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EVALUATION OF ANTIOXIDATIVE PROPERTIES OF HOLY BASIL AND GALANGAL AND THEIR APPLICATION TO INCREASE THE OXIDATIVE STABILITY OF COOKED GROUND PORK

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Introduction

Lipid oxidation is one of great concern to the meat during the storage and processing of foods, because it can lead to the development of unpleasant tastes and off-flavors as well as changes in color, rheological properties, and solubility, and potential formation of toxic compounds such as 4-hydroxy-nonenal (Addis, 1989). Ground meat tends to become brown and rancid more rapidly than whole muscle retail cuts (Ho, McMillin, & Huang, 1996). Because of its relatively high content of unsaturated fatty acid, pork oxidizes more rapidly than either beef or lamb (Pearson, Love, & Shorland, 1977). Additionally, thermal processes can promote lipid oxidation by disrupting cell membranes and releasing prooxidants, thereby inducing "warm-over flavor (WOF)", which rapidly develops in cooked meat products during refrigerated storage and subsequent reheating (Sato & Hegarty, 1971). Therefore, it is essential to control lipid oxidation to delay the development of those WOF, because this is one of the major reasons for spoilage of meat-based products.

The most common synthetic antioxidants used in raw and precooked ground poultry, beef and pork products during storage to retard lipid oxidation are butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate (Shahidi, Rubin, & Wood, 1987; St. Angelo, 1996; McCarthy, Kerry, Kerry, Lynch, & Buckley, 2001a, 2001b). However, the demand for natural antioxidants has recently increased because of the toxicity and carcinogenicity of synthetic antioxidants (Branen, 1975; Ito, Fukushima, Hasegawa, Shibata, & Ogiso, 1983). Thus, there is an increasing interest in finding natural herbal plants that show high antioxidant activity.

Several research studies have documented the effectivesness of antioxidative components in herbal plants such as flavonoids, phenolic or polyphenolic and related compounds for the prevention of lipid oxidation in meat and meat products (Cuvelier, Richard, & Berset, 1996; Botsoglou, Christaki, Fletouris, Florou-Paneri, & Spais, 2002; Ahn, Grün, & Fernando, 2002; Fernández-López, Sevilla, Sayas-Barberá, Navarro, Marín, & Pérez-Alvarez, 2003). The antioxidant activity of phenolic compounds in herbs and spices is mainly due to their redox properties and chemical structure, which can act as reducing agent, free radical scavenger, Fe^{2+}-chelator or quenchers of the formation of

singlet oxygen (Zheng & Wang, 2001; Pizzale, Bortolomeazzi, Vichi, Uberegger, & Conte, 2002).

The culinary herbs and spices such as lemon grass, ginger, galangal and holy basil are widely used in Thai cooking (Cousminer & Hartman, 1996; Uhl, 1996). Galangal (*Alpinia galanga*), a rhizome closely related to the ginger family, has been one of the most important ingredients in Thai curry paste and commonly used as flavoring (Uhl, 1996). Basil is generally used in Italian, Southeast Asian, and Mediterranean food for its clean flavor and sweet scented herbal bouquet. There are many types of basil, which vary in size, color and flavor. The most commonly used types in Thai cooking are holy basil (*Ocimum sanctum* Linn), which has spicy and lemony notes (Uhl, 1996). Several researchers have reported that galangal and basil extracts showed antioxidant activity in a model system (Barik, Kunda, & Dey, 1987; Wang, Chen, Liu, & Guo, 1997; Cheah & Abu-Hasim, 2000; Javanmardi, Stushnoff, Locke, & Vivanco, 2003). However, a few studies are available about its application in meat systems.

Objectives

1. To study the total phenolic content, antioxidant activity against a β-carotene-linoleic acid emulsion system, DPPH scavenging activity, superoxide anion scavenging activity, Fe^{2+} chelating activity and reducing power of ethanolic extracts from Holy basil and Galangal.
2. To study the effectiveness of natural antioxidant including dried Holy basil and dried Galangal powder and ethanolic extracts from Galangal and Holy basil as inhibitor of lipid oxidation in cooked ground pork.
3. 2.3.To compare the effectiveness of a commercial antioxidant mixture of citric acid, ascorbic acid and α-tocopherol and natural antioxidants from Holy basil and Galangal.

Methodology

Materials

Fresh Holy basil (*Ocimum sanctum* Linn) leaves and Galangal (*Alpinia galanga*) rhizomes, imported from various locations in Thailand, were purchased from Asian supermarkets in Vienna, Austria. Samples were cleaned, washed with water, cut into small pieces, dried overnight in an air dryer (Memmert-GmbH+Co.KG, type UM 200-800, Germany) at 40˚C, ground to a particle size of 25 mesh by using a grinder (Moulinex, Type MCU 1A, France), and stored at –20˚C in an airtight container until use. Pork meat was obtained from a local market in Vienna, Austria.

