

Effects of Lemon Balm on the Oxidative Stability and the Quality Properties of Hamburger Patties during Refrigerated Storage

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Abstract

This study was performed to investigate the effects of lemon balm (*Melissa officinalis* L.) on various quality and antioxidant activity of hamburger patties. Lemon balm extract (LBE) showed the highest amount of total polyphenol (801.00 mg TAE/g DW) and flavonoids (65.05 mg RA/g DW). The IC₅₀ value of DPPH hydroxyl scavenging of LBE was 132 µg/mL. The hamburger patties were prepared by 0% (N), 0.1% (L1), 0.5% (L2), and 1.0% (L3) of the lemon balm powder. The addition of lemon balm powder increased the chewiness value, but did not affect the hardness, cohesiveness, and springiness values. Lemon balm powder had positive effects on sensory evaluation of patties. The pH of all patties decreased with longer storage period. 2-Thiobarbituric acid value, volatile basic nitrogen content, and the total microbial counts of hamburger patties in the L3 group were lower, compared to those of the normal (N group). In conclusion, the L3 group had significantly delayed lipid peroxidation compared to other treatment groups. However, the addition of lemon balm powder into patties showed no significantly influence on proximate composition, calorie contents, water holding capacity and cooking loss of patties. Therefore, lemon balm might be a useful natural antioxidant additive in meat products.

Keywords: lemon balm, hamburger patty, thiobarbituric acid value, antioxidant effect, quality

Introduction

Modernized society has led to a lot of changes in the structure and style of dietary habits, along with qualitative improvements of daily life, concomitant with advances in technology and the information industry. Food consumption patterns have been gentrified and diversified due to changes in nuclear family dynamics, the increase of women entering public affairs, the development of the food service industry, and the westernization of food culture, and there have been increases in the use of convenience food, processed food, fast food, and ready-to-eat food due to their convenience. In particular, the preference and consumption of fast food, such as hamburgers and pizza, has extended from youths to middle-aged and elderly adults (Choi *et al.*, 2013; Oh and Lim, 2011; Song *et al.*, 2000).

The hamburger, which is a prototypical fast food, is

made by inserting a cooked patty composed of pulverized livestock meat between breads. Hamburger patties use mostly beef, pork, or chicken as the main materials, and about 20-30% animal fat is added (Miller *et al.*, 1987), or it is made using the meat containing attached fat (Cross *et al.*, 1980). With meat products prepared using livestock meat such as the hamburger patty, a warmed-over flavor (WOF) due to lipid oxidation during processing and storing is generated, and the quality can be deteriorated (Murphy *et al.*, 1998). This WOF is generated more rapidly in cooked meat products and ground meat than in uncooked meat products (El-Alim *et al.*, 1999). In the grinding process of livestock meat, the cell walls of muscle are destroyed, and rapid degradation via interactions of pro-oxidant substances such as unsaturated fatty acid and non-heme occurs, and lipid oxidation is promoted (Tichivangana and Morrissey, 1985). Additionally, oxidative products, such as various types of alcohols, aldehydes, and ketones, generated by the oxidation impair DNA *in vivo* may even cause cancer, and this is also related to cellular aging (Mukai and Goldstein, 1976; Shamberger *et al.*, 1974; Shamberger *et al.*, 1977). The palatability of meat products has been improved, and phenolic synthetic

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antioxidants have been widely used (Garacía-Íñiguez de Ciriano *et al.*, 2010; Hernández-Hernández *et al.*, 2009; Kong *et al.*, 2010; Lara *et al.*, 2011). However, their use is being limited because of safety concerns (Barnen, 1975). As a result, studies on natural antioxidants to replace synthetic antioxidants have been actively conducted, and there have been attempts to develop hamburger patties and meat products that have better health properties, as well as quality and storage improvement by adding natural functional materials having antioxidant and antibacterial effects (Jung *et al.*, 2004; Lara *et al.*, 2011; McCarthy *et al.*, 2001; Park *et al.*, 2011).

Lemon balm (*Melissa officinalis* L.) is an herb with fresh, sweet, and strong lemon flavoring. It is a perennial plant of *Tubiflorae, lamiaceae* and is also a famous bee plant. From ancient times, in the West, dried lemon balm leaves have been used often as valuable spices and herbs. Lemon balm flavors induce calm and comfortable feelings and act to lower blood pressure by lowering the heart-beat (Dastmalchi *et al.*, 2008; Lamaison *et al.*, 1990). Additionally, lemon balm has been used to treat diseases such as depression, anxiety headaches, memory deterioration, and menstrual pain. It has also been used as an antidote for detoxifying poisonous mushroom. Dried lemon balm extract has been used to improve blood circulation. Antibacterial, anti-hormonal, antioxidant, and antiviral activities have also been reported (Gruenwalkd *et al.*, 2000; Sweetman, 2002; Yang *et al.*, 2009). For these reasons, lemon balm is a good potential source of functional components, including polyphenolic compounds (rosmarinic acid, caffeic acid, and protocatechuic acid), flavonoids (luteolin), monoterpenoid aldehydes, essential oils (citral), sesquiterpenes, and tannins (Jang *et al.*, 2011; Lamaison *et al.*, 1990). Therefore, many studies have investigated the use of lemon balm as natural antioxidants in food products (Berasategi *et al.*, 2011; Garacía-Íñiguez de Ciriano *et al.*, 2010; Lara *et al.*, 2011).

