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Antioxidant and antibacterial activities of Thai culinary herb and spice extracts, and application in pork meatballs

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Abstract

Twenty-two local Thai culinary herbs and spices normally used for cooking were screened for their antioxidant properties by using FRAP, DPPH and TBARS assays. Four ethanolic extracts, i.e., holy basil, Vietnamese coriander, turmeric and green peppercorn, having high antioxidant properties were selected for freeze-drying and used in the pork meatball batter. The batter of pork meatball was prepared with 0.2% (w/w) freeze-dried ethanolic extract of each selected herb and spice. The meatball model was obtained by cooking the batter stuffed in plastic casing and cut into 2.5 cm per piece. The meatballs were aerobically and vacuum packaged and stored at 4°C for 9 days. Antioxidant and antimicrobial efficacies of the extracts in the meatball samples were performed every 3 days. It was found that holy basil, Vietnamese coriander and green peppercorn showed stronger antioxidant effect in the pork meatballs than did turmeric throughout 9 days of storage period in both aerobic and vacuum conditions ($p<0.05$). Regarding the standard for the marginal acceptable microbial counts of ≤ 5.0 log cfu/g sample, holy basil and green peppercorn extracts provided up to 9 days shelf life for the meatballs packaged in both aerobic and vacuum conditions while those made with Vietnamese coriander and turmeric extracts had the shelf life of about 6-9 days and control meatballs had shelf life less than 6 days.

Introduction

Nowadays, in response to recent claims that synthetic antimicrobials and antioxidants have the potential to cause toxicological effects and consumers are demanding for more natural (organic) foods. Short term acute effects from synthetic additives are unlikely while cancer and reproductive problems resulting from the long term consumption of them (Branen *et al*., 1990). It is an obliging of the industry to use, in some means, and include natural preservatives in foods such as herbs and spices and their extracts. Herbs and spices have been used as food additives since ancient times, not only as flavoring agents but also as natural food preservatives. Some studies have demonstrated that shelf life and food quality can be improved by using herb and spice extracts in some stages of production. The main effects of these compounds are to retard microbial growth and lipid oxidation during storage. Nevertheless, more research is needed to determine herb and spice efficacies, particularly for their antimicrobial and antioxidant, in food products during processing and storage, and their effects on other product quality parameters. Many herbs and spices have been investigated for their antioxidant properties for at least 50 years. For instance, rosemary, oregano, sage, clove, cinnamon, coriander and others belonging to the Labiatae family, exhibited antioxidant properties (Wu *et al*., 2004). Commonly used kitchen herbs and spices such as garlic, shallot bulb (Leelarungrayub *et al*., 2006), ginger (Kikuzaki *et al*., 1993), cumin, chili, onion (Tang *et al*., 2007) holy basil and galangal (Juntachote *et al*., 2005) were reported to exhibit strong antioxidant activity and used in meat products. Antimicrobial of herbs and spices and their essential oils have been as well reported. Major essential oil components were reported to have strong effect on microbial growth inhibition including eugenol in clove, cinnamon, all spice and basil (Davidson, 1999) and found to affect the growth of *Listeria monocytogenes, Bacillus cereus, Campylobacter jejuni, Escherichia coli* 0157:H7, *Salmonella* spp, *Aeromonas hydrophila* and *Enterobacter aerogenes* (Friedman *et al*., 2002) and fungi such as *Aspergillus* spp. and *Penicillium* spp. (Vazquez *et al*., 2001). In addition, Anetol, the major volatile compound of anise seed and thymol which contain in thyme, have been shown to have inhibitory activities against *Aspergillus* spp. and aflatoxin production (Hirasa *et al.*, 1998). Allicin, one of the active principals of garlic was found to inhibit *Escherichi coli, Candida albicans, Entamoeba histolytica* and *Giardia lamblia* (Ankri *et al*., 1999).

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Thailand is in the tropical region; there are wide varieties of herbs and spices available which some of them are used as culinary cooking ingredients. There are very few experiments reported the use of local Thai herbs and spices in meat and meat products published in terms of antimicrobial and antioxidant activities. Therefore, it is very of interesting to search for those culinary herbs and spices which have good antioxidant and antimicrobial efficacy to use for improving storage quality of meat and meat products. The outcome results of those herbs and spices and their extracts may be effectively used to replace the synthetic food preservatives. In addition, the effects of herb and spice used on some physicochemical and sensory properties of meat products are also very much of interesting. Since very few investigations are found to reveal the efficacies of Thai culinary herbs and spices on meats and meat products, therefore, this experiment was set up for this purpose. The objective of this study was to evaluate antioxidant capacities of culinary herbs and spices commonly used in Thai cuisine cooking in order to screen and select those having relatively high antioxidant activities of which an antimicrobial activity was also tested to extend the shelf life of pork meatballs aerobically and vacuum packaged during storage at 4°C.