Preparation of herbal extracts

In the preliminary study, antioxidative properties such as total phenolic content, reducing power, and antioxidant activity of Holy basil and Galangal were influenced by

extraction conditions. Optimum conditions for extraction of antioxidants from Holy basil and Galangal in this study are followings:

Dried Holy basil powder (4.50±0.05 g dry basis) were extracted by stirring with 50 ml of ethanol and water (3:1, v/v) at 75˚C and 300 rpm for 30 min, whereas, dried Galangal powder (4.5 g dry basis) was extracted with 50 ml of ethanol and water (1:1, v/v) at 50˚C for 1 h. Each extract was then filtrated through filter paper (595 ½ folded filters, ∅125 mm, Ref. No. 10311644, Schleicher&Schuell GmbH, Germany); the filtrates were collected and dried using a rotary evaporator (Büchi rotavapor (R), Switzerland) at 40˚C for 15 min, filled in a plastic bottle and stored at –20˚C until use.

Total phenolic content

The total phenolic content was measured by the method described by Weurman and Swain (1955). The phenolic extracts (0.5 ml) was added to 5 ml of distilled water and vortexed for 1 min, then 1 ml of Folin and Ciocalteu's Phenolic Reagent was added and mixed well by using vortex mixture (Bender& Bobein AG, Model K-550-GE, Switzerland). After 5 min, 1 ml of saturated sodium carbonate solution was added and the mixture was vortexed again. The sample was allowed to develop color for 1 hr. The absorbance was measured at 640 nm by using a spectrophotometer (Hitachi U-1500 spectrophotometer). A standard curve was prepared at the same time with chlorogenic acid at concentration ranging from 0–100 µg/ml. The quantity of total phenolic content in the sample was calculated as chlorogenic acid equivalent by using the standard curve.

Antioxidant activity

Antioxidant activity based on coupled oxidation of β-carotene and linoleic acid emulsion system was evaluated by some modification of the method described by Taga, Miller, and Pratt (1984). The β-carotene (2 mg) was dissolved in 20 ml of chloroform. A 3 ml aliquot of the solution was put into a 50 ml beaker and 40 mg linoleic acid and 400 mg Tween 20 were added. Chloroform was removed by purging with nitrogen. Oxygenated distilled water (100 ml), which was generated by aerating air bubble into distilled water for 1 h, was added into the β-carotene emulsion and mixed well by using a vortex mixer (Bender & Bobein AG, Model K-550-GE, Switzerland). Aliquots (3 ml) of the oxygenated β-carotene emulsion and 0.12 ml of ethanolic extracts at concentration 1 mg/ml were placed in capped culture tubes and mixed thoroughly. The tubes were immediately placed in a water bath and incubated at 50˚C. Oxidation of β-carotene emulsion was monitored spectrophotometrically by measuring absorbance at 470 nm in a Hitachi U-1500 spectrophotometer. Absorbance was measured at 0, 10, 20, 30 and 40 min. A control was prepared by using 0.12 ml of ethanol instead of the ethanolic extracts. Degradation rate of the extracts was calculated according to first order kinetics using equ. 1 (Al-Saikhan, Howard, & Miller, 1995).

$$\ln (a/b) \times 1/t = \text{sample degradation rate} \qquad \text{(equ. 1)}$$

ln = natural log

a = initial absorbance (470 nm) at time zero

b = absorbance (470 nm) at time 40 min

t = time (min)

The antioxidant activity (AA) was expressed as % inhibition relative to the control using equ. 2:

$$AA = \frac{\text{Degradation rate of control} - \text{Degradation rate of sample} \times 100}{\text{Degradation rate of control}} \quad \text{(equ. 2)}$$

DPPH scavenging activity

The effect of the ethanolic extracts on the content of 2,2-diphenyl-2-picrylhydrazyl radical ($DPPH^{\bullet}$) was estimated according to the modified method of Hatano, Kagawa, Yasuhara, and Okuda (1988). An aliquot (0.5 ml) of the $DPPH^{\bullet}$ solution (50 mg/ml) was diluted in 4.5 ml of methanol, and 0.1 ml of the ethanolic extracts at various concentrations was added. The mixture was shaken vigorously and allowed to stand for 45 min in the dark. The decrease in absorbance was measured at 515 nm against a blank (without extract) in a Hitachi U-1500 spectrophotometer. The decrease in absorbance depends on the antioxidant and radical concentration, the molecular structure of the antioxidant and its kinetic behavior (Brand-Willams, Cuvelier, & Berset, 1995).

From a calibration curve obtained with different amounts of ethanolic extracts, the ED_{50} was calculated. The ED_{50} was defined as the concentration of an antioxidant extracts which was required to quench 50% of the initial $DPPH^{\bullet}$ under the experimental conditions given.

Superoxide anion scavenging activity

Measurement of superoxide anion scavenging activity of the ethanolic extracts was based on the method described by Liu, Ooi, and Chang (1997). Superoxide anions were generated in a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system by oxidation of NADH and assayed by reduction of nitroblue tetrazolium (NBT).

