties. However, PV of beef patties were increased during storage time and reached to the highest values after 8 days of storage with significant increases ($p < 0.05$). At the same level of extract used compared to BHT showed that there was comparable efficacy in retardation of lipid oxidation as exhibited by the similar PV ($p < 0.05$).

Moreover, MLE 400 ppm at the same packaging condition exhibited lower PV compared to all samples. This result showed that MLE possessed radical scavenging antioxidant activity in order to inhibit primary oxidation in beef patties. In different packaging condition at same level of extract used, there was significant difference among the samples ($p < 0.05$). This result was in agreement with (Kang, et al., 2013) which MAP successfully retarded the lipid oxidation in beef patties. Among all of the samples treated, M400M peroxide value was remained the lowest up to the 8 day of refrigerated storage.

Thiobarbituric acid reactive substances (TBARS) value

The result of TBARS value determination from all samples during 8-days refrigerated storage are represented in Table 6 and Fig. 2 below. There were 8 different treatments in total applied in refrigerated pre-cooked beef patties which were: 1) control/no additives and stored in air; 2) control/no additives and stored in MAP; 3) BHT 200 ppm and stored in air; 4) BHT 200 ppm and stored in MAP; 5) Melinjo leaf extract (MLE) 200 ppm and stored in air; 6) Melinjo leaf extract (MLE) 200 ppm

and stored in MAP; 7) Melinjo leaf extract (MLE) 400 ppm and stored in air; 8) Melinjo leaf extract (MLE) 400 ppm and stored in MAP. Each of them were referred as CA, CM, BA, BM, M200A, M200M, M400A, and M400M respectively.

During the same day of the storage, on the starting day there was no significant differences among the samples ($p > 0.05$) due the absence secondary oxidation products formation However, as increasing time of the storage, there were significant differences among the samples on the same treatment ($p< 0.05$). These occurrences exhibited increasing level of TBARS value each 2 days of analysis as effects of rapid auto-oxidation in beef patties. No additives patties in air packaging exhibited the highest increases of TBARS value and significantly difference from all samples that treated with additives of extract or BHT and MAP ($p< 0.05$). This showed that both additives and MAP were having their role to inhibit the lipid oxidation of refrigerated beef patties during storage.

Addition of the same level of extract used compared to BHT showed that there were similar results in retardation of lipid oxidation as exhibited by the similar TBARS value ($p< 0.05$). In addition, the highest level of extract used under the same packaging condition exhibited lower TBARS compared to all samples.

However, beef patties packed inside the MAP packaging with absence of oxygen were reported to experienced lipid oxidation during increasing time ($p< 0.05$). This was happening due to the oxygen trapped inside the beef patties when preparation such as grinding, and cooking, thus providing internal pro-oxidant substances inside the meat system then accelerating the increases of TBARS value.

The addition of MLE was successfully retard the secondary oxidation of beef patties during refrigerated storage because it has reducing and chelating mechanism of oxidation. Campo, et al., (2006) reported that TBARS value within 0.002 mg MDA/ kg could be considered as the limiting threshold of acceptability of oxidized beef products. That condition could be achieved within M400M treatment up to 8 days, however several methods must be applied in pre-cooked beef patties to ensure its quality preservation during storage. The lowest TBARS values in pre-cooked beef patties during refrigerated storage was showed in M400M up to 8 days.

Total viable count (TVC)

The microbiological growth of refrigerated beef patties with different treatments were examined by total viable count (TVC) to determine the aerobic and mesophyll bacterial that causing spoilage in meat products. TVC are often used as microbiological analysis of food product to determine the shelf-life due to microbial spoilage. The values were represented by log value in Table 7 and Fig. 3 below.

It was observed that the TVC of all samples were increased with increasing time of storage at 4^{o}C ($p< 0.05$). The TVC of CA and BA beef patties increased rapidly from an initial value of 10^4 to more than 10^8 CFU/ g within 8 days of storage and was generally higher than that CM ($p< 0.05$). The similar log value showed by sample without addition of extract and addition of BHT as indicated by the p-value was happened due to absence of anti-microbial properties in the beef patties system, thus accelerating the microbial growth. This results also showed that the beef patties during storage without addition of extracts and/or packaging with MAP had highest microbial growth among all samples ($p< 0.05$).

However, the addition of extracts and/or MAP showed retardation of microbial growth during 8-days storage. According to FDA, the amount of TVC allowed in refrigerated pre-cooked beef patties are log values of 6 or 10^6 CFU/g. Among all samples, the addition of extract individually in concentration of 400 ppm successfully retard the microbial growth up to 8 days ($p<0.05$).

In contrast with the control, maximum viable counts of samples packaged under MAP conditions were typically below 10^6 CFU/ g and remained at this level for 1 week. The lower TVC indicated that CO_2 at just 30% concentration was sufficient to retard the growth of bacteria. According to a microbiological acceptability limit of 10^6 CFU/g for aerobic plate count (Food and Drug Administration, 2013), the shelf-life of samples stored in air and MAP was estimated to be 6 and 8 days respectively. This happened due to presence of carbonic acid inside the meat system produced by the presence of CO_2 in the food packaging system which exhibited antimicrobial properties (Maqsood et al., 2013).