Materials and Methods

Chemicals

2,2-Diphenyl-1-picrylhydrazy (DPPH), butylated hydroxyl-anisole and soybean phosphatidylcholine were purchased from Sigma Chemical Co., Ltd. (St. Louis, MO, USA), TPTZ (2,4,6-tripyridyl-S-tri-azine) and 2-thiobarbituric acid were purchased from Fluka (Buchs, Switzerland). Gallic acid was purchased from Fluka (Madrid, Spain). Trichloroacetic acid was purchased from Qrec (Auckland, New Zealand). Other chemicals and solvents used in this experiment were analytical grade, purchased from Sigma-Aldrich Co., Ltd. (Steinheim, Germany).

Preparation of herb and spice extracts

Twenty-two indigenous herbs and spices commonly used in Thai cooking cuisine were selected for this study as shown in Table 1. They were purchased from local markets in Nakhon Ratchasima and Sakon Nakhon Provinces, Thailand. The herbs and spices were cleaned, cut into small pieces, freeze-dried (LYOVAC GT2, GEA Lyophil GmbH, Hürth, Germany), finely ground (Retsch ZM 1000, Retsch GmbH, Haan, Germany) and stored at -20°C for further application.

Ethanolic extraction of dried herbs and spices were performed by mixing 50 g of freeze-dried power of each herb and spice in 750 ml of 95% ethanol at room temperatures for 24 h. After extraction, the extract was filtered through a Whatman No.1 filter paper, the residue was re-extracted twice with 750 ml and 500 ml of 95% ethanol. The pooled supernatant was evaporated to dryness using a rotary evaporator at 40°C (Rotavapor-R114, BÜCHI, Flawil, Switzerland). The extract was stored at -20°C until use.

Determination of total phenolic content

Total phenolic content (TPC) was estimated according to the Folin-Ciocalteu method (Matthaus, 2002). To 100 µl sample (1000 µg/ml in ethanol) and 2 ml of 2% (w/v) Na_2CO_3 was added. After 2 min, 100 µl of 0.1 N Folin-Ciocalteu reagent was added, mixed well and incubated for 30 min at room temperature and then, the absorbance was measured at 760 nm. The TPC was calculated and expressed as an equivalent of gallic acid (µg gallic acid equivalents /g extract) using gallic acid as chemical standard.

Determination of total flavonoid content

Total flavonoid content (TFC) of each herb and spice extract was determined using the modified of Zhishen *et al.* (1999) with some modification. An aliquot of 0.5 ml of diluted extract (2000 µg /ml in ethanol) or standard solution of catechin (µg catechin equivalents/g extract) was added to 2 ml of deionized distilled water and 0.15 ml of 5% $NaNO_2$, mixed well and kept for 6 min and then, 0.15 ml of 10% $AlCl_3$ was added. After 6 min, 1 ml of 1 M NaOH solution was added and the total volume was made up to 5 ml with water and mixed well and then, the absorbance was measured against prepared reagent blank at 510 nm. The TFC was calculated from the standard calibration curve based on concentration of catechin solutions and expressed as an equivalent of catechin (µg catechin equivalents/g extract).

Free radical scavenging activity by DPPH method

Free radical scavenging activity of the extract was determined using 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH) according to the method described by Sanchez-Moreno *et al.* (1999) with slight modification. Stock solution of each herb and spice extract and trolox used as a positive control were prepared in the concentrations of 0 to 1000 µg/ml in ethanol. Each diluted extract of 75 ml was added to 2.925 ml of DPPH solution (0.025 g/l in ethanol). The reaction mixture was incubated in the dark for 30 min and the absorbance at 515 was measured. The remaining DPPH concentration in the reaction

Table 1. Indigenous Thai culinary herbs and spices collected and used for evaluation of their antioxidant activities and total phenolic contents (TPC), total flavonoid contents (TFC), FRAP, DPPH and TBARS values

