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Antioxidative effect of added dried Holy basil and its ethanolic extracts on susceptibility of cooked ground pork to lipid oxidation

T. Juntachote [a,c,*], E. Berghofer [a], S. Siebenhandl [a], F. Bauer [b]

[a] Department of Food Science and Technology, Division of Food Technology, University of Natural Resources and Applied Life Sciences, Muthgasse 18, A-1190 Vienna, Austria
[b] Institute of Meat Hygiene, Meat Technology and Food Science, University of Veterinary Medicine Vienna, Veterinärplatz, A-1210 Vienna, Austria
[c] Department of Food Technology, Faculty of Technology and Community Development, Thaksin University, 140 Moo 4, Tambon Khao Roop Chang, Muang district, Songkhla 90000, Thailand

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Abstract

Efficacy of varying concentrations of dried Holy basil powder and its ethanolic extracts in retarding oxidative rancidity was tested with cooked ground pork. The development of lipid oxidation products during 14 days refrigerated storage (5 °C) was evaluated by means of thiobarbituric acid-reactive substances (TBARS) value, peroxide value (POV), conjugated diene and hexanal content. In the amounts studied, dried Holy basil powder and its ethanolic extract significantly ($P < 0.05$) inhibited the formation of TBARS, peroxide, conjugated dienes and hexanal in a dose-dependent manner. The oxidation process was significantly ($P < 0.05$) influenced by the type of antioxidant and its concentration. Addition of dried Holy basil powder to cooked ground pork was more effective in inhibiting lipid oxidation than its ethanolic extracts. Additionally, TBARS values and hexanal contents in cooked ground pork were highly correlated ($r^2 = 0.95$; $P < 0.05$) with each other.

Keywords: Holy basil; Natural antioxidant; Oxidative stability; Cooked ground pork

1. Introduction

A modern trend towards production of precooked, refrigerated ready-to-eat products has made the control of lipid oxidation increasingly important (Mielche & Bertelsen, 1994). Chilled raw meat is usually oxidatively stable, but mincing, cooking and other processes prior to refrigerated storage disrupt muscle cell membranes, facilitating the interaction of unsaturated fatty acids with pro-oxidant substances such as non-heme iron, and thereby accelerate lipid oxidation leading to rapid quality deterioration and development of rancidity (Tichivangana & Morrissey, 1985). Pork meat, in particular, oxidizes more rapidly than either beef or lamb because of its relatively high content of unsaturated fatty acid (Pearson, Love, & Shorland, 1977). Incorporation of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tertiary*-butyl hydroquinone (TBHQ), and propyl gallate (PG) into foods can retard lipid oxidation. However, the use of synthetic antioxidants in food products is under strict regulation due to the potential health hazards caused by such compounds (Hettiarachchy, Glenn, Gnanasambandam, & Johnson, 1996). This situation has created a necessity for scrutinizing naturally occurring antioxidant substances that may be used in foods in place of synthetic antioxidants. In the past few years, various plant materials containing phenolic compounds have been demonstrated to be effective antioxidants in model systems. Flavonoids, the most potent antioxidative compounds of plant phenolics occur in vegetable, fruits, berries, tea leaves and herbs (Kandaswami & Middleton, 1997; Shahidi & Wanasundara, 1992; Skrede & Wrolstad, 2002). Antioxidant properties of herbs and spices are apparently related to their

* Corresponding author. Tel.: +43 1 360066250; fax: +43 1 360066251.
E-mail address: tjuntachote@hotmail.com (T. Juntachote).

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phenolic content, suggesting that their antioxidant action is similar to that of synthetic phenolic antioxidants (Lai et al., 1991).