In these experiments the superoxide anion was generated in 3 ml of Tris-HCl buffer (16 mM, pH 8.0) containing 1 ml of NBT (50 µM) solution, 1 ml of NADH (78 µM) solution and ethanolic extracts at concentration 1.0 mg/ml. The reaction started by adding 1 ml of phenazine methosulphate (PMS) solution (10 µM) to the mixture. The reaction mixture was incubated at 25˚C for 5 min and its absorbance at 560 nm was recorded against blank samples in a Hitachi U-1500 spectrophotometer. A lower absorbance of the reaction mixture indicated a higher superoxide anion scavenging activity. Superoxide anion scavenging activity (SASA) was calculated using equ. 3.

$$\text{SASA [\%]} = \left(1 - \frac{\text{Absorbance of sample at 560 nm}}{\text{Absorbance of control at 560 nm}}\right) \times 100 \qquad \text{(equ. 3)}$$

Chelating activity on Fe^{2+}

The chelating activity of the ethanolic extracts on ferrous ions Fe^{2+} was measured according to the method of Decker and Welch (1990). A 1 ml of ethanolic extracts at concentrations 1.0 mg/ml was mixed with 3.7 ml of deionized water. The mixture was left for reaction with $FeCl_2$ (2 mM, 0.1 ml) and ferrozine (5 mM, 0.2 ml) for 10 min at room temperature, and then the absorbance was measured at 562 nm in a Hitachi U-1500 spectrophotometer. A lower absorbance indicates a higher chelating power. Chelating activity was calculated according to equ. 4.

$$\text{Chelating activity [\%]} = \left(1 - \frac{\text{Absorbance of sample at 562 nm}}{\text{Absorbance of control at 562 nm}}\right) \times 100 \qquad \text{(equ. 4)}$$

Reducing power

The reducing power of the ethanolic extracts was measured according to the method of Oyaizu (1986). A 0.5 ml of ethanolic extracts at concentration 1 mg/ml was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1 %). The mixture was incubated at 50˚C for 20 min. TCA (10 %: 2.5 ml) was added. The mixture was centrifuged at 650x g for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride and the absorbance was measured at 700 nm in a Hitachi U-1500 spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power.

Preparation of pork meat samples

The pork was trimmed to remove bone, skin and visible fat, cut into small pieces and then divided into six portions for each experiment prior to the addition of the test compounds. The pork of each portions was mixed with salt (2%) and the antioxidants according to the following formulation: (1) control (no antioxidant); (2) commercial antioxidant mixture of 0.3 % citric acid + 0.5 % ascorbic acid + 0.02% α-tocopherol; (3) 0.1 % ethanolic extracts of Holy basil; (4) 0.345 % dried Holy basil powder; (5) 0.1 % ethanolic extract of Galangal; (6) 0.350 % dried Galangal powder. Each portion was mixed for 2 min in a cutter (Dianawerk Model 65020), Austria. The concentration of dried Holy basil and dried Galangal powder and their ethanolic extracts were set according to the results obtained from preliminary experiments. The concentration of dried herb powder is based on the concentration of ethanolic extracts. Each portion of

ground pork was placed in polyethylene and then evenly spread to a thickness of 1 cm. All samples was packed bag under vacuum and heated on an open electric water bath until the final internal temperature of 80°C (measure with a thermocouple). After cooling down to room temperature, cooked ground pork of each portion was divided into smaller portion (about 100 g). Each sample was individually overwrapped tightly with oxygen-permeable cling film. The samples were displayed in a refrigerator (5°C) for 14 days. The samples in each experiment were evaluated at 0, 3, 7, 10, and 14 days of storage. Six replications at day 0 and 14 and duplication at day 3, 7 and 10 of storage from each treatment were sampled and then separately chopped in a microblender to obtain homogeneneous samples. The samples in each treatment were analyzed TBARS value, conjugated diene, hexanal content and peroxide value.

Conjugated dienes

The formation of conjugated dienes was determined according to the procedure described by Sirinivasan, Xiong, and Decker (1996) with some modifications. Meat samples (0.5 g) were suspended in 5 ml of distilled water and homogenized to form a smooth slurry. A 0.5 ml of aliquot of this suspension was mixed with 5 ml of extracting solution (3:1 hexane:isopropanol) for 1 min. After centrifugation at 2000 g for 5 min, the absorbance of the supernatant was read at 233 nm. The concentration of conjugated diene was calculated using the molar extinction coefficient of 25,200 $M^{-1}cm^{-1}$ and the results was expressed as μmole per mg of meat sample.

Thiobarbituric acid reactive substances (TBARS)

Lipid oxidation was assessed by the 2-thiobarbituric acid (TBA) method of Witte, Krause, and Bailey (1970). In brief, ten grams of sample were homogenized in 50 ml of 10% (w/w) trichloroacetic acid. After homogenization the mixture was transferred to measuring flask and adjusted to 50 ml with distilled water. The dispersion was filtered through a folded filter paper (MN 615 ¼, ∅150 mm, Cat. No. 531015, Macherey-Nagel GmbH & Co. KG., Germany). The supernatant (5 ml) was mixed with 5 ml 2-thiobarbituric acid (2.88 g l^{-1} H_20) and heated in a boiling water bath for 10 min to develop the rose-pink color by reaction between malondialdehyde and 2-thiobarbituric acid ($C_4H_4N_2O_2S$) and cooled to room temperature. The absorbance was measured at 532 nm, against a blank prepared with 5 ml distilled water and 5 ml TBA-reagent, using a UV-VIS spectrophotometer (Hitachi U-1100). Thiobarbituric acid reactive substances (TBARS) were calculated from a standard curve (8⁻50 nmol) of malondialdehyde (MDA), freshly prepared by acidification of TEP (1,1,3,3-tetraethoxypropane). The TBARS value was calculated as mg of malondialdehyde per kg of sample.