Meanwhile, addition of extract at same packaging condition was also successfully retarded the growth of mesophillic bacteria. Addition of MLE 200 ppm showed quite satisfactory retardation of microbial growth about 1 log in 2 days. The lowest microbial growth was showed on M400M treatment. This result showed that *Gnetum gnemon* mature leaves extract within 400 ppm possessed antimicrobial activity in conjunction with MAP condition.

Psychrophilic bacterial count (PBC)

The microbiological growth of refrigerated beef patties with different treatments were examined by psychrophilic bacterial count (PBC) to determine the aerobic and psychrophilic bacterial that causing spoilage in meat products. The values were represented by log value in Table 8 and Fig. 4 below.

The psychrophilic bacterial count (PBC) of all samples were increased with increaing time of storage at 4^{o}C ($p< 0.05$), the same trend was produced compared to TVC (Fig. 11). The PBC of CA beef patties increased rapidly from an initial value of 10^4 to more than 10^8 CFU/ g within 8 days of storage and was generally higher than that CM ($p< 0.05$). The lower PBC showed that CO_2 at just 30% concentration was sufficient to retard the growth of psychrophylic bacteria. There was no significant differences between control and addition of BHT at the

same packaging conditions due BHT was acting only as antioxidant, not antimicrobial agent. However, MLE showed both activities at both 200 ppm and 400 ppm extract used.

In different treatment, MLE at same packaging condition successfully retarded the growth of mesophilic bacteria. Addition of MLE 200 ppm showed quite satisfactory retardation of microbial growth about 1 log in 2 days. The lowest microbial growth was showed on M400M treatment. This result showed that *Gnetum gnemon* mature leaves extract within 400 ppm possessed antimicrobial activity in conjunction with MAP condition. According to a microbiological acceptability limit of 10^6 CFU/g for aerobic plate count (Food and Drug Administration, 2013), the shelf-life of samples stored in air and MAP was estimated to be 4 and 8 days respectively. Lastly, individual addition of MLE 400 ppm was sufficient to prolong the shelf-life of refrigerated beef patties up to 8 days from control which only 4 days.

Conclusions

Gnetum gnemon mature leaves extract possessed high total phenolic content and antioxidant activity due to appropriate ethanol concentration used (60% v/v) and enhancement of them through ultrasonic-assisted extraction at optimum parameters (20 minute and 70% amplitude). *Gnetum gnemon* leaves extract (200 ppm) showed the comparable efficacy in retardation of lipid oxidation to BHT at same level as indicated by similar PV and TBARS value ($p>0.05$) of beef patties during refrigerated storage. At the same level of extract used, patties packaged under modified atmosphere packaging (MAP) (30% CO_2/70% N_2) showed the lowest lipid oxidation and microbial growth than those packaged in air. The increment of PV, TBARS value, TVC, and PBC of patties stored under MAP in combination with *Gnetum gnemon* leaves extract (400 ppm) were retarded and the value of all quality indices remained the lowest among other treatments up to 8 days of refrigerated storage ($p<0.05$), and it was the best treatment instead of others. Thus, *Gnetum gnemon* mature leaves could be utilized as natural antioxidant and natural anti-microbial agent in pre-cooked beef patties during refrigerated storage in conjunction with MAP.

Conflict of Interest

All the authors declare that they have no conflict of interest.

References

1 Bastos, S., Pimenta, M., Pimenta, C., Reis, T., Nunes, C., Pinheiro, A., Leal, R., 2014. Alternative fat substitues for beef burger: technological and sensory characteristics. Journal of Food Science and Technology, 51 (9), pp. 2046-2053.

2 Bender, A., 1992. Meat and meat products in human nutrition in developing countries. Rome: Food and Agriculture Organization of The United Nations.

3 Benzie, I. and Strain, J., 1996. The feric reducing ability of plasma (FRAP) as a measure of "antioxidant"power: The FRAP assay. Journal of Analytical Biochemistry, 239, pp. 70-76.

4 Bevis, E., 2012. Home Cooking and Eating Habits: Global Survey Strategic Analysis, London: Euromonitor International.

5 Binsan, W. et al., 2008. Antioxidative activity of Mungoong, an extract paste, from the cephalothorax of white shrimp (Litopenaeus vanamei). Journal of Food Chemistry, 106, pp. 185-193.

6 Campo, M. et al., 2006. Flavour perception of oxidation in beef. Journal of Meat Science, 72, pp. 303-311.

7 Chang, L., Xiao, F. and Jian, M., 2015. Optimization of Ultrasonic-assisted Extraction of Flavonoid Compounds and Antioxidants from Alfalfa Using Response Surface Method. Journal of Molecules, 20, pp. 15550-15571.

8 Chotphruethipong, L., Benjakul, S. and Kijroongrojana, K., 2017. Optimization of extraction of antioxidative phenolic compounds from cashew (Anacardium accidentale L.) leaves using response surface methodology. Journal of Food Biochemistry, pp. 1-10

9 Dayana, W., Syahida, A., Radzali, M., Maziah, M., and Shukor, M., 2011. Antioxidant activities of different parts of Gnetum gnemon L. Journal of Plant Biochemistry and Biotechnology. 20 (2), pp. 234-240.