The objective of the this study was to investigate the effects of addition of different levels of lemon balm to hamburger patties on retarding lipid oxidation as well as evaluating the various quality parameters during storage at 4°C.

Materials and Methods

Sample preparation

The lemon balm used in experiments was purchased from Muan herb botanic garden (Muan herb farm, Korea) in August 2012. The moisture was removed by using the

salad spinner (Caous, WINDAX, Korea) after washing 2 times. The lemon balm was dried using a lyophilizer (ED 8512, Ilshin, Korea), triturated using a grinder (HR 2904, Philips Co., Netherlands), and then was stored until used for experiments in a deep freezer at -70°C. Fresh beef and pork muscles were purchased in a local market. All fat and visible connective tissue were removed from the fresh pork muscles.

Sample extraction

One hundred grams of lemon balm was added to 1.5 L of 80% ethanol, and then, it was extracted 3 times for 3 h in a 65°C heating mantle (Mtops ms-265, Korea) with a reflux cooler and filtered with qualitative filter paper (No. 2, Advantec, Japan). Solvent was removed from the remainder of the sample in a 40°C water bath with rotary vacuum evaporator (EYELA VACUUM NVC-1100, Japan). After that, it was decompressed, concentrated, lyophilized, and stored until use in a -70°C deep freezer.

Assay of total polyphenol content

The analysis of total polyphenol content was performed according to the slightly modified method of Folin-Denis (1912). Briefly, a sample aliquot of 1 mL extract (1 mg/mL of distilled water) and 2 mL of Folin reagent were put into a test tube and allowed to stand for 3 min at room temperature, and then, 2 mL of 10% Na₂CO₃ was added into that test tube. The solution in the tube was thoroughly mixed and allowed to stand for 40 min at 30°C, and the absorbance of the solution was measured at 760 nm using a UV-spectrophotometer (Shimadzu UV-1601 PC, Japan). The standard curve was plotted from samples with a final concentration of 0, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL using tannic acid, and total polyphenol content of the sample was obtained from this calibration curve. The results were expressed as mg of tannic acid equivalent (TAE) per gram dry weight (DW).

Assay of total flavonoid content

Total flavonoids were measured according to the Davis method with slight modifications (Chae *et al.*, 2002). Briefly, a sample aliquot of 1 mL extract (1 mg/mL of distilled water) and 2 mL of diethylene glycol were added to a test tube, and then 20 µL of 1 N NaOH was added into that test tube. The solution in the tube was incubated for 1 h in a 30°C water bath, and the absorbance of the solution was measured at 420 nm using a UV-spectrophotometer (Shimadzu UV-1601PC, Japan). The standard curve was plotted from samples with a final concentration

of 0, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL using rutin, and total flavonoid content of the sample was obtained from this calibration curve. The results were expressed as mg of rutin equivalent (RE) per gram DW.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity of lemon balm ethanol extract was determined according to the modified method described by Blois (1958). Briefly, 1 mL of lemon balm ethanol extract and 1 mL of 0.2 mM DPPH were added to a test tube and mixed for 30 min at 37°C, and the absorbance of the mixture was measured at 517 nm using a UV-spectrophotometer (Shimadzu UV-1601PC, Japan). At the same time, antioxidant activity was measured by the same method using ascorbic acid (Sigma Co., USA), which is a natural antioxidant, as well as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which are synthetic antioxidants, as a positive control groups. The DPPH radical scavenging activity of each sample was calculated using the following formula: (absorbance of sample addition group/absorbance of the control group) × 100. BHA, BHT and ascorbic acid were used at 0.5 mg/mL. Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage against sample concentration.

Antioxidant index measurement

The antioxidant index was measured using Rancimat (Metrohm Model 679, Switzerland) according to the method of Joo and Kim (2002). The lemon balm ethanol extract was added in soybean oil (Sigma Co., USA) so that the content, completely removing the solvent in them, became 1 mg/mL, and then the extract and fat of the sample were mixed well using an ultrasonic processor (UCX-750, USA). For Rancimat measurement conditions, 3.0 g of lemon balm ethanol extract was put into a reaction vessel and added to 70 mL of distilled water, and the oxidation stability was compared with an air flow rate of 20 L/h at 110°C. All measurements were presented as the average value of the values obtained from experiments repeated three times. BHA and BHT, which are synthetic antioxidants, and ascorbic acid, which is a natural antioxidant, were added to soybean oil, and they were compared as positive controls.