Several herbs and spices such as black papper, propolis (Dessouki, El-Dashlouty, El-Ebzary, & Heikal, 1980), rosemary (Cuvelier, Berset, & Richard, 1994) and oriental herbs (Kim, Kim, Kim, Oh, & Jung, 1994) have been reported to provide significant protection in freshly cooked meat and were effective in retarding lipid oxidation. There are several varieties of basil grown in many regions of the Orient. Most of the Oriental basils have a clove-like flavour that is generally stronger than that of Western basils. Holy basil (*Ocimum sanctum* Linn), known as "bai gkaprow" in Thailand, has dark green leaves with reddish purple stems and a purplish cast on the younger leaves, while the milder white basil has medium-green leaves with very light green, almost white, stems. The leaves of both varieties are smaller than sweet basil, and are slightly hairy and jagged around the edges. Holy basil is commonly used as a flavouring ingredient in Thai stir-fried dishes and some Thai spicy soups because of its spicy and lemony notes (Uhl, 1996). Recently, we showed that ethanolic extracts of Holy basil exhibited strong antioxidant activity against a β-carotene linoleic system, superoxide anion scavenging activity, Fe^{2+}-chelating activity and reducing power, and also acted as radical scavenger and lipoxygenase inhibitors (Juntachote & Berghofer, 2005). However, little or no work has been undergone to study the effect of the Holy basil plant itself as a natural antioxidant in preventing lipid oxidation in meat systems.

The objective of this study therefore was to study the effectiveness of both Holy basil powder and its ethanolic extracts in preventing/minimizing lipid oxidation in cooked ground pork as measured by thiobarbituric acid-reactive substances (TBARS), peroxide value (POV), conjugated dienes and hexanal content during storage at 5 °C.

2. Materials and methods

2.1. Materials

Holy basil (known as "bai gkaprow" in Thailand), imported from various locations in Thailand, was 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). Dried Holy basil powder was stored at −20 °C in an airtight container until use. Pork meat was obtained from a local market in Vienna, Austria.

2.2. Chemicals

2-Heptanone, TEP (1,1,3,3-tetraethoxypropane), 2-thiobarbituric acid and trichloroacetic acid (TCA) were obtained from Sigma (Sigma–Aldrich GmbH, Sternheim, Germany). All other chemicals and solvents used were of analytical grade and were obtained from Sigma (Sigma–Aldrich GmbH, Sternheim, Germany).

2.3. Preparation of Holy basil extracts

Dried Holy basil powder (4.50 ± 0.05 g dry basis) was extracted by stirring with 50 ml of ethanol and water (3:1, v/v) at 75 °C and 300 rpm for 30 min. The extracts were then filtrated through filter paper (595 1/2 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. Dried powder of ethanolic extracts obtained was transferred into plastic dark bottles and stored at −20 °C. Dilutions of 0.02%, 0.05%, and 0.10% (w/w) were prepared by dissolving the dried extracts in the extraction solvent on the day of experiments.

2.4. Preparation of pork meat samples

Fresh pork belly meat was trimmed to remove connective tissues, skin and visible fat, and cut into approximately 2-cm^3 pieces. The pork meat contained 30.57% fat as determined by the Soxhlet extraction method (AOAC method 960.39). The pork meat was divided into seven portions, for each experiment prior to the addition of the test compounds. Each portion of pork meat (1500 g) was mixed with salt (2%), and then Holy basil was added (w/w) according to the following formulation: (1) control (no antioxidant added); (2) 0.02% ethanolic extracts (3) 0.05% ethanolic extracts; (4) 0.10% ethanolic extracts; (5) 0.07% dried powder; (6) 0.18% dried powder; (7) 0.35% dried powder, whereas the amount of ethanolic extracts added to the cooked ground pork was equivalent to the original (unextracted) dried Holy basil powder.

Each sample was finely ground for 2 min with a laboratory bowl cutter (bowl speed 30 rpm, 4 knives, maximum capacity 2 kg, 1 mm height between bowl and knives, Dianawerk Model 65020, Austria). Ground pork was packed into polyethylene bags and evenly spread to a thickness of 1 cm. All samples were vacuum-packaged and then heated on an open electric water bath until a final internal temperature of 80 °C (measured with a thermocouple) was reached. After cooling down to room temperature with running cold water for 10 min, the cooked pork samples were divided into smaller portions (about 150 g each) and wrapped in oxygen-permeable cling film. The samples were displayed under daylight conditions for 14 days using a refrigerator at 5 °C equipped with a glass door. Oxidative changes were evaluated in triplicate (3 separate packages for each sample treatment) after 0, 7, and 14 days storage. Each sample was separately chopped in a microblender to obtain a homogeneous sample and then analyzed for its TBARS value, peroxide value conjugated dienes and hexanal content.