Herbs and spices	Scientific name	Part of plant	TPC (µg gallic acid equivalents / g extract	TFC (µg catechin equivalents /g extract)	FRAP value (µg trolox equivalents / g extract)	DPPH (IC_{50})(µg/ml)	TBARS (IC_{50})(µg/ml)
1. Green shallot	*Alliumcepa* var. aggregatum	Leaves	33.67±1.51 o	30.88±0.81 k	134.17±6.19 n	5,302.23±156.49 b	1,961.34±82.81 h
2. Coriander	*Coriandrum sativum* Linn.	Leaves/ branches	72.67±3.86 k	34.80±0.75 i	283.83±13.38 j	2,742.04±188.61 h	1,425.19±54.46 j
3. Dill	*Anethum graveolens* Linn.	Leaves/ branches	80.33±4.37 j	25.30±0.66 l	269.50±14.12 j	2,433.08±152.29 j	2,042.83±54.40 g
4. Garden parsley	*Eryngium foetidum* Linn.	Leaves	92.50±4.47 i	33.95±0.54 j	380.50±17.87 gh	1,887.30±164.66 n	2,423.86±80.38 d
5. Kaffir lime	*Citrus hystrix* DC	Leaves	116.00±5.90 g	24.79±0.39 l	355.67±14.93 h	3,657.08±125.66 f	2,150.92±80.92 f
6. Celery	*Apium graveolens* Linn.	Leaves/ branches	55.17±2.74 m	13.38±0.32 n	184.17±9.86 l	5,769.64±145.33 a	1,314.88±12.05 k
7. Holy basil	*Ocimum sanctum* Linn.	Leaves	148.33±7.50 e	49.58±0.29 d	671.83±31.17 d	1,258.96±38.08 p	887.95±14.31 o
8. Sweet basil	*Ocimum basilicum* Linn.	Leaves.	114.00±5.79 g	41.68±0.43 f	485.67±22.63 f	2,065.47±155.23 l	1,975.81±93.76 h
9. Vietnamese coriander	*Polygonum odoratum* Lour.	Leaves	389.00±18.37 b	62.24±0.82 b	3,395.00±104.85 a	380.40±25.95 s	808.54±17.19 p
10. Lemon balm	*Melissa officinalis* Linn.	Leaves	139.83±6.14 f	39.50±0.60 g	610.33±25.75 e	1,634.67±144.01 o	1,563.48±61.76 i
11. Lemon basil	*Ocimum basilicum* L.f. var. citratum Back.	Leaves.	89.50±4.23 i	36.98±0.63 h	387.23±16.78 g	2,553.57±127.51 i	2,937.56±55.11 b
12. Ginger	*Zingiber officinale* Roscoe	Tubers	31.17±1.17 o	4.20±0.07 r	278.11±10.40 j	2,873.75±178.38 g	1,998.70±57.05 gh
13. Galangal	*Alpiniagalanga* Linn.	Tubers	140.33±7.03 f	11.27±0.12 o	322.50±12.68 i	2,456.14±153.63 j	1,315.82±50.85 k
14. Finger root	*Boesenbergia pundurata* (Roxb) Schitr	Tubers	167.33±8.55 d	17.96±0.17 m	327.67±16.19 i	1,636.52±55.66 o	1,068.40±10.29 m
15. Garlic	*Allium sativum* Linn.	Bulbs	39.83±1.75 n	1.21±0.01 s	142.56±7.75 n	2,272.32±156.24 k	2,899.26±52.73 b
16. Shallot	*Allium ascalonicum* Linn.	Bulbs	42.33±2.03 n	1.59±0.01 s	147.33±7.47 mn	3,824.09±195.91 e	2,744.67±66.56 c
17. White curcuma	*Curcuma mangga* Val.and.Zijp.	Tubers	59.67±2.21 l	48.89±0.12 e	238.17±11.94 k	2,311.60±182.97 k	1,234.44±33.65 l
18. Turmeric	*Curcuma longa* Linn	Tubers	579.83±21.47 a	129.62±0.47 a	1,357.80±51.47 c	628.71±37.93 q	347.57±17.60 r
19. Green peppercorn	*Piper nigrum* Linn.	Young fruits	187.67±9.75 c	58.30±0.05 c	2,254.67±93.27 b	527.94±20.82 r	909.64±29.90 o
20. Kaffir lime (skin)	*Citrus hystrix* DC	Fruit peels	110.17±5.49 h	8.25±0.05 q	388.67±12.34 g	5,048.36±187.19 c	2,252.26±51.17 e
21. Long red chili	*Capsicum annuum* L.var.grossum	Tubers	34.50±2.17 o	10.01±0.06 p	171.17±8.47 kl	4,452.44±132.89 d	4,424.44±39.52 a
22. Lemon grass	*Cymbopogon citratus* Stapf.	Stems	61.33±3.37 l	33.95±0.45 j	264.83±11.47 jk	1,969.38±92.58 m	997.59±19.18 n
23. Trolox	-	-	-	-	-	294.97±17.62 t	427.59±16.01 q

Within column, different letters are significantly different ($p<0.05$), each value in the table was expressed as mean ± standard deviation (n = 6).

mixture was calculated from the DPPH standard curve and the remaining DPPH (%) was calculated using the following equation:

$$\text{Scavenging activity (\%)} = \left[1 - \left(\frac{\text{absorbance}_{\text{sample}}}{\text{absorbance}_{\text{control}}}\right)\right] \times 100$$

The DPPH radical scavenging activity (%) was plotted against the extract concentration (µg/ml) to determine the concentration of extract necessary to decrease DPPH radical scavenging activity by 50% (IC_{50}).

Ferric reducing antioxidant power (FRAP Assay)

The FRAP assay was determined according to the method described by Katalinic *et al.* (2004) and Woraratphoka *et al.* (2007) with slight modification.

Working solution of FRAP reagent was prepared by mixing 10 volumes of 1.0 mol/l acetate buffer, pH 3.6 with 1 volume of 10 µmol/l TPTZ (2,4,6-tripyridyl-S-tri-azine) in 40 µmol/l hydrochloric acid and with 1 volume of 20 µmol/l ferric chloride. A 100 µl of the sample extract (1000 µg /ml in ethanol) was mixed with 3 ml of FRAP reagent and an absorbance was measured at 593 nm after 8 min. The antioxidant efficiency of the sample solution was calculated with reference to the standard curve of known concentrations (0 to 1000 µg /ml) of trolox. The FRAP of the sample was expressed as trolox equivalent (µg trolox equivalents/g extract).