Preparation of hamburger patties

The manufacture of the hamburger patty with the addition of lemon balm powder was performed in a prelimi-

Table 1. Formula of patties added with lemon balm powder (%)

Ingredients	Treatment ¹⁾				
	N	C	L1	L2	L3
Beef	70.0	70.0	70.0	70.0	70.0
Pork	10.0	10.0	10.0	10.0	10.0
Soybean oil	3.0	3.0	3.0	3.0	3.0
Onion	5.0	5.0	5.0	5.0	5.0
Garlic	2.0	2.0	2.0	2.0	2.0
Bread powder	5.3	5.3	5.3	5.3	5.3
Egg	3.0	3.0	3.0	3.0	3.0
Salt	1.5	1.5	1.5	1.5	1.5
Black pepper	0.2	0.2	0.2	0.2	0.2
Total	100.0	100.0	100.0	100.0	100.0
Ascorbic acid	-	0.05	-	-	-
Lemon balm powder	-	-	0.1	0.5	1.0

¹⁾N (Normal: no lemon balm powder added), C (Control: ascorbic acid 0.05% added), L1 (lemon balm powder 0.1% added), L2 (lemon balm powder 0.5% added), L3 (lemon balm powder 1.0% added).

nary experiment by referring to the study of Oh and Lim (2011) and the previous study of Lee and Cho (2012), and the mixing ratios of the materials used in the hamburger patties are listed in Table 1. The beef and pork meat were ground using a meat chopper (M-12S, Hankook Fugee Industries Co., Korea), on which the holes plate of 8-mm diameter were mounted after removing the connective tissue and excess fat. The samples with ground onion, garlic, bread crumbs, cooking oil, egg, salt, and pepper were mixed in with the ground beef and pork were in the normal group. The treatment groups with added 0.1%, 0.5%, and 1% lemon balm powder were noted as L1, L2, and L3. Ascorbic acid, which is widely used as an antioxidant in processed foods, was used as a positive control group and the 0.05% concentration of ascorbic acid was selected on the basis of previous studies (Choi *et al.*, 2011). Each 100 g mixture was molded in a shape with a diameter of 10.0 cm and thickness of 1.2 cm using a patty molding machine, and this experiment was performed by opening a package of patties from each experimental group on 0, 5, 10, and 15 d, while storing the patties for 15 d in vacuum-sealed packaging (FJ-500XL, Fugee Tech, Korea) using unheated nylon/PE film in the refrigerator. The sample analysis of each experimental item was performed in triplicate.

Proximate composition

The analysis of proximate composition of sample was conducted in accordance with Association of Official Analytical Chemists (AOAC) method (1990), and moisture

was analyzed by drying using heat at atmospheric pressure and 105°C. Crude protein was analyzed using the micro-Kjeldahl method, crude fat was analyzed using the Soxhlet extraction method, crude ash was analyzed using the ashing method, and calories were analyzed using a calorimeter (PARR 1351 Bomb Calorimeter, USA).

Measurement of water holding capacity (WHC)

The measurement of WHC was performed in accordance with the method of Laakkonen *et al.* (1970). A 12-mL tube with fine holes, the sample of 0.5 ± 0.05 g, and the combined weight of the sample and tube were measured individually. The sample was heated for 20 min in an 80°C water bath (HB-205SW, Hanbaek Scientific Co., Korea), and the ratio (%) of the sample weight after heating to that before heating was calculated, after cooling at room temperature for 10 min and centrifuging at 4°C at 6,710 g for 10 min.

Measurement of cooking loss

To examine cooking loss, the samples were preheated on an open flame for 3 sec using a home pan, and a hamburger patty was inserted into each sample and heated until the temperature in the center of the patty reached 72°C. Then, further heat treatment was conducted for 15 min. The samples were transferred in a wire net, and then the weights of the samples were measured after cooling for 30 min. The weights of patties before and after the heat treatment measured and then the reduced weight was expressed as the cooking loss (%).

Texture profile analysis

The texture properties of each cooked hamburger patty were measured by the Rheometer (Compac-100, Sun Scientific Co., Japan) and analyzed using Rheology Data System version 2.01. When measuring the test items above, the table speed was 110 mm/min, the graph interval was 20 m/s, and the load cell (max) was 10 kg. The texture properties of sample were expressed as hardness (g), cohesiveness, springiness, and chewiness (g).