10 Decker, E., and Welch, B., 1990. Role of ferritin as a lipid oxidation catalyst in muscle food. Journal of Agricultural and Food Chemistry, 38, pp. 674-677.

11 Fasseas, M. K., Mountzouris, K. C., Tarantilis, P. A., Polissiou, M., and Zervas, G., 2007. Antioxidant activity in meat treated with oregano and sage essential oils. Journal of Food Chemistry 106, pp. 1188-1194.

12 Food and Drug Administration, 2013. Revised guidelines for the assessment of microbiological quality of processed foods, Muntinlupa City: FDA.

13 Hasegawa, H., 1987. Determination of total viable count (TVC). Singapore: Marine Fisheries Research Department.

14 Hossain, M. et al., 2012. Optimization of ultra sound assisted extraction of antioxidant compounds from marjoram (Origanum majorana L.) using response surface methodology. Ultrasonics Sonocheistry, 19, pp. 582-590.

15 Kandhasamy, S. and Sun, C., 2013. Free radical scavenging activity from different extracts of leaves of Bauhinia valii Wight and Arn.. Saudi Journal of Biological Sciences, 20 (4), pp. 319-325.

16 Lim, T., 2012. Edible Medicinal and Non-Medicinal Plants. London: Springer.

17 Maqsood, S., Benjakul, S. and Shahidi, F., 2013. Emerging Role of Phenolic Compounds as Natural Food Additives in Fish and Fish Products. Critical Reviews in Food Science and Nutrition, pp. 162-179.

18 M. et al., 2013. The Effect of Modified Atmosphere Packaging and Addition of Rosemary Extract, Sodium Acetate and Calcium Lactate Mixture on the Quality of Pre-cooked Hamburger Patties during Refrigerated Storage. Asian-Australasian Journal of Animal Sciences Vol. 26, No.1, pp. 134-142.

19 Parhusip, A., and Sitanggang, A. 2011. Antimicrobial activity of melinjo seed and peel extract (Gnetum gnemon) Against Selected Pathogenic Bacteria. Journal of Microbiology Indonesia, 5 (3), pp. 103-112.

20 Ramadhan, K., Huda, N. and Ahmad, R., 2011. Physicochemical characteristics and sensory properties of selected Malaysian commercial chicken burgers. International Food Research Journal, 18, pp. 1349-1357.

21 Richards, M. and Hultin, H., 2002. Contributions of blood and blood components to lipid oxidation in fish muscle. Journal of Agricultural and Food Chemistry, 50, pp. 555-564.

22 Rodrigues, S. and Pinto, G., 2007. Ultrasound extraction of phenolic compounds from coconut (Cocosnucifera) shell powder. Journal of Food Engineering, 80 (3), pp. 869-872.

23 Rojas, M. and Brewer, M., 2007. Effect of Natural Antioxidants on Oxidative Stability of Cooked, Refrigerated Beef and Pork. Journal of Food Science, 72 (4), pp. 282-288.

24 Shahidi, F. and Wanasundara, P., 1992. Phenolic antioxidants. Critical Revews in Food Science and Nutrition, 32, pp. 67-103.
25 Shantha, N. and Decker, E., 1994. Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. Journal of American Oil Chemist Society 77, pp. 421-424.
26 Slinkard, K. and Singleton, V., 1997. Total phenol analyses: Automation and comparison with manual methods. American Journal of Efnology and Viticulture, 28 (1), pp. 49-55.
27 Spencer, E., Frank, E. and Mcintosh, N., 2005. Potential effects of the next 100 billion hamburgers sold by McDonalds. American Journal of Preventive Medicine, 28, pp. 379-381.
28 Steel, R. G. D. and Torrie, J. H., 1980. Principle and Procedure of Statistics. New York: McGraw-Hill.
29 Susan, B., 2008. Preserving Beef Quality with Natural Antioxidants, Denver: National Cattlemen's Beef Association.
30 Vaityhiyanathan, S., Naveena, B., Muthukumar, M., Girish, P., and Kondaiah, N., 2011. Effect of dipping in pomegranate (Punica granatum) fruit juice phenolic solution on the shelf life of chicken meat under refrigerated storage. Journal of Meat Science, 88, pp. 409-414.
31 Vega, J. and Brewer, M., 1995. Detectable odor thresholds of selected lipid oxidation compounds in a meat model system. Journal of Food Science, 60 (3), pp. 592-595.
32 Wei, Z. and Shiow, Y., 2001. Antioxidant Activity and Phenolic Compounds in Selected Herbs. Journal of Agricultural Food Chemistry, 49, pp. 5165-5170.
Yogesh, K., and Ali, J., 2014. Antioxidant potential of thuja (Thuja occidentalis) and peach (Prunus persia) seeds in raw chicken ground meat during refrigerated storage. Journal of Food Science and Technology, 51, pp. 1547-1553.