2.5. Total phenolic content

The total phenolic content of the ethanolic extracts of Holy basil was measured by the method described by Weurman and Swain (1955). The ethanolic extracts of Holy basil (0.5 ml) were added to 5 ml of distilled water and vortexed for 1 min, after which 1 ml of Folin and Ciocalteu's Phenolic reagent was added and mixed in well using a vortex mixer (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 colour for 1 h. The absorbance was measured at 640 nm using a spectrophotometer (Hitachi U-1500 Spectrophotometer, San Jose, CA, USA). A standard curve was prepared at the same time with chlorogenic acid at concentrations ranging from 0 to 100 μg ml^{-1}. The quantity of total phenolic content in the sample was calculated as chlorogenic acid equivalent by using the standard curve.

2.6. Analysis of conjugated dienes

The formation of conjugated dienes was determined according to the procedure described by Sirinivasan, Xiong, and Decker (1996) with some modifications. Cook ground pork samples (0.5 g) were suspended in 5 ml of distilled water and homogenized to form a smooth slurry. A 0.5 ml 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 dienes was calculated using the molar extinction coefficient of 25,200 M^{-1} cm^{-1} and the results were expressed as μmol mg^{-1} meat sample.

2.7. Analysis of thiobarbituric acid reactive substances (TBARS)

Lipid oxidation was assessed by the 2-thiobarbituric acid (TBA) method of (Witte, Krause, & Bailey, 1970). In brief, 10 g of sample were homogenized in 50 ml of 10% (w/w) trichloroacetic acid. After homogenisation, the mixture was transferred to a measuring flask and adjusted to 50 ml with distilled water. The dispersion was filtered through a folded filter paper (MN 615 1/4, ∅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_2O) and heated in a boiling water bath for 10 min to develop the rose-pink colour by the reaction between malondialdehyde and 2-thiobarbituric acid. The absorbance was measured at 532 nm after cooling, against a blank prepared with 5 ml distilled water and 5 ml TBA-reagent, using a UV–VIS spectrophotometer (Hitachi U-1100, San Jose, CA, USA). TBARS were calculated from a standard curve (8–50 nmol) of malondialdehyde (MDA), freshly prepared by acidification of TEP (1,1,3,3-tetraethoxypropane). TBARS values were calculated as mg of malondialdehyde (MDA) per kg sample.

2.8. Analysis of peroxide value

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

2.9. Analysis of 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 separated on a high polarity DB-5MS fused silica capillary column (15 m × 0.248 mm internal diameter, 0.25 μm film, J & W Scientific Inc., CA, USA). Operating conditions for GC were: helium flow 2 ml min^{-1}, initial oven temperature 40 °C for 3 min; increase to 220 °C at 20 °C min^{-1}, and held at 220 °C for 2 min. The injector and flame ionization detector (FID) temperatures were adjusted to 250 °C and held at this temperature throughout the analysis. Total run time was 15 min.

For head space (HS) analysis, 3 g of homogenized cooked ground pork sample were transferred to 10 ml glass vials, capped with teflon-lined septa, crimped and then placed in an HS-6 magazine assembly to preheat at 60 °C for 30 min to equilibrate. 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.

2.10. Statistical analysis

Statistical assessment was carried out with the software system of SPSS for Windows (Version 9) (SPSS Inc., Chicago, IL, USA). The results of TBARS value, peroxide value, conjugated dienes 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 values and hexanal contents.