Analysis of peroxide value

Peroxide value was determined according to AOAC method 965.33 (Association of official Analytical Chemists, 1995) and expressed as meq O_2/ kg meat.

Hexanal content

A Fisons GC 8000 gas chromatograph and HS-6 headspace sampler (Fisons Instruments SpA, Milan, Italy) were used. The volatiles in cooked ground pork samples were seperated on a high polarity DB-5MS fused silica capillary column (15 m x 0.248 mm internal diameter, 0.25 μm film, J & W Scientific Inc., California, U.S.A.) was used. Operating conditions for GC were: helium flow 2 ml min^{-1}, initial oven temperature 40 ^{O}C for 3 min, raised to 220 ^{O}C at 20 ^{O}C/min, and held at 220 ^{O}C for 2 min. The injector and flame ionization detector (FID) temperature were adjusted to 250 ^{O}C and held at this temperature throughout the analysis. Total run time was 15 min.

For head space (HS) analysis, 3 g portion of minced cooked ground pork sample were transferred to 10 ml glass vials, capped with Teflon-lined septa, crimped and then placed in HS-6 magazine assembly to preheat at 60 ^{O}C for a 30 min equilibration. Chromatogram peak areas were expressed as integrator count units. Comparing relative retention time of GC peaks with those of commercially available standards tentatively identified the volatile compounds. Quantitative determination of hexanal was accomplished using 2-heptanone as an internal standard.

Statistical analysis

The statistical assessment was carried out with the program system of SPSS for Windows (Version 9). The results of TBARS value, peroxide value, conjugated diene and hexanal content were analyzed using one-way analysis of variance (ANOVA). Differences were considered significant at the $P < 0.05$ level. Comparison of treatment mean was based on Duncan's multiple range test (Montgomery, 1991). Furthermore, a correlation procedure (Pearson's correlation coefficient) was performed to evaluate any relationship between the TBARS value and hexanal content.

Results & Discussion

Antioxidant properties of ethanolic extracts from Holy basil and Galangal

Table 1 shows the total phenolic content, antioxidant activity against a β-carotene-linoleic acid emulsion system, DPPH scavenging activity, Fe^{2+} chelating activity, superoxide anion scavenging activity and reducing power of ethanolic extracts from Holy basil and Galangal. So far as plant phenolic constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, it was reasonable to determine their total amount in the selected plant extracts. From the result, ethanolic extracts of Galangal contained less total phenolic content than ethanolic extracts of Holy basil. The antioxidant activity, which reflected the ability of both extracts to inhibit the bleaching of β-carotene, was measured. The results indicated that ethanolic extracts from Holy basil exhibited greater antioxidant activity compared to ethanolic extracts from Galangal. Radical scavengers were evaluated by their reactivity toward a stable free radical, DPPH. DPPH radical scavenging activity (EC_{50}) of ethanolic extracts from Holy basil was better than that of ethanolic extracts from Galangal. Superoxide radical is

known to be very harmful to cellular components as a precursor of the more reactive oxygen species, contributing to tissue damage and various diseases (Halliwell & Gutteridge, 1999). The results showed that ethanolic extracts from Holy basil had higher superoxide radical-scavenging activity than ethanolic extracts from Galangal. Metal chelating capacity was significant since it reduce the concentration of the catalyzing transition metal in lipid oxidation (Duh, Tu, & Yen, 1999). Fe^{2+} chelating activity of ethanolic extracts from Holy basil was better than that of ethanolic extracts from Galangal. Additionally, the power of certain antioxidant is associated with their reducing power (Jayaprakasha, Singh, & Sakariah, 2001), which is associated with the presence of reductones (Duh, 1998). The results revealed that ethanolic extract from Holy basil had higher reducing power than ethanolic extracts from Galangal.

The results of the present work indicated that ethanolic extracts from Holy basil and Galangal possess high antioxidant activity, reducing power, free radical scavenging activity, Fe^{2+} chelating activity and superoxide anion scavening activity. This was postulated to be due to the diversity and complexcity of natural mixtures of antioxidant phenolic compounds in both extracts. Plant phenols and polyphenolic compounds are widely distributed in natural herb and spice extracts, and they have been found to possess significant antioxidant activity (van Acker et al., 1996; Zheng & Wang, 2001). However, it is rather difficult to characterize every compound and assess or compare their antioxidant activities. Generally, each herb contained different phenolic compounds, and each of these compounds differing amounts of antioxidant activity.