Sensory evaluation

After explaining the rules, sensory evaluation was conducted using a group consisting of ten students with food-related majors and graduate students who were already knowledgeable about the sensory test. The color, springiness, flavor, juiciness, and overall acceptability were evaluated on a 5-point scale, with “extremely like” being a 5, “moderately like” being a 3, and “extremely dislike” being

a 1 point, with respect to each evaluation item. The sample was heated and cooked until the center temperature of hamburger patty reached 72°C by pan-frying, and then it was tested on a white dish after cutting it into $2 \text{ cm} \times 2 \text{ cm} \times 1.5 \text{ cm}$ pieces. Drinking water was provided between each sample.

Determination of pH

The pH values of meat patties were assessed according to the Khalil method (2000). Briefly, 10 g of sample was homogenized for 30 sec using a stomacher (400 Lab blender, Seward, England) in 100 mL of distilled water. Then, the pH of sample was measured by pH-meter (WTW pH 720, Germany).

2-Thiobarbituric acid (TBA) value

The TBA values were measured according to the modified extraction method described by Witte *et al.*, (1970). Briefly, 10 g of each sample, 15 mL of cold 10% perchloric acid, and 25 mL distilled water were added in this sample. After homogenizing the mixture at 10,000 rpm for 10 sec in a homogenizer (AM-Series), the homogenate was filtered using qualitative filter paper No. 2. After adding and completely mixing 5 mL of the filtrate solution and 5 mL of 0.02 M TBA solution, the solution was allowed to stand for 16 h in a cool, dark place. The absorbance was measured at 529 nm using a Spectrophotometer (DU-650, Beckman, USA). 1,1,3,3,-Tetraethoxypropane (Sigma-Aldrich, USA) was used as standard for TBA assay. TBA values were expressed as milligram malonaldehyde (MA) per 1 kg sample (mg MA/kg), and the used standard curve equation was $y = 0.1975x - 0.0011$ ($r=0.999$), where y = absorbance for a given x , the TBA value.

Volatile basic nitrogen (VBN) value

The VBN contents were measured by microdiffusion analysis (Short, 1954) using a Conway unit. For each sample, 10 g of sample was added to 90 mL of distilled water. After homogenizing at 10,000 rpm for 30 s in a homogenizer (AM-Series), the homogenate was filtered using qualitative filter paper No. 2. Then, 1 mL of filtrate was placed in the outer compartment of a Conway unit, and 1 mL of 0.01 N boric acid and 3 drops of indicator (0.066% methyl red + 0.066% bromocresol green) were added in the inner compartment. Glycerin was applied as a gluing material, and the lid was closed. Then, 1 mL of 50% K_2CO_3 was injected in the outer compartment. After this, it was immediately sealed, and after horizontally stirring the vessel, the boric acid in the inner compartment

was titrated with 0.02N H₂SO₄, after incubating at 37°C for 120 min. The VBN value was expressed as mg (mg%) per 100 g sample using the following formula: $VBN = \{(a-b) \times F \times 28.014 \times 100\} / \text{amount of sample}$, where a is the amount of injected sulfuric acid (mL), b is the amount of sulfuric acid injected in the blank (mL), and F is the standardized index of 0.02 N H₂SO₄. The constant 28.014 describes the amount required to consume 1 mL 0.02N H₂SO₄.

Microbiological analysis

For this analysis, 10 g samples were taken aseptically from each treatment, transferred to sterile plastic pouches, and homogenized with 90 mL of 0.1% peptone solution in a stomacher (400 Lab Blender, Seward, London, England) for 1.5 min. Serial 10-fold dilution were prepared from each dilution by pouring 1 mL in fluid agar, and these were then incubated (APHA, 1992) for 48 h at 37°C by inoculating on plate count agar (PCA) medium. The results were expressed as log of colony forming units (CFU)/g.

Statistical analysis

Three replications of this study were performed and measured of all parameters were made in duplicate. The results from these tests were examined with an analysis of variance using the SAS program (2002), and its significance was verified at the level of 5% using Duncan's multiple range test.

Results and Discussion

Total polyphenol and total flavonoid contents of LBE

Lipid components existing in food or biomembranes *in vivo* are oxidized by free radicals, which induce changes in food quality of food and cause aging in the human body. In order to prevent lipid oxidation, phenolic compounds, which are natural antioxidants, have been used (Nijveldt *et al.*, 2001). Total polyphenol and total flavonoid contents of LBE are shown in Table 2. Total polyphenol and total flavonoids contents of LBE were 801.00 ± 9.24 mg TAE/g DW and 65.05 ± 2.43 mg RE/g, respectively. Flavonoids, a class of low-weight phenolics compound, are contained in the edible plant resources in quantity and have various functional biological effects, such as cardiovascular disease prevention, as well as anti-inflammatory, anti-allergic, antiviral, antioxidant, immune enhancement, and capillary potentiation activities (Kawaguchi *et al.*, 1997). Thus, in this study, lemon balm, which con-

Table 2. Contents of total polyphenol and total flavonoids in lemon balm ethanol extract

Sample	Total polyphenol content (mg TAE/g DW)	Total flavonoids content (mg RE/g DW)
LBE	801.00±9.24 ¹⁾	65.05±2.43

¹⁾All values are expressed as mean±SE of triplicate determinations.