3. Results and discussion

3.1. Changes of TBARS values during storage of cooked ground pork

TBARS analysis measures the formation of secondary products of lipid oxidation, mainly malondialdehyde, which may contribute off-flavour to oxidized oil (Rossel, 1994). Table 1 summarises the effect of various concentra-

Table 1
TBARS values of cooked ground pork treated with varying amounts (w/w) of dried Holy basil powder and its ethanolic extracts during storage at 5 °C[A]

Antioxidant	TBARS value (mg MDA/kg meat) Storage (day)		
	0	7	14
Control	$1.26^{a,x} \pm 0.06$	$4.66^{a,y} \pm 0.07$	$5.36^{a,z} \pm 0.04$
Ethanolic extract 0.10%	$0.99^{e,x} \pm 0.09$	$3.15^{d,y} \pm 0.08$	$3.57^{d,z} \pm 0.08$
Ethanolic extract 0.05%	$1.12^{b,x} \pm 0.03$	$3.65^{c,y} \pm 0.07$	$4.12^{c,z} \pm 0.05$
Ethanolic extract 0.02%	$1.18^{b,x} \pm 0.02$	$4.23^{b,y} \pm 0.10$	$4.54^{b,z} \pm 0.07$
Dried powder 0.35%	$0.83^{d,x} \pm 0.09$	$2.44^{f,y} \pm 0.03$	$3.19^{f,z} \pm 0.05$
Dried powder 0.18%	$0.98^{e,x} \pm 0.04$	$2.83^{e,y} \pm 0.05$	$3.45^{e,z} \pm 0.06$
Dried powder 0.07%	$1.11^{b,x} \pm 0.04$	$3.65^{c,y} \pm 0.07$	$4.10^{c,x} \pm 0.10$

[A] Different letters (a–f) within a column are significantly different ($P < 0.05$). Different letters within a row (x–z) are significantly different ($P < 0.05$).

tions of dried Holy basil powder and its ethanolic extracts on TBARS values of cooked ground pork during refrigerated storage (5 °C) for 14 days. Initial (day 0) TBARS values for all treatments were significantly lower than those of control cooked ground pork ($P < 0.05$). This suggests that the studied antioxidants retarded lipid oxidation during and immediately after cooking. The results agree with those reported by Ahn, Grün, and Fernando (2002) and by Fernández-López et al. (2003). Sato and Hegarty (1971) reported that non-heme iron was the active catalyst in cooked meats. Chen, Pearson, Gray, Fooladi, and Ku (1984) demonstrated 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 acids, are responsible for the initial development of oxidation in cooked meat products during storage.

In general, the storage period had a significant influence on the development of lipid oxidation in cooked ground pork resulting in extensive increase in TBARS values during the 14 days of refrigeration. TBARS values of all treatment samples were considerably lower ($P < 0.05$) than that of the control sample for each day studied during the storage period, thus indicating high protection of dried Holy basil powder and its ethanolic extracts against lipid oxidation in cooked ground pork. The oxidative stability effect of Holy basil presumably may be related to their polyphenol content and structure. The total phenolic content of Holy basil was 4720 mg chlorogenic acid/100 g of dried herb. Javanmardi, Stushnoff, Locke, and Vivanco (2003) reported that the amount of phenolic content and antioxidant activity of basil (*Ocimum basilicum* L.) accessions from Iran ranged from 22.9 to 65.5 mg GAE/g of dry material and from 10.8 to 35.7 μM Trolox. Typical phenolic compounds that possess antioxidant activity have been characterized as phenolic acids and flavonoids (Kähkönen et al., 1999). Both groups are known as free radical scavengers and, as was shown in a previous study, that ethanolic extracts of Holy basil exhibited strong antioxidative properties (Juntachote & Berghofer, 2005).

The type of antioxidant and the level of antioxidant added influenced the oxidative stability of cooked ground pork. Generally, an increase in efficacy in minimizing lipid oxidation was observed with increasing the concentration of dried Holy basil power and its ethanolic extracts. This is in agreement with findings by other authors (Lai et al., 1991) who reported that the oxidative stability of restructured chicken nuggets was influenced by the type of antioxidant and its concentration. They observed a strong linear relationship between amount of rosemary extract in the nuggets and TBARS values.

Overall mean TBARS values of cooked ground pork treated with ethanolic extracts from Holy basil were higher than the TBARS values of powder-treated ones. This was probably due to loss of some antioxidative compounds during the ethanolic antioxidant extraction. Abd El-Alim, Lugasi, Hóvári, and Dworschák (1999) found ethanolic extracts of sage, basil, thyme and ginger at concentrations of 200 mg ml^{-1} much more effective for the inhibition of lipid oxidation than the corresponding dried spices (10 g/kg). Since the authors did not give any indication about the comparability of both concentrations, no conclusion about the efficacy of extracted herbs can be drawn.