Thiobarbituric acid reactive substances (TBARS) assay

TBARS assay was monitored for lipid oxidation by the method modified by Maikhunthod *et al.* (2005). Stock solution of sample extract and trolox (positive control) were prepared and diluted to obtain the concentrations from 0 to 1000 µg/ml in ethanol. The extract solution was dissolved in soybean phosphatidylcholine liposome suspension to give various concentrations. After 10 min, sodium ascorbate and $FeCl_3$ solution were added, incubated in a 37°C water bath for 30 min, and then 2 ml of thiobarbituric acid (TBA) reagent (0.02 M in water) was added. The reaction tube was heated in boiling water bath for 15 min, cooled and centrifuged at 4000xg for 15 min. The absorbance of supernatant was measured at 532 nm. Concentration of malondialdehyde (MDA) in oxidation system was calculated from the MDA standard curve. The percentage of inhibition was calculated as follow:

$$\% \text{ inhibition} = \frac{(\text{MDA in absence of ext}) \ (\text{MDA in presence of ext})}{\text{MDA in absence of ext}} \times 100$$

The antioxidant activity of extract was expressed as amount of extract used in the system to obtain 50% inhibition of oxidation (IC_{50}).

Application of selected herb and spice extracts in pork meatballs

Top four herb and spice extracts having high antioxidant capacity screened from twenty-two culinary herbs and spices were selected for mixing in the pork meatball preparation. The selected herbs and spices were cut into small pieces, freeze dried and finely ground. The extract was obtained from ethanolic extraction of dried herb or spice at room temperatures for 24 h. The extract was filtered and evaporated to dryness in a rotary evaporator at 40°C and kept at -20°C until use.

Pork meatball batter model was prepared using 5 kg lean ground pork, 2% salt, 0.25% sodium phosphate, 15% ice, 2% tapioca starch and 0.2% herb/spice extract. After chopping, the meatball batter was stuffed in a 20 mm diameter plastic casing, cooked in hot water at 70°C for 30 min, cooled in chilled water and cut into pieces with the length of about 2.5 cm. The meatballs were aerobically and vacuum packaged in polyethylene bag and stored at 4°C. The meatball samples were randomly taken every 3 days during 9 days of storage for microbial enumeration and antioxidant activity determination.

TBARS assay in pork meatballs

Lipid oxidation of the pork meatball sample was determined for TBARS values by Buege *et al.* (1978) with slight modification. A 5 g of sample was homogenized in 15 ml of deionized distilled water. Meatballs homogenate (1 ml) was transferred into a test tube and 50 µl of 7.2% butylated hydroxyanisole and 2 ml of 20 mM 2-thiobarbituric acid (TBA) in 15% trichloroacetic acid (TCA) solution were added. The reaction tube was heated in boiling water bath for 15 min, cooled and centrifuged at 4000xg for 15 min. The absorbance of supernatant was measured at 532 nm. TBARS value (mg MDA/kg sample) of the sample was calculated from the concentration of malondialdehyde (MDA) in oxidation system using MDA as chemical standard.

Determination of hexanal content

Hexanal content of the meatball samples was determined by the method of Ahn *et al.* (2007) with slight modification. One gram of sample was weighed into a 22 mL headspace vial and 3 mL of deionized distilled water was added. The vial was crimped with aluminum cap with Teflon septa after purging with nitrogen. The sample was equilibrated in the headspace autosampler (Tekmar HT3, Teledyne Tekmar, Mason, OH, USA) at a platen temperature of 75°C (the sample temperature was 75°C when the equilibrium was reached). After thermal equilibration was filled and equilibrated for 0.3 min, the carrier gas (helium) back flushed the loop and carried the compound through the heated transfer line (150°C) into the Gas Chromatography (GC) (CP-3800 GC, Varian Inc., Walnut Creek, CA, USA). The released volatiles were automatically injected and separated on the GC capillary column (CP8924, 30 m×0.32 mm×0.25 µm) with a split injection ratio of 1:10 at 220°C and flow rate of carrier gas of 2.0 mL/min. The oven temperature was programmed from 35°C for 5 min, increased 45°C at 8°C/min and increased to 200°C at 40°C/min for 6 min and FID was set

Table 2. TBARS values (mg MDA/kg sample) of pork meatballs added herb and spice extracts and stored at 4°C for 9 days (mean±SD)