Table 3. Antioxidative activities of lemon balm ethanol extract

Sample ¹⁾	Antioxidative activity	
	DPPH radical scavenging activity IC ₅₀ (µg/mL)	IP ²⁾ (Antioxidant index) ³⁾
LBE	131.92±8.67 ^{4)a5)}	13.22±0.41 ^a (1.94)
BHA	94.16±6.47 ^b	13.77±0.51 ^a (2.00)
BHT	99.05±4.87 ^b	13.47±0.51 ^a (1.97)
Ascorbic acid	42.34±2.16 ^c	15.32±0.25 ^a (2.24)
Control	-	6.83±0.50 ^b (1.00)

¹⁾LBE: 80% ethanol extract from lemon balm, BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene, Control: soybean oil without lemon balm ethanol extract.

²⁾Induction period (IP) of oil was determined by test of Rancimat at 110°C.

³⁾Antioxidant index was expressed as IP of oil containing sample/IP of soybean oil.

⁴⁾All values are expressed as mean±SE of triplicate determinations.

^{a-c}Means with different letters in the same column are significantly different ($p < 0.05$) by Duncan's multiple range test.

tains abundant polyphenols and flavonoids, can be assumed to have natural antioxidant availability.

Antioxidative activities of LBE

Antioxidant activities of LBE by DPPH radical scavenging activity and by Rancimat assay are shown in Table 3. The IC₅₀ values of DPPH radical scavenging of LBE, BHA, BHT, and ascorbic acid were 132, 94, 99, and 42 µg/mL, respectively. This result is in agreement with the finding of Dastmalchi *et al.* (2008) where they reported that the content of total polyphenol and the IC₅₀ value of DPPH radical scavenging of LBE were 268 mg GAE/g DW and 134 µg/mL. These results are consistent with other reports that higher phenolic compound content leads to more DPPH radical scavenging activity (Kwak *et al.*, 2005). The antioxidant index was measured by Rancimat assay in order to find out the level of inhibition of lipid oxidation by LBE, which showed an antioxidant index in 1.0 mg/mL of 1.94. The antioxidant indices of BHA, BHT, and ascorbic acid in 1.0 mg/mL were 2.00, 1.97, and 2.24, respectively. The antioxidant index of LBE was also high, and this property was similar to those of BHA, BHT, and ascorbic acid.

Proximate composition, caloric content, WHC and cooking loss of hamburger patties added with lemon balm powder

Proximate composition and caloric content of hamburger patties were not significantly different with the addition of lemon balm (data not shown). These results agreed with a study by Mohamed and Mansour (2012) that used natural plants such as rosemary and marjoram in the formulation of beef patties and reported no significant differences among the groups. There were no significant differences between the WHC and cooking loss of normal sample and treated samples (data not shown). In this study, the addition of lemon balm powder did not significantly affect the proximate composition, WHC, and cooking loss, perhaps due to the low amount of lemon balm powder used.

Texture properties of hamburger patties added with lemon balm powder

Results from the texture properties analysis of hamburger patties refrigerated for 15 d are shown in Table 4. The hardness, cohesiveness, and springiness of hamburger patties tended to reduce with increased lemon balm powder, but there was no significant difference. For chewiness, the hamburger patties added with 1% lemon balm powder (L3 group) had the highest value compared to the other samples ($p < 0.05$). Lara *et al.* (2011) obtained similar results of increasing chewiness with the addition of

lemon balm. Hwang *et al.* (1998) have reported that the changes in springiness and chewiness of the patty upon the addition of algae resulted from the binding capacity of the dietary fiber within the algae. Thus, chewiness was thought to have changed because the binding capacity of the patty upon the addition of lemon balm powder was increased.