The impact of chlorophylls and their derivatives as pro-oxidants has been studied by Endo, Usuki, and Kaneda (1985). He and Shahidi (1997) stated that the antioxidant effect of green tea extracts in white muscles of mackerel might be markedly influenced by the presence of chlorophyll and other impurities. Although TBARS values of the treated samples in the present study increased during the storage period, there is no clear evidence that chlorophylls or their derivatives exhibited a pro-oxidant effect. Holy basil administered either as extract or dried powder at the highest concentration resulted, significantly, in the lowest TBARS values (Table 1).

After 14 days of storage, TBARS value of the control sample was 1.68-fold higher (5.36 mg MDA/kg meat) and that of the sample treated with 0.10% ethanolic extract was 1.12-fold higher (3.57 mg MDA/kg meat) than the sample treated with 0.35% dried Holy basil powder (3.19 MDA/kg meat).

Additionally, from our results based on sensory tests (data not shown), it should be noted that the dose of dried Holy basil powder and its ethanolic extracts used in this work did not modify any sensory quality of the cooked ground pork.

3.2. Changes of peroxide values during storage of cooked ground pork

The peroxide value (POV) measures primary products of lipid oxidation and is used to express the oxidative state of

Table 2
Peroxide values (POV) of cooked ground pork treated with varying amounts (w/w) of dried Holy basil powder and its ethanolic extracts during storage at 5 °C[A]

Antioxidant	POV (meq of active O_2/kg meat) Storage (day)		
	0	7	14
Control	$1.64^{a,x} \pm 0.08$	$9.07^{a,y} \pm 0.30$	$21.75^{a,z} \pm 0.32$
Ethanolic extract 0.10%	$0.87^{d,x} \pm 0.10$	$6.42^{d,y} \pm 0.11$	$13.89^{d,z} \pm 0.12$
Ethanolic extract 0.05%	$0.94^{cd,x} \pm 0.04$	$6.85^{c,y} \pm 0.10$	$14.29^{c,z} \pm 0.26$
Ethanolic extract 0.02%	$1.09^{b,x} \pm 0.04$	$7.42^{b,y} \pm 0.14$	$14.77^{b,z} \pm 0.12$
Dried powder 0.35%	$0.74^{e,x} \pm 0.05$	$5.98^{e,y} \pm 0.08$	$13.18^{e,z} \pm 0.09$
Dried powder 0.18%	$0.86^{d,x} \pm 0.05$	$6.47^{d,y} \pm 0.08$	$13.97^{d,z} \pm 0.10$
Dried powder 0.07%	$1.02^{bc,x} \pm 0.28$	$7.17^{b,y} \pm 0.08$	$14.63^{b,z} \pm 0.09$

[A] Different letters (a–e) within a column are significantly different ($P < 0.05$). Different letters within a row (x–z) are significantly different ($P < 0.05$).

lipid-containing foods. POVs of all samples were below 25 meq of active O_2/kg meat, which is considered the maximum limit of acceptability in fatty foods (Evranuz, 1993; Narasimhan, Raghuver, Arumngham, Bhat, & Sen, 1986). Similar to the TBARS value, POV gradually increased for all treatments during storage time (Table 2), but values were significantly lower than that of the corresponding control sample ($P < 0.05$) for each storage time studied. As was found for the TBARS values, the reduction in POV of cooked ground pork was a dose-dependent within the ethanolic extract samples and within the dried powder samples. This is in accordance with results of Wanasundara and Shahidi (1998), who reported that green tea extracts effectively inhibited the formation of peroxide in marine oil in a dose-dependent manner.

The addition of dried Holy basil powder led to lower POVs compared to the counterpart samples prepared with ethanolic extracts. At days 7 and 14, the POV did not differ significantly ($P < 0.05$) between the lowest concentrations of extract (0.02%) and powder (0.07%), and between the highest concentration of ethanolic extract (0.10%) and medium concentration of dried powder (0.18%).