Changes of TBARS values during storage of cooked ground pork

The 2-thiobarbituric acid reactive substances values (TBARS) represent the content of secondary lipid oxidation products, mainly aldehydes (or carbonyls), which contribute to off-flavors in oxidized in meat and meat products. The effect of the antioxidants on TBARS value of cooked ground pork over 14 days of refrigerated storage is shown in Fig 1. The analysis of variance for the TBARS data indicates that the TBARS values were significantly affected ($P < 0.05$) between the control sample and those treated with the various antioxidants throughout storage. The overall lipid oxidation was drastically accelerated as storage progressed due to the denatured structure of the muscles by cooking and aerobic storage conditions. The TBARS value of commercial antioxidant mixture dried Galangal and dried Holy basil powder and ethanolic extracts from Galangal and Holy basil were considerably lower ($P < 0.05$) than the control, thus indicating high protection of meat against lipid oxidation. The ability of dried Galangal and dried Holy basil powder and their ethanolic extracts to inhibit lipid oxidation probably maybe related to the ability of their polyphenol content and structure. Hettiarachchy, Glenn, Gnanasambandam, and Johnson (1996) and Akamittath, Brekke, and Schanus (1990) found that the effectiveness of synthetic and natural antioxidants in controlling lipid oxidation in meat products. Cheah and Abu Hasim (2000) reported that 10 % Galangal extracts were as affective as 0.10 % α-tocopherol and 0.02 % BHT inhibiting/minimising lipid oxidation in raw beef during storage at 4 ^{O}C.

Initial (day 0) TBARS vaules for all antioxidant-containing samples were significantly ($P < 0.05$) lower than those for the control. This result suggests that these antioxidants retarded lipid oxidation during and immediately after cooking. The results agree with that reported by Ahn, Grün, and Fernando (2002); Fernández-López, Sevilla,

Sayas-Barberá, Navarro, Marín, and Pérez-Alvarez (2003). Sato and Hegarty (1971) reported that non-heme iron was the active catalyst in cooked meats. Chen, Pearson, Gray, Fooladi, and Ku (1984) exhibited that iron was released from heme pigments during cooking and proposed that the resultant increase in non-heme iron was responsible for lipid oxidation. Moreover, phospholipids are the primary substrates of lipid oxidation and are membrane components in close contact with the catalysts of lipid oxidation, which are located in the aqueous phase of the muscle cell (Gandemer, 1998). Gray and Pearson (1987) demonstrated that the membrane phospholipids, which are high in polyunsaturated fatty acid are responsible for the initial development of oxidation in cooked meat products during storage.

The results of this study indicated that the dried herb powder were much more effective on inhibition of lipid oxidation than ethanolic extracts from herbs. This is postulated to be due to decomposition of some active compounds, which had efficiency in oxidation prevention during the ethanolic extraction. It also could be because all antioxidative compounds in herbs could not be extracted by extracting solvent. This disagrees with the results of Abd El-Alim, Lugasi, Hóvári, and Dworschák (1999) who found that the ethanolic extracts of sage, basil, thyme and ginger at concentration 200 mg/ml were much more effective on inhibition of lipid oxidation than the dried spices at concentration 10 g/kg.

In addition, it was observed that the addition of Galangal was more effective than addition of Holy basil in retarding lipid oxidation throughout the storage period. Using the dried herbs, it was observed that Galangal was more effective than Holy basil in inhibiting the lipid oxidation. Thus, TBARS values did not correlate with total phenolic content, antioxidant activity against a β-carotene-linoleic acid emulsion system, DPPH scavenging activity, Fe^{2+} chelating activity, superoxide anion scavening activity and reducing power of both herbs. It could be due to the pro-oxidant effect of Holy basil, by the presence of chlorophyll. Endo, Usuki, and Kaneda (1985) have also reported that chlorophylls and their derivatives promote oxidation of lipids during storage. He and Shahidi (1997) have shown that antioxidant effect of green tea extracts in white muscles of mackerel might be markedly influenced by the presence of chlorophyll and other impurities.

It was found that TBARS value in cooked ground pork treated with dried Galangal powder was less than 1.63 mg MDA kg^{-1} sample on day 7. The threshold of TBARS values of oxidized flavor in cooked beef was between 0.5–1.0 and perceived by trained panelists (Tarladgis, Watts, & Younathan, 1960) and between 0.6–2.0 by inexperienced panelists (Greene & Cummuze, 1982). With this threshold as indicator dried Galangal powder added at a concentration of 0.35 % (w/w) almost controlled the development of lipid oxidation in cooked ground pork.

The efficiency of the sample treated with the various antioxidants in inhibiting lipid oxidation throughout refrigerated storage is in the following order: commercial antioxidant mixture (0.3 % citric acid + 0.5 % ascorbic acid + 0.02% α-tocopherol) > dried Galangal powder > dried Holy basil powder > Galangal extracts > Holy basil extracts > control. At the end of storage time (14 days) treatments added with dried Galangal and dried Holy basil powder and ethanolic extracts from Holy basil and Galangal resulted in significantly lower ($P < 0.05$) TBARS values compared to the control, which indicates that addition of Holy basil and Galangal exhibited antioxidant

properties. Beyond that, extension of shelf life without further oxidative changes would be possible with addition of dried Galangal and dried Holy basil powder and ethanolic extracts from Holy basil and Galangal.

Changes of peroxide value during storage of cooked ground pork

Peroxide values were used as indices to assess the level of lipid oxidation in cooked ground pork during storage at 5 ^{o}C (Fig. 2). The peroxide value of the control sample and those treated with the various antioxidants throughout storage increased during 14 days refrigerated storage. The increase of POV in cooked ground pork during storage may results from catalysis of intracellular compounds, the destruction of the cell structure by NaCl and processing.