Storage time (days)	CON	HOB	VNC	TMR	GPP
Aerobically packaged					
0	1.36±0.05 Ad	0.20±0.01 Dd	0.26±0.03 Cd	0.61±0.04 Bd	0.18±0.04 Dc
3	1.53±0.06 Ac	0.31±0.03 Dc	0.41±0.05 Cc	0.90±0.06 Bc	0.23±0.02 Ebc
6	1.73±0.08 Ab	0.35±0.02 Db	0.44±0.03 Cb	1.00±0.99 Bb	0.25±0.03 Eb
9	2.09±0.17 Aa	0.43±0.03 Da	0.52±0.03 Ca	1.25±0.95 Ba	0.34±0.06 Ea
Vacuum packaged					
0	1.30±0.11 Ad	0.13±0.01 CDd	0.17±0.02 Cd	0.51±0.02 Bd	0.11±0.02 Dd
3	1.54±0.08 Ac	0.15±0.01 Dc	0.25±0.03 Cc	0.61±0.03 Bc	0.14±0.01 Dc
6	1.64±0.09 Ab	0.18±0.03 Db	0.31±0.04 Cb	0.76±0.03 Bb	0.17±0.01 Db
9	1.82±0.12 Aa	0.25±0.03 Da	0.36±0.04 Ca	0.90±0.04 Ba	0.23±0.03 Da

Within packaging condition; uppercase letters indicate significant difference in the row ($p < 0.05$), lowercase letters indicate significant difference in the column ($p<0.05$), n = 6. CON = control, HOB = holy basil, VNC = Vietnamese coriander, TMR = turmeric, and GPP = green peppercorn

at 250°C. Hexanal concentration (mg of hexanal/kg sample) was quantitated using pure hexanal for standard curve and calculated.

Microbial enumeration

Total viable counts (TVC) and lactic acid bacterial counts (LAB) were performed using PetrifilmTM (3M, St. Paul, MN, USA.) and incubated at 37°C for 24 h for TPC or incubated at 35°C for 24 h for LAB. The number of colony count was expressed as log cfu/g sample.

Statistical analysis

Statistical analysis was evaluated in Completely Randomized design (CRD) using SPSS for Windows and means comparison by Duncan's Multiple Range Tests (DMRT) were analyzed (Montgomery, 1991). Two replications of the experiment were performed with triplicate analyses per replication. Statistical difference was determined at $p \leq 0.05$.

Results and Discussion

Total phenolic content and total flavonoid content

Total phenolic content (TPC) is very important due to its exhibition of antioxidant activity. The amount of TPC measured by the Folin-Ciocalteu method is a rapid and widely-used assay (Kähkönen *et al.*, 1999). In this work, the TPC of 22 herb and spice extracts tested varied from 31.17-579.83 µg gallic acid equivalents /g extract ($p<0.05$) as shown in Table 1. Turmeric extract contained the highest level of TPC (579.83 µg gallic acid equivalents /g extract) followed by Vietnamese coriander, green peppercorn, finger root and holy basil extracts while the extract considering contained the lowest amounts was from ginger (31.17 µg gallic acid equivalents /g extract). Flavonoid is the largest class of phenolic compounds, and it is ubiquitous in the plants. The basic flavonoid structure is the flavan nucleus, which consists of 15 carbon atoms arranged in three rings (C6-C3-C6) with different oxidation and antioxidant activity and influences phenoxyl radical stability (Wojdylo *et al.*, 2007). Total flavonoid contents (TFC) exhibit antioxidant activity and their mechanisms of action are through free radical scavenging or chelating process (Kessler *et al.*, 2003). In this study, the flavonoid contents ranged 1.21-129.62 µg catechin equivalents/g extract ($p<0.05$) as shown in Table 1. Turmeric extract contained the highest amount of TFC (129.62 µg catechin equivalents/g extract) followed by Vietnamese coriander (62.24 µg catechin equivalents/g extract), green peppercorn (58.30 µg catechin equivalents/g extract) and holy basil (49.58 µg catechin equivalents/g extract).

Antioxidant capacity of culinary herbs and spices

In this study, the antioxidant activities of the extract of culinary herbs and spices were focused on phenolic and flavonoid compounds and three common assays used to evaluate their antioxidant capacities were based on different radicals and mechanisms of reaction.

DPPH assay is a free radical compound that has been widely used to determine the free radical-scavenging ability of various samples (Amarowicz *et al.*, 2004). The color turns from purple to yellow followed by the formation of DPPH upon absorption of hydrogen from an antioxidant (Moon *et al.*, 2009). The DPPH free radical-scavenging activity of 22 Thai local culinary herbs and spices are presented in Table 1, expressed as IC_{50} values ranging from 380.40 to 5,769.64 µg/ml ($p<0.05$) while the Trolox, which was used as a standard reference, showed the

Table 3. Hexanal contents (mg/kg sample) of pork meatballs added herb and spice extracts, aerobically and vacuum packaged, stored at 4°C for 9 days (mean ± SD)