After 14 days storage, POV of the control meat sample was 1.65-fold (21.75 meq of active O_2/kg meat) higher than that of the sample treated with 0.10% ethanolic extract (13.89 meq of active O_2/kg meat) and the sample treated with 0.35% dried Holy basil powder (13.18 meq of active O_2/kg meat).

3.3. Changes in conjugated dienes during storage of cooked ground pork

Dried Holy basil powder and its ethanolic extracts were able to decrease the formation of conjugated dienes in cooked ground pork during storage (Table 3). All treatments had lower concentrations of conjugated dienes ($P < 0.05$) compared to the control sample. However, initially (day 0), no significant difference ($P < 0.05$) in the formation of conjugated dienes between the control sample and the sample treated with 0.02% ethanolic extract was observed. The concentration of conjugated dienes decreased as storage time progressed. The results are in agreement with those reported by Peña-Ramos and Xiong (2003). The formation of conjugated dienes, which relates to the production of hydroperoxides, occurs at the early stages of lipid oxidation (Frankel, 1996). Conjugated diene hydroperoxides are expected to decompose to secondary products, which occurred in this study, where the concentration of conjugated dienes in cooked ground pork decreased during storage concomitant with increasing formation of TBARS and hexanal.

Dried Holy basil powder resulted in lower concentrations of conjugated dienes compared to its ethanolic extract upon initial treatment. However, at 7 and 14 days of storage, no significant differences ($P > 0.05$) in the formation of conjugated dienes were found between 0.05% ethanolic extract and 0.18% dried Holy basil powder, nor between 0.02% ethanolic extract and 0.07% dried Holy basil powder. At the end of the storage period, 0.35% dried Holy basil powder and 0.10% ethanolic extract of Holy basil inhibited the conjugated dienes production by 20% and 15%, respectively, compared to the respective control sample.

3.4. Changes in hexanal content during storage of cooked ground pork

Hexanal content, which is a major breakdown product of linoleic acid oxidation (Frankel, 1996), has been used

Table 3
Conjugated diene of cooked ground pork treated with varying amounts (w/w) of dried Holy basil powder and its ethanolic extracts during storage at 5 °C[A]

Antioxidant	Conjugated diene (μmol mg^{-1} meat) Storage (day)		
	0	7	14
Control	$0.793^{a,x} \pm 0.011$	$0.762^{a,y} \pm 0.006$	$0.685^{a,z} \pm 0.010$
Ethanolic extract 0.10%	$0.748^{cde,x} \pm 0.012$	$0.636^{d,y} \pm 0.008$	$0.547^{e,z} \pm 0.008$
Ethanolic extract 0.05%	$0.763^{bcd,x} \pm 0.010$	$0.673^{c,y} \pm 0.009$	$0.623^{c,z} \pm 0.007$
Ethanolic extract 0.02%	$0.784^{ab,x} \pm 0.009$	$0.726^{b,y} \pm 0.003$	$0.660^{b,z} \pm 0.015$
Dried powder 0.35%	$0.744^{e,x} \pm 0.009$	$0.607^{e,y} \pm 0.010$	$0.577^{d,z} \pm 0.006$
Dried powder 0.18%	$0.754^{cde,x} \pm 0.008$	$0.665^{c,y} \pm 0.008$	$0.622^{c,z} \pm 0.011$
Dried powder 0.07%	$0.769^{bc,x} \pm 0.006$	$0.714^{b,y} \pm 0.010$	$0.665^{b,z} \pm 0.007$

[A] Different letters (a–e) within a column are significantly different ($P < 0.05$). Different letters within a row (x–z) are significantly different ($P < 0.05$).