On the other hand, the peroxidation of lipid also may be facilitated by oxygen during storage. Nevertheless, all treated samples had significantly ($P < 0.05$) effected with lower peroxide value compared to the control. The addition of both dried powders and ethanolic extracts from Holy basil and Galangal in cooked ground pork markedly inhibited the lipid peroxidation by decreasing POV, probably due to the phenolic constituents in Galangal and Holy basil function as antioxidants by terminating free radical chain-type reaction.

At the end of the storage (14 days), the effectiveness of the cook ground pork treated with the various antioxidants was in the following decreasing order: commercial antioxidant mixture > dried Galangal powder > dried Holy basil powder > Galangal extracts > Holy basil extracts > control. However, no significant differences ($P > 0.05$) were found between cooked ground pork treated with dried Galangal and dried Holy basil powder and also between cooked ground pork treated with ethanolic extracts from Galangal and Holy basil extracts on day 14. However, samples treated with Holy basil had higher POV than those treated with Galangal.

Changes of conjugated diene during storage of cooked ground pork

The development in conjugated diene was assessed on the basis of the hydroperoxides formed in extracted lipid of the cooked ground pork. All treated sample was able to decrease the formation of conjugated diene in cooked ground pork (Fig. 3). The concentration of conjugated diene increased significantly ($P < 0.05$) for all treatment with exception of the sample treated with ethanolic extracts from Holy basil during the first 3 days of storage and then decreased with a further storage time increased beyond 14 days.

This result was in agreement with Peña-Ramos and Xiong (2003) who report that the concentration of conjugated diene significantly increased on the first day, followed by decrease thereafter for cooked pork patties treated with whey and soy protein hydrolysates. Frankel (1998) noted that the formation of conjugated diene, which parallels the production of hydroperoxides, occurs in the early stages of lipid oxidation. Conjugated hydroperoxides are expected to decompose to the secondary products, and from the results, it is noteworthy that the decrease in conjugated dienes was accompanied by an increase in TBARS in cooked ground pork. Additionally, cooked ground pork treated with dried Galangal and dried Holy basil powder had lower concentration of conjugated diene compared to cooked ground pork containing ethanolic extracts from Galangal and Holy basil. No significant difference ($P > 0.05$) in conjugated diene was found between all treatments and control on day 3 and 7.

At the end of the storage period (14 days), cooked ground pork treated with synthetic antioxidants mixture was the most susceptible treatment to inhibit lipid oxidation, evidenced by the lowest conjugated diene, followed by cooked ground pork treated with dried Galangal powder, dried Holy basil powder, Galangal extracts and Holy basil extracts. However, no significant ($P > 0.05$) differences in conjugated diene were detected between cooked ground pork treated with commercial antioxidants mixture, dried Galangal and dried Holy basil powder on day 14.

Changes of hexanal content during storage of cooked ground pork

Hexanal has been used to follow the course of lipid oxidation and off-flavor development in cooked food (Dupuy, Bailey, St Angelo, Legendre, & Vercelotti, 1987). The analysis of variance for the hexanal content data indicates that hexanal content for all treatment was significantly ($P < 0.05$) lower after cooking on day 0 than that of the control (Fig. 4). While significant increases in hexanal content were observed for all samples treatments throughout the storage period, the hexanal content for all antioxidant-containing treatments was consistently lower than that for the control.

At the end of the storage (14 days), the effectiveness of the cooked ground pork treated with various antioxidants was in the following decreasing order: commercial antioxidant mixture > dried Galangal powder > dried Holy basil powder > Galangal extracts > Holy basil extracts > control. Increasing in hexanal content in all treatments during storage may indicate persistent formation of aldehyde in the meat. Oxidation of linoleic acid and further oxidation of preformed volatiles have been considered to be responsible for the abundant occurrence of hexanal in food systems (Barbut, Josephson, & Maurer, 1985). Matthews (1971) noted that large amounts of hexanal were formed from the further oxidation of 2,4, -decadienal.

On the other hand, hexanal has been previously tested as qualitatively comparable to the TBARS test (Brunton, Cronin, Monahan, & Durcan, 2000; Brunton, Cronin, & Monahan, 2001; Beltran, Pla, Yuste, & Mor-Mur, 2003), and its determination is faster and easier than conventional methods for evaluating lipid oxidation. In the present study, changes in hexanal content were similar to changes in TBARS value. TBARS values and hexanal contents correlated well ($r^2 = 0.87$; $P< 0.05$) during storage period. This results was in agreement with findings by Ahn, Grün, and Fernando (2002) who report that hexanal contents in cooked ground beef treated with ActiVin^{TM}, BHA/BHT, rosemary and α-tocopherol increased in the same manner as the TBARS values.