Sensory evaluation of hamburger patties added with lemon balm powder

The sensory properties of hamburger patties containing lemon balm powder are shown in Table 5. For the color of cooked hamburger patty, the groups L1, L2, and L3 were evaluated higher than the normal group (N), and as lemon balm addition increased, the results became significant, with the L3 and the positive control groups having the highest score. The color of the meat product significantly affects the sensory properties (Benedini *et al.*, 2008). Generally, sensory properties of meat products with added herbs can be a disadvantage with regard to color. In this study, the color of patties became slightly dark red due to the addition of lemon balm powder, but it scored positively in preference evaluation. However, Berasategi *et al.* (2011) and Lara *et al.* (2011) reported that sensory evaluation for color did not reveal any significant difference between the control and the lemon balm treated samples. Springiness and juiciness were not significantly different among the groups. However, regarding flavor and total

Table 4. Textural properties of patties prepared with different levels of lemon balm powder

Items	Treatments ¹⁾				
	N	C	L1	L2	L3
Hardness (g)	2054.00±97.86 ²⁾	2724.00±265.62	2134.00±206.61	2276.00±57.84	2404.00±116.99
Cohesiveness	35.14±1.41	39.00±3.14	34.03±1.04	35.27±1.49	37.21±1.50
Springiness	53.84±3.98	56.05±3.02	54.51±0.49	55.83±2.21	54.10±2.01
Chewiness (g)	279.60±37.27 ^b	281.31±35.64 ^b	370.01±68.25 ^{ab}	397.21±49.49 ^{ab}	486.40±45.57 ^a

¹⁾See the legend of Table 1.

²⁾All values are expressed as mean±SE of triplicate determinations.

^{a,b}Means in the same row not sharing a common letter are significantly different ($p < 0.05$) by Duncan's multiple range test.

Table 5. Sensory evaluation of patties prepared with different levels of lemon balm powder

Sensory characteristics ²⁾	Treatments ¹⁾				
	N	C	L1	L2	L3
Color	2.43±0.20 ^{3)c}	4.54±0.31 ^a	3.09±0.28 ^{bc}	3.93±0.23 ^{ab}	4.54±0.24 ^a
Springiness	3.50±0.19	3.59±0.20	3.66±0.18	4.07±0.17	3.84±0.28
Flavor	3.57±0.20 ^b	3.59±0.17 ^b	4.03±0.25 ^{ab}	4.61±0.29 ^a	3.91±0.31 ^{ab}
Juiciness	3.61±0.26	3.63±0.27	3.87±0.15	4.11±0.28	3.93±0.25
Total acceptability	3.31±0.20 ^b	3.41±0.48 ^b	4.11±0.17 ^{ab}	4.72±0.27 ^a	3.71±0.38 ^{ab}

¹⁾See the legend of Table 1.

²⁾1: dislike extremely, 3: neither like nor dislike, 5: like extremely.

³⁾All values are expressed as mean±SE of triplicate determinations.

^{a-c}Means in the same row not sharing a common letter are significantly different ($p < 0.05$) by Duncan's multiple range test.

acceptability, the patties in lemon balm-treated groups received higher scores than the normal (N) and positive control (C) groups. In particular, the L2 group, in which 0.5% lemon balm powder was added, was most highly preferred, and the normal group (N) was relatively lower preferred. Thus, the flavor and total acceptability of the patties with added lemon balm powder were highly favored because the fresh and sweet taste of lemon balm removed the unique flavor and aroma of beef and pork by acting positively on the patties. These results agree with Kong *et al.* (2010). They reported that the spice-treated patties, such as clove, rosemary, and cassia bark, had significantly lower discoloration, rancidity and off-flavour scores, but significantly higher overall acceptability scores than those of control sample, indicating that spices were able to improve the sensory quality of cooked patties.

Changes in pH of patties added with lemon balm powder

Table 6 shows the pH changes in uncooked hamburger patties added with lemon balm powder during refrigeration for 15 d. The pH values immediately after preparing the normal group (N), the positive control group (C), and the treatment groups (L1, L2, and L3) were 6.03, 5.98, 5.91, 5.92, and 5.95, but they were 4.67, 4.56, 4.55, 4.58, and 4.52, respectively, with 15 d of storage, decreasing over the storage period. The positive control group (C) and the treatment groups (L1, L2, and L3) had lower pH values compared to the normal group (N), and the decreasing pH was not affected by the addition levels of lemon balm powder. Lara *et al.* (2011) found that pH values were significantly lower in pork patties with lemon balm throughout the storage period, which could be attributed to the fact the active compound in this extract is an acid (rosmarinic acid in lemon balm, with a pH of 4.25). Similar trends in pH values were reported in studies by Kim (2011), in which pine needle extract was added to sausage. In addition, Langlois and Kemp (1974) and Go-

ddar *et al.* (1996) have reported that, overall, pH decreased as the storage period of the ground meat increased and originated with the generation of lactic acid, due to the growth of microorganisms.