Storage time (days)	CON	HOB	VNC	TMR	GPP
Aerobically packaged					
0	2.04±0.14 Ad	0.12±0.01 Cd	0.24±0.04 Bd	0.13±0.01 Cd	0.07±0.01 Cd
3	3.47±0.39 Ac	0.19±0.01 Bc	0.38±0.03 Bc	0.39±0.03 Bc	0.14±0.02 Bc
6	5.09±0.44 Ab	0.25±0.02 Cb	0.85±0.06 Bb	0.65±0.03 Bb	0.25±0.01 Cb
9	6.79±0.89 Ac	0.37±0.06 Ca	1.38±0.12 Ba	0.90±0.07 BCa	0.50±0.03 Ca
Vacuum packaged					
0	0.96±0.24 Ad	0.07±0.01 Bd	0.09±0.01 Bd	0.10±0.01 Bd	0.05±0.01 Bc
3	2.44±0.40 Ac	0.13±0.03 Bc	0.30±0.04 Bc	0.28±0.05 Bc	0.06±0.01 Bc
6	3.99±0.34 Ab	0.21±0.02 Cb	0.50±0.08 Bb	0.47±0.07 Bb	0.19±0.04 Cb
9	5.35±0.45 Aa	0.30±0.01 Da	0.66±0.07 BCa	0.71±0.05 Ba	0.34±0.03 CDa

Within packaging condition; uppercase letters indicate significant difference in the row ($p < 0.05$), lowercase letters indicate significant difference in the column ($p < 0.05$), n = 4. CON = control, HOB = holy basil, VNC = Vietnamese coriander, TMR = turmeric, and GPP = green peppercorn

most active with the IC_{50} values of 294.97 µg/ml. Significant difference of radical scavenging activities among all 22 culinary herb and spice extracts were found ($p<0.05$). The Vietnamese coriander extract showed the strongest inhibition activity with the IC_{50} value of 380.40 µg/ml ($p<0.05$), followed by green peppercorn (527.94 µg/ml), turmeric (628.71 µg/ml) and holy basil (1,258.96 µg /ml).

The FRAP assay is a measure of antioxidant activity according to their reducing ability/antioxidant power of the herb and spice extracts. When a Fe^{3+}-TPTZ complex is reduced to the Fe^{2+} formed by an antioxidant under acidic conditions, an intense blue color with absorption maximum develops at 593 nm (Moon *et al*., 2009). In this study, the FRAP values of the extracts of all culinary herb and spice extracts used ranged from 134.17 to 3,395.00 µg trolox equivalents/g extract ($p<0.05$) as shown in Table 1. Vietnamese coriander, green peppercorn, turmeric and holy basil extracts were found to possess very high FRAP values.

TBARS assay has been commonly used to measure lipid oxidation. This method measures the malonaldehyde (MDA) formed after lipid hydroperoxide decomposition. The sample reaction was characterized as the color complex formed due to the condensation adducted between thiobarbituric acid (TBA) and MDA. The result was expressed as percentage of inhibition and concentration of 50% of inhibition activity was determined as IC_{50} values, µg MDA/ml extract. The TBARS values as IC_{50} values of 22 culinary herb and spice extracts ranged from 347.57 to 4,424.44 µg/ml ($p<0.05$) (Table 1). Turmeric extract was found to have the strongest antioxidant activity in term of TBARS value (347.57 µg MDA/ml) and found to be better than trolox (427.59 µg MDA/ml) ($p>0.05$) followed by Vietnamese coriander, holy basil and green peppercorn extracts.

The results of antioxidant activities of herbs and spices are mainly contributed by the active compounds present in them especially phenolic compounds and flavonoids. 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 plant extracts. Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenolic (Agrawal, 1989). Due to the fact that antioxidant activities are influenced by many factors, it cannot be fully described by only a single method for evaluation antioxidant mechanisms for the plant extracts (Maisuthisakul *et al*., 2008).

In this study, the antioxidant activities of all culinary herb and spice extracts were determined using FRAP, DPPH and TBARS assay. Among 22 herbs and spices, it could be concluded that Vietnamese coriander and turmeric possessed the highest antioxidant activities and had high TPC and TFC (Table 1). Overall, spices contained a high amount of TPC tended to have high scavenging activity. However, finger root, which had slightly higher TPC than holy basil, had lower scavenging activity than did holy basil. Prior *et al*. (2005) suggested that some of inorganic substances may also interact with Folin-Ciocalteu reagent, giving an inaccurate result of the TPC of the samples. In general, antioxidant activity of phenolic and flavonoid depends on the structure and substitution pattern of hydroxyl groups. Nanasombat *et al.* (2009)

reported 20 extracts of Thai local vegetables (20 species) and found that the extract from Vietnamese coriander *(Polygonum odoratum)* had the highest phenolic content and highest antioxidant activity evaluated by DPPH method. Significant amounts of flavonoids existed in its leaves such as rutin was the most abundant constituent (3.77% w/w dry extract), followed by catechin (0.34%), quercetin (0.079%), kaempferol (0.009%) and isorhamnetin (0.007%). Rutin in combination with other flavonoids may cause strong antioxidant activity. Sun *et al*. (2011) reported that rutin was significant in scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH•).

The active principles in turmeric are group of phenolic compounds, including curcumin which was found at the highest level (17.61 mg/g) followed by demethoxycurcumin (3.91 mg/g) and bis-methoxycurcumin (3.88 mg/g) (Puangsombat *et al*., 2011). Curcumin, is well known for its strong antioxidant activity group of phenolic compounds (Miquel *et al*., 2002). The antioxidant properties of turmeric have been assessed by various lipid peroxidation assays as well as DPPH radical scavenging and metal chelating methods. The results clearly show that essential oils of both fresh and dry rhizomes were more effective than synthetic antioxidants while the activities of oleoresins were comparable to BHT but higher than BHA (Singh *et al*., 2010). Zaeoung *et al*. (2005) also reported the strong antioxidant activity of methanol extract of turmeric against the DPPH radical with % inhibition in the range of 86-92%.

Antioxidant activity of components in green peppercorn obtained are 3,4-dihydroxyphenyl ethanol glucoside (0.076 mg/ml), 3,4-dihydroxy-6-(N-ethylamino) benzamide (0.27 mg/ml) and phenolic acid glycosides (0.12 mg/ml), suggesting a high radical scavenging activity of these phenolics (Orav *et al*., 2004; Chatterjee *et al*.,2007). Chatterjee *et al.,* (2007) demonstrated the various fractions from green peppercorn tested for their antioxidant activity, phenolic compounds from pepper showed the lower EC_{50} values. Among the pepper phenolics, 3,4-dihydroxyphenyl ethanol (hydroxytyrosol) glucoside exhibited the highest activity approximately 60% higher than standard trolox.

Phytochemical investigations on holy basil leaf extract have been shown to possess potent antioxidant. Phenolic compounds found in holy basil extract are eugenol, cirsilineol, isothymucin, isothymonin, apigenin and vosamarinic acid and flavonoids compounds are orientin and vicenin (Yanpallewar *et al.,* 2004). Pharmacologically active compounds isolated from the rhizomes of finger root are flavonoid compound (pinostrobin), flavanones (pinostrobin, pinocembrin and alpinetin) and chalcones (cardamonin and boesenbergin A) (Mahmood *et al*., 2010). Javanmardi *et al*. (2003) reported that the amount of phenolic content and antioxidant activity of basil (*Ocimumbasilicum* L.) obtained from Iran, typical phenolic compounds that possess antioxidant activity have been characterized as phenolic acids and flavonoids (Kähkönen *et al*., 1999). Both groups were reported by Juntachote *et al*. (2005) of having strong antioxidative properties.

Many studies have shown good positive linear correlation between antioxidant capacity and total phenolic contents of herbs and spices. Moreover, these results have also suggested that phenolic compounds are responsible for their antioxidant capacity (Zheng *et al*., 2001; Lu *et al*., 2011). However, the results of Shan *et al*. (2005) indicated that several factors including the number of the tested samples, the ranges of the tested values, different antioxidant assay methods, and specific conditions of samples, all could greatly influence the correlative relationship. Therefore, phenolic compounds in herbs and spices could be the major contributor to their antioxidant capacity.

Generally, antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups. Some of the studies reported lack of correlation between total flavonoids content and with antioxidant activity in terms of DPPH, ABTS and reducing power assays (Andarwulan *et al.*, 2010). However, Mustafa *et al.* (2010) showed the correlation between the antioxidant activity measured by DPPH assay with those of total phenolic compounds and flavonoid contents of the 21 plants. The results revealed strong correlation between free radical scavenging activity with those of total phenolic compounds (R=0.8613) and total flavonoids (R=0.8430).

Antioxidant activities of selected herb and spice extracts used in pork meatballs

Based on the highest antioxidant activities assessed in terms of DPPH activity, FRAP and TBARS values, four herb and spice extracts were selected to incorporate in pork meatball batter, i.e. holy basil (*Ocimum sanctum* Linn.), Vietnamese coriander (*Polygonum odoratum* Lour.), turmeric (*Curcuma longa* Linn.) and green peppercorn (*Piper nigrum* Linn.). All four selected Thai culinary herb and spice extracts clearly exhibited antioxidant capacity in the pork meatballs providing longer retardation compared with control ones in both aerobic and vacuum package during storage at 4°C.

Such natural extracts contain high level of bioactive phenolic compounds to inhibit lipid oxidation in the meatball products.

The effects of adding selected herb and spice extracts, packaging conditions and storage times on the lipid oxidation on the TBARS values of pork meatballs are shown in Table 2. At all storage times, the pork meatballs with those selected herb and spice extracts showed significantly lower TBARS values than control ones in both packaging conditions ($p<0.05$). Among pork meatballs with added selected herb and spice extracts, turmeric extract had higher TBARS values in the pork meatballs than did other herb and spice extracts ($p<0.05$) in aerobic condition, followed by Vietnamese coriander, holy basil and green peppercorn extracts, consecutively. Storage period had significant influence on the development of lipid oxidation in the pork meatballs resulting in intensive increase in TBARS values with prolonging storage times at refrigerator temperatures.

Storage under vacuum packaging conditions, holy basil and green peppercorn extracts provided the lowest TBARS values in the pork meatballs followed by Vietnamese coriander and turmeric extracts when compared with control samples ($p<0.05$). The highest TBARS values were found in the meatballs with turmeric extract ($p<0.05$). Oxidative rancidity measured as TBARS for all samples increased during storage while the highest increases in TBARS over time were found in control samples in both packing conditions. It was obvious that in addition to herb and spice extracts in the meat products, vacuum packaging could also help retarding lipid oxidation. Obviously, TBARS values of aerobically packaged meatballs increased with increasing storage time. The presence of oxygen was the most critical factor in influencing lipid oxidation of food products aerobically packaged (Nam *et al*., 2003). The pork meatballs added green peppercorn and holy basil extracts were more effective than Vietnamese coriander and turmeric at reducing the formation of TBARS values throughout refrigerated storage in both packaging conditions ($p<0.05$). Therefore, The TBARS values did not correlate with total phenolic compounds, total flavonoid content and antioxidant activities assessed in terms of DPPH activity, FRAP and TBARS values. Similarly, Juntachote *et al*. (2006) found that the TBARS values of holy basil and galangal extracts in cooked ground pork did not correlate with total phenolic content, antioxidant activity against a β-carotene-linoleic acid emulsion system, DPPH scavenging activity and reducing power. Galangal extract had lower total phenolic content and antioxidant activity than holy basil extract but the TBARS values; galangal extract was more effective than holy basil extract in retarding lipid oxidation throughout storage ($p<0.05$).

Hexanal content is one of major breakdown products of linoleic acid oxidation (Frankel, 1996), it has been used to determine lipid oxidation and off-flavor development in cooked foods (Dupuy *et al.*, 1987). The hexanal contents of pork meatballs packaged in aerobic and vacuum conditions during storage at 4°C are shown in Table 3. Similar to the TBARS results, under an aerobic condition, development of lipid oxidation in the pork meatballs was more intense with increasing storage time resulting in intensive increase in hexanal contents. The pork meatballs with added selected herb and spice extracts had significantly ($p<0.05$) lower hexanal contents throughout storage times (day 9) compared with control samples. However, among pork meatballs with selected herb and spice extracts, the pork meatballs with Vietnamese coriander extract had the highest hexanal contents than did the meatballs with other herb and spice extracts ($p<0.05$). However, at the end of storage, Vietnamese coriander extract give antioxidant activity similarly to turmeric extract ($p>0.05$) but lower than holy basil and green peppercorn extracts ($p<0.05$). This was in agreement with Juntachote *et al.* (2007) who reported that ethanolic extracts provided good antioxidant activity in cooked ground pork. But the activity of ethanolic extract was found less intense than that of dried holy basil powder.

The stability of cooked, refrigerated pork meatballs is also influenced by packaging systems. Air removal enables to extend the shelf life of foodstuffs (Škrinjar *et al*., 2009). In vacuum condition, hexanal contents of pork meatballs with selected herb and spice extracts had significantly ($p<0.05$) lower than those of control samples throughout storage times. Nevertheless, at day 0 and 3 of storage, hexanal contents of pork meatballs with all selected herb and spice extracts were not significantly different ($p>0.05$) but difference was found at day 6 and 9. Higher hexanal contents were observed from the meatballs added Vietnamese coriander and turmeric extracts when compared with those with holy basil and green peppercorn extracts ($p<0.05$). However, hexanal contents of all pork meatballs made with all four selected culinary herb and spice extracts and stored under vacuum condition also increased with increasing storage time ($p<0.05$). Similar trend between volatile compounds and TBARS values were reported positively correlated and the highest correlation was found for hexanal and TBARS values. Juntachote *et al.*, (2007) reported that

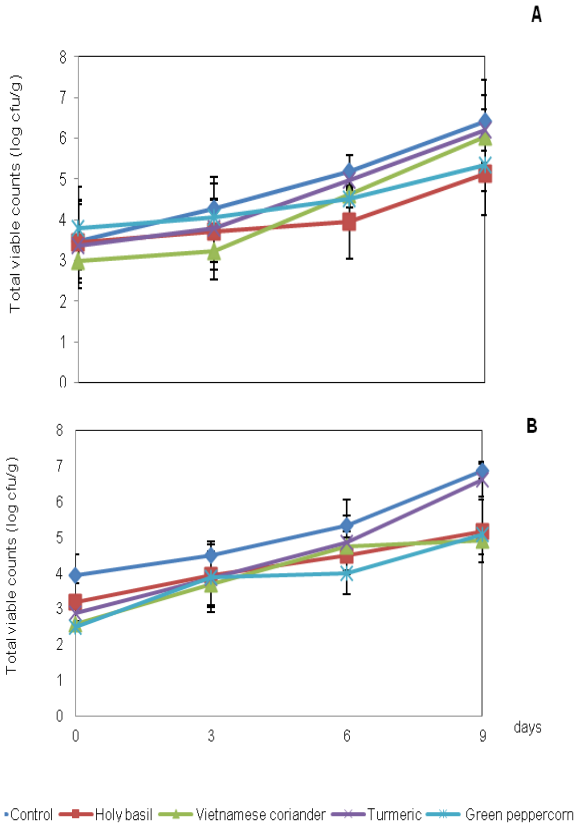

Figure 1. Total viable counts (TVC; log cfu/g) of pork meatballs added herb and spice extracts and stored at 4°C for 9 days; n=4; A = aerobically packaged and B = vacuum packaged.