Conclusions

The findings of this study demonstrated that ethanolic extracts of Holy basil showed better total phenolic content, antioxidant activity against a β-carotene-linoleic acid emulsion system, DPPH scavenging activity, Fe^{2+} chelating activity, superoxide anion scavenging activity and reducing power than ethanolic extracts of Galangal. Additionally, the addition of commercial antioxidant mixture, dried Holy basil and Galangal powder or ethanolic extracts of Holy basil and Galangal were all inhibitory of lipid oxidation in cooked ground pork by reducing TBARS value, conjugated diene, POV and hexanal content compared to control during refrigerated at 5 ^{O}C for 14 days. Commercial

antioxidant mixture of 0.3 % citric acid + 0.5 % ascorbic acid + 0.02% α-tocopherol was identified as being the most effective antioxidant in retarding lipid oxidation in cooked ground pork. Dried powder of Galangal and Holy basil was more potent than ethanolic extracts of Galangal and Holy basil in suppressing lipid oxidation. Furthermore, addition of Galangal in cooked ground pork was more effective with elimination of lipid oxidation compared to addition of Holy basil. TBARS value and hexanal contents were well correlated.

Galangal and holy basil are culinary herbs and spices without any known toxic effect and has increasing use in preparation of ethnic food. This study brings attention to the antioxidant potential of dried powder and ethanolic extracts of Galangal and Holy basil as natural antioxidants for improving oxidative stability in meat products during storage. However, dechlorophyllization of ethanolic extracts from Holy basil may be necessary to avail them for application to meat products in which the original color of the ethanolic extracts might be of concern or when chlorophyll might act as pro-oxidant. Moreover, application of herbs antioxidant might be limited if the sensory quality of the meat products were affected, which needs further investigation.

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Tables and Figures

Table 1. Total phenolic content, antioxidant activity against a β-carotene-linoleic acid emulsion system, DPPH scavenging activity, Fe^{2+} chelating activity, superoxide anion scavenging activity and reducing power of ethanolic extracts from Holy basil and Galangal

	Holy Basil	Galangal
Total phenolic content [mg $(100g)^{-1}$]	4712.45±10.87	2288.39±8.76
Antioxidant activity[b] (%)	97.35±0.65	96.94±0.31
DPPH scavenging activity (ED_{50})[C] (mg/ml)	0.34±0.02	0.41±0.01
Fe^{2+} chelating activity [b] (%)	51.75±0.19	24.17±0.35
Superoxide anion scavenging activity [b] (%)	87.36±0.23	65.84±0.37
Reducing power[b] (absorbance 700 nm)	2.344±0.009	0.564±0.007

[a]Values are means±standard deviation of three replicate analyses.

[b]The data concerning the antioxidant activity, Fe^{2+} chelating activity, superoxide anion scavenging activity and reducing power were obtained with solution containing 1 mg of extracts/ml of extracting solvent.

[C]ED_{50} is the concentration of Holy basil and Galangal extracts to quench 50% $DPPH^{\bullet}$ under the chosen experimental conditions.

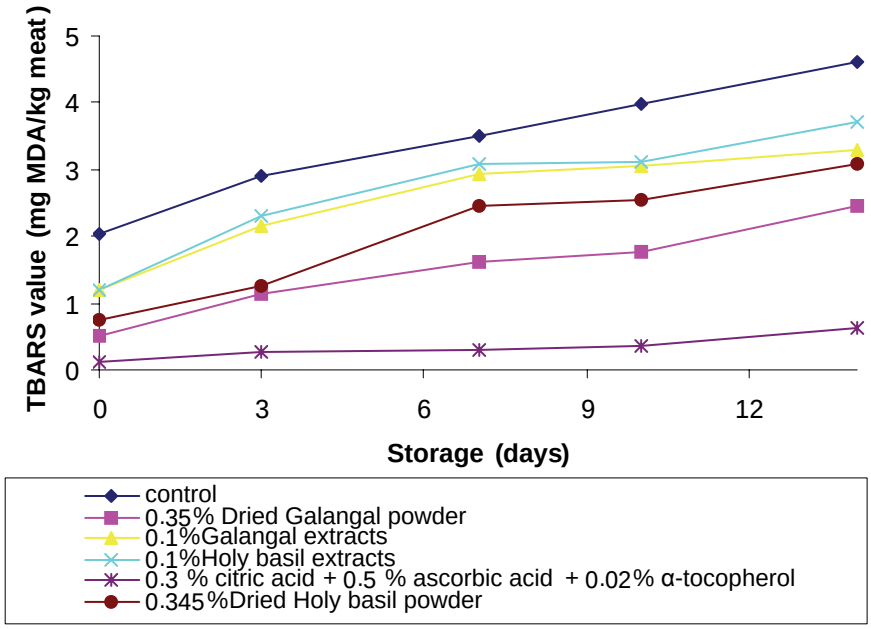

Fig. 1. Changes in TBARS value of cooked ground pork treated with different antioxidants during storage at 5 °C