Changes in the TBA values of patties added with lemon balm powder

The TBA values in uncooked hamburger patties added with lemon balm powder during refrigeration over a 15-d period are shown in Table 7. The TBA values of the positive control group (C) and groups added with lemon balm powder (L1, L2, and L3) were significantly lower than those in the normal group (N) on the day of preparation, with a steady increase in TBA values during storage, but the groups added with lemon balm powder (L1, L2, and L3) had lower rates of increase compared to the normal group (N). On d 15 of storage, the normal group (N) had the highest TBA value, 0.60 mg malonaldehyde/kg, and the positive control group (C) and groups added with lemon balm powder (L1, L2, and L3) had significantly lower TBA values compared to the normal group (N), with values of 0.35, 0.56, 0.47, and 0.45 mg malonaldehyde/kg, respectively. Also, lipid oxidation in the L3 group was not inhibited as much as the positive control group (C). Vuorela *et al.* (2005) have reported that they evaluated the degree of inhibition of lipid oxidation by measuring the amount of hexanal formed during storage by adding phenolic compounds extracted from various plants in the pork patty, and the more phenolic compounds that were added, the more the lipid oxidation was inhibited. In this study, phenolic compounds (Lamaison *et al.*, 1990) such as flavonoids, terpenic acid, carnosic acid, carnosol, and volatile oil, were high in lemon balm and inhibited the lipid oxidation in hamburger patties.

Changes in VBN values of patties added with lemon balm powder

The VBN contents in uncooked hamburger patties with

Table 6. pH changes of total aerobic counts for patties prepared with different levels of lemon balm powder during 15 d of storage at 5°C

Storage time (d)	Treatments ¹⁾					
	N	C	L1	L2	L3	
pH	0	6.03±0.01 ^{2)abA}	5.98±0.01 ^{bA}	5.91±0.01 ^{dA}	5.92±0.01 ^{dA}	5.95±0.01 ^{cA}
	5	5.60±0.04 ^{ab}	5.31±0.03 ^{bB}	5.27±0.03 ^{bB}	5.23±0.02 ^{bcB}	5.13±0.03 ^{cB}
	10	4.74±0.01 ^{ac}	4.64±0.01 ^{bc}	4.62±0.01 ^{bcC}	4.63±0.01 ^{bcC}	4.60±0.01 ^{cC}
	15	4.67±0.01 ^{ac}	4.56±0.01 ^{bcC}	4.55±0.01 ^{cD}	4.58±0.01 ^{bd}	4.52±0.01 ^{dD}

¹⁾See the legend of Table 1.

²⁾All values are expressed as mean±SE of triplicate determinations.

^{a-d}Means in the same row not sharing a common letter are significantly different ($p<0.05$) by Duncan's multiple range test.

^{A-D}Means in the same column not sharing a common letter are significantly different ($p<0.05$) by Duncan's multiple range test.

Table 7. Changes of thiobarbituric acid (TBA) for patties prepared with different levels of lemon balm powder during 15 d of storage at 5°C

	Storage time (d)	Treatments ¹⁾				
		N	C	L1	L2	L3
TBA (mg MA/kg)	0	0.29±0.01 ^{2)ad}	0.18±0.01 ^{cd}	0.24±0.01 ^{cc}	0.26±0.01 ^{bd}	0.21±0.01 ^{dd}
	5	0.40±0.01 ^{ac}	0.26±0.01 ^{cc}	0.34±0.01 ^{bb}	0.27±0.01 ^{cc}	0.26±0.02 ^{cc}
	10	0.46±0.01 ^{ab}	0.32±0.01 ^{cAB}	0.33±0.01 ^{cb}	0.39±0.01 ^{bb}	0.30±0.01 ^{cb}
	15	0.60±0.01 ^{aA}	0.35±0.01 ^{eA}	0.56±0.01 ^{bA}	0.47±0.01 ^{cA}	0.45±0.01 ^{dA}

¹⁾See the legend of Table 1.

²⁾All values are expressed as mean±SE of triplicate determinations.

^{a-d}Means in the same row not sharing a common letter are significantly different ($p<0.05$) by Duncan's multiple range test.

^{A-D}Means in the same column not sharing a common letter are significantly different ($p<0.05$) by Duncan's multiple range test.

Table 8. Changes of volatile basic nitrogen (VBN) for patties prepared with different levels of lemon balm powder during 15 d of storage at 5°C

	Storage time (d)	Treatments ¹⁾				
		N	C	L1	L2	L3
VBN (mg%)	0	5.95±0.09 ^{2)abd}	5.31±0.09 ^{bd}	6.59±0.27 ^{aC}	6.68±0.37 ^{aC}	6.59±0.16 ^{ad}
	5	10.57±0.32 ^{abC}	9.47±0.16 ^{bc}	13.59±1.35 ^{ab}	10.84±1.35 ^{abb}	9.38±0.51 ^{bc}
	10	15.33±0.64 ^{ab}	11.67±0.27 ^{bb}	14.87±0.66 ^{aAB}	13.59±0.42 ^{ab}	11.39±0.16 ^{bb}
	15	17.80±0.24 ^{aA}	15.05±0.48 ^{bA}	17.62±0.09 ^{aA}	17.25±0.33 ^{aA}	15.76±0.27 ^{bA}

¹⁾See the legend of Table 1.