Table 4
Hexanal content of cooked ground pork treated with varying amounts (w/w) of dried Holy basil powder and its ethanolic extracts during storage at 5 °C[A]

Antioxidant	Hexanal content (mg/100 g meat) Storage (day)		
	0	7	14
Control	$0.020^{a,x} \pm 0.002$	$0.376^{a,y} \pm 0.005$	$0.417^{a,z} \pm 0.008$
Ethanolic extract 0.10%	$0.013^{cd,x} \pm 0.003$	$0.249^{f,y} \pm 0.007$	$0.316^{d,z} \pm 0.005$
Ethanolic extract 0.05%	$0.015^{bc,x} \pm 0.002$	$0.297^{d,y} \pm 0.007$	$0.347^{c,z} \pm 0.005$
Ethanolic extract 0.02%	$0.018^{ab,x} \pm 0.001$	$0.332^{b,y} \pm 0.004$	$0.375^{b,z} \pm 0.006$
Dried powder 0.35%	$0.010^{d,x} \pm 0.002$	$0.234^{g,y} \pm 0.009$	$0.305^{e,z} \pm 0.008$
Dried powder 0.18%	$0.014^{bc,x} \pm 0.002$	$0.277^{e,y} \pm 0.009$	$0.325^{d,z} \pm 0.007$
Dried powder 0.07%	$0.017^{ab,x} \pm 0.012$	$0.312^{c,y} \pm 0.007$	$0.366^{b,z} \pm 0.007$

[A] Different letters (a–g) within a column are significantly different ($P < 0.05$). Different letters within a row (x–z) are significantly different ($P < 0.05$).

to follow the course of lipid oxidation and off-flavour development in cooked foods (Dupuy, Bailey, St Angelo, Legendre, & Vercelotti, 1987). In general, the hexanal contents increased as storage progressed, however, the type of antioxidant and its concentration was found to influence the hexanal content significantly ($P < 0.05$) (Table 4). Holy basil effectively inhibited the formation of hexanal in a dose-dependent manner. In addition, hexanal contents of samples treated with dried Holy basil powder were higher than those of samples treated with its ethanolic extracts. Within the investigated storage period, the significantly ($P < 0.05$) highest levels of hexanal content were found for the control samples, to which no antioxidant had been added. Notably, initially (day 0), no significant differences ($P > 0.05$) in the hexanal content were observed among the control sample, sample treated with 0.02% ethanolic extract and sample treated with 0.07% dried powder. Moreover, at the end of the storage period no significant differences ($P > 0.05$) in hexanal content were found between 0.10% ethanolic extract of Holy basil and 0.18% dried Holy basil powder, nor between 0.02% ethanolic extract of Holy basil and 0.07% dried Holy basil powder. After 14 days of storage, the control sample contained 0.417 mg/100 g meat hexanal, which was 1.3-fold higher than the sample treated with 0.35 % dried Holy basil powder (0.305 mg/100 g meat).

Data obtained from this study have shown that changes in hexanal content were similar in trends to changes in TBARS value. TBARS values and hexanal contents correlated well during the storage period ($r^2 = 0.95$; $P < 0.05$), which has also been reported by others (Ahn, Jo, Du, Olson, & Nam, 2000; Ahn et al., 2002; Du, Ahn, Nam, & Sell, 2001; Jensen, Flensted-Jensen, Skibsted, & Bertelsen, 1998).

4. Conclusions

According to the present results, dried Holy basil powder and its ethanolic extracts exhibited a protective effect against lipid oxidation in cooked ground pork during storage at 5 °C for 14 days in a dose-dependent manner. Ethanolic extracts of Holy basil were less effective than dried Holy basil powder in controlling oxidative stability. Dried Holy basil powder at a concentration of 0.35% (w/w) was the most effective in retarding lipid oxidation in cooked ground pork during the investigated storage period. In addition, TBARS values for the various cooked ground pork sample were well correlated with hexanal contents ($r^2 = 0.95$; $P < 0.05$) for the respective samples.

These findings have demonstrated that Holy basil, a culinary herb without any known toxic effects, may be considered a potential natural antioxidant for stabilization of lipid oxidation in meat and meat products. However, the application of this natural antioxidant at levels higher than in the present study might effect the sensory quality of meat and meat products. Additionally, dechlorophyllization may be necessary to avail them for applications in meat and meat products in which the original colour of the dried powder or crude extract might be of concern or when chlorophyll might act as a pro-oxidant.

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