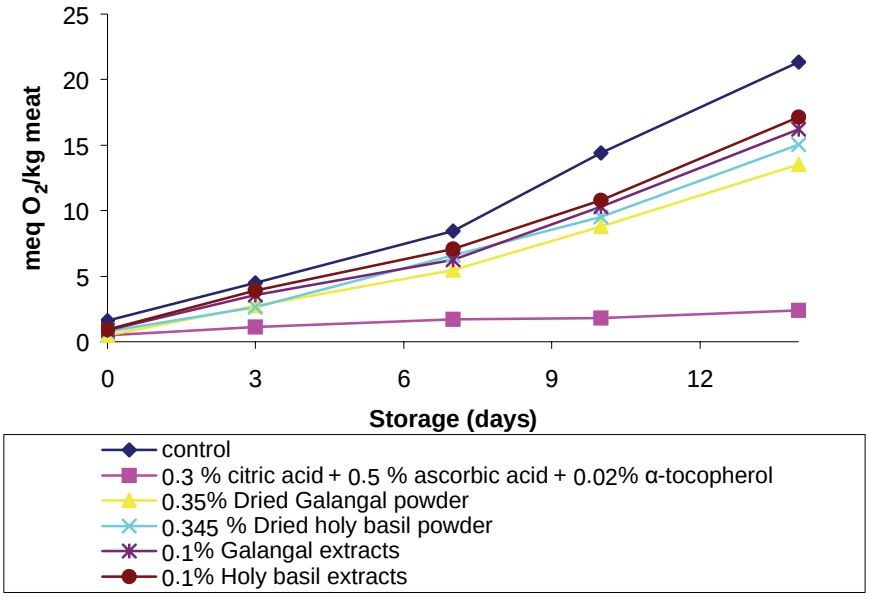

Fig. 2. Changes in peroxide value of cooked ground pork treated with different antioxidants during storage at 5 °C

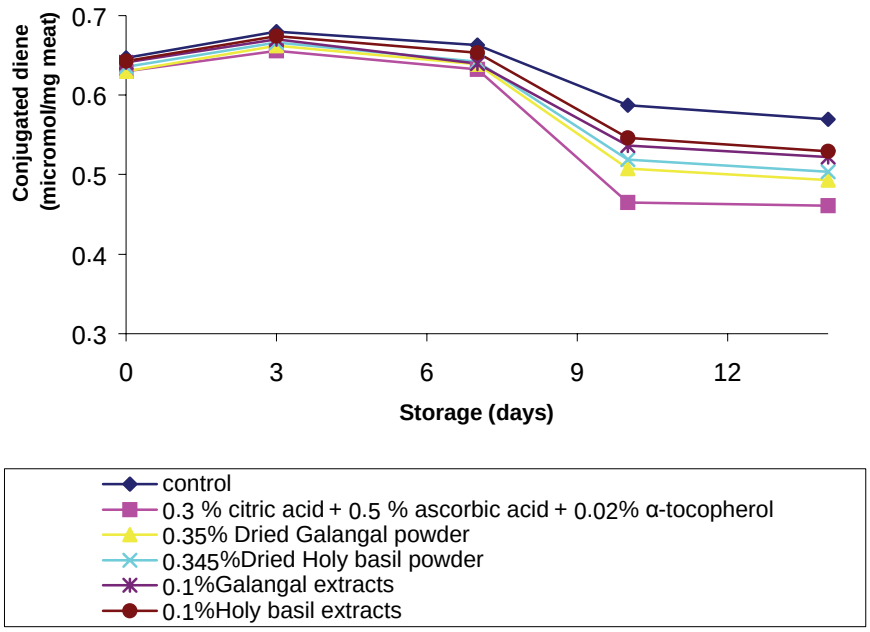

Fig. 3. Changes in conjugated diene of cooked ground pork treated with different antioxidants during storage at 5 °C

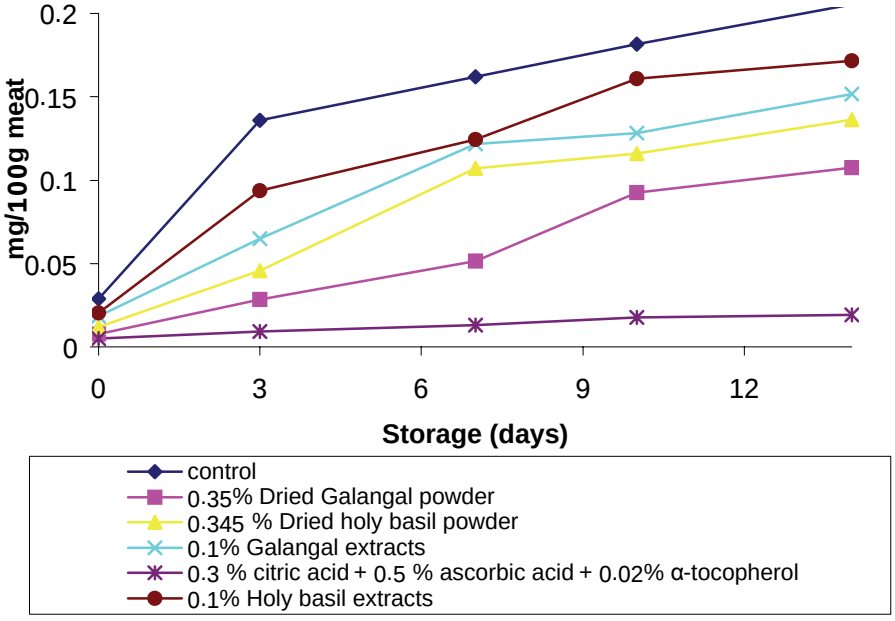

Fig. 4. Changes in hexanal content of cooked ground pork treated with different antioxidants during storage at 5 °C