²⁾All values are expressed as mean±SE of triplicate determinations.

^{a-d}Means in the same row not sharing a common letter are significantly different ($p<0.05$) by Duncan's multiple range test.

^{A-D}Means in the same column not sharing a common letter are significantly different ($p<0.05$) by Duncan's multiple range test.

lemon balm powder that were refrigerated for 15 d are shown in Table 8. The VBN content on the day of preparation was 5.3-6.68 mg%, but increased to 15.05-17.80 mg% on d 15 of storage. The L3 group, except for immediately after manufacturing with 5 d of storage, had significantly lower values compared with the normal group (N) and had the VBN content similar to the positive control group (C). The VBN content of raw meat and packed meat is regulated at 20 mg% or less in the Korea Food Sanitation Act (2002). In this study, the VBN content of hamburger patties during storage period was maintained at 20 mg% or less. There was no corruption of protein, and freshness was maintained. The L3 group could inhibit the increase in VBN content compared to the normal group (N) due to antioxidant and antibacterial effects (Gruenwalkd *et al.*, 1999; Sweetman, 2002). Thus, the addition of lemon balm powder will be effective in maintaining meat quality by effectively inhibiting the creation of VBN.

Changes in total aerobic bacteria added with lemon balm powder

The total plate counts in uncooked hamburger patties added with lemon balm powder during refrigeration over a 15-d period are shown in Table 9. In general, total plate counts significantly increased in linear fashion in all patties. However, on d 10 and 15, the L3 group had signifi-

cantly lower total plate counts compared to the normal group (N) and had a similar level to the positive control group (C). These groups tended to show inhibition of the growth of microbes, with approximately 0.5-1 log levels lower than the normal group (N) upon the addition of 1% lemon balm powder or more. In general, when the total aerobic bacteria counts in meat reached 7-8 Log CFU/g, corruption and off-odor occurred (Egan *et al.*, 1980). Analyzing the stored hamburger patties after 15 d, the normal (N), L1, and L2 groups had values of 7.69, 7.40, and 7.18, respectively, verifying that deterioration was occurring. Because the positive control group (C) and the L3 group showed inhibition of microbial proliferation, these groups were safe from bacteria during storage. The L3 group had inhibited proliferation of the total aerobic bacteria compared to the normal group (N) due to antiseptic action and antibacterial effects (Gruenwalkd *et al.*, 1999; Sweetman, 2002) of lemon balm powder. Therefore, the addition of lemon balm powder extended the storage period by inhibiting corruption and deterioration by microorganisms.

Conclusion

The aims of this study were to examine the antioxidant properties of lemon balm in order to evaluate the potential use of lemon balm as a functional food material and

Table 9. Changes of total aerobic counts (TPC) for patties prepared with different levels of lemon balm powder during 15 d of storage at 5°C

	Storage time (d)	Treatments ¹⁾				
		N	C	L1	L2	L3
Log CFU/g	0	3.93±0.09 ^{2)D}	4.01±0.01 ^D	3.94±0.08 ^D	3.92±0.01 ^D	4.03±0.02 ^D
	5	5.39±0.01 ^{aC}	4.95±0.02 ^{cC}	5.34±0.01 ^{aC}	5.02±0.02 ^{bcC}	5.05±0.02 ^{bcC}
	10	6.53±0.06 ^{abB}	5.90±0.01 ^{cB}	6.29±0.11 ^{abB}	6.16±0.07 ^{bcB}	5.93±0.05 ^{cB}
	15	7.69±0.21 ^{aA}	6.40±0.12 ^{bA}	7.40±0.01 ^{aA}	7.18±0.01 ^{aA}	6.48±0.01 ^{bA}

¹⁾See the legend of Table 1.

²⁾All values are expressed as mean±SE of triplicate determinations.

^{a-d}Means in the same row not sharing a common letter are significantly different ($p<0.05$) by Duncan's multiple range test.

^{A-D}Means in the same column not sharing a common letter are significantly different ($p<0.05$) by Duncan's multiple range test.

to investigate the antioxidant properties of lemon balm in ground hamburger patties stored for 15 d at 4°C. The high total polyphenol and total flavonoids contents, and high antioxidant activity were exhibited in the lemon balm. The patties made with addition lemon balm had significantly lower total aerobic bacteria counts, TBA and VBN values compared to the normal sample. Lemon balm-treated patties had also significantly higher flavor and total acceptability scores than those of the normal sample. In conclusion, lemon balm has been indicated to protect against lipid oxidation and to improve the sensory attributes of ground hamburger patties. Therefore lemon balm is a useful natural antioxidant with strong potential to be used in meat products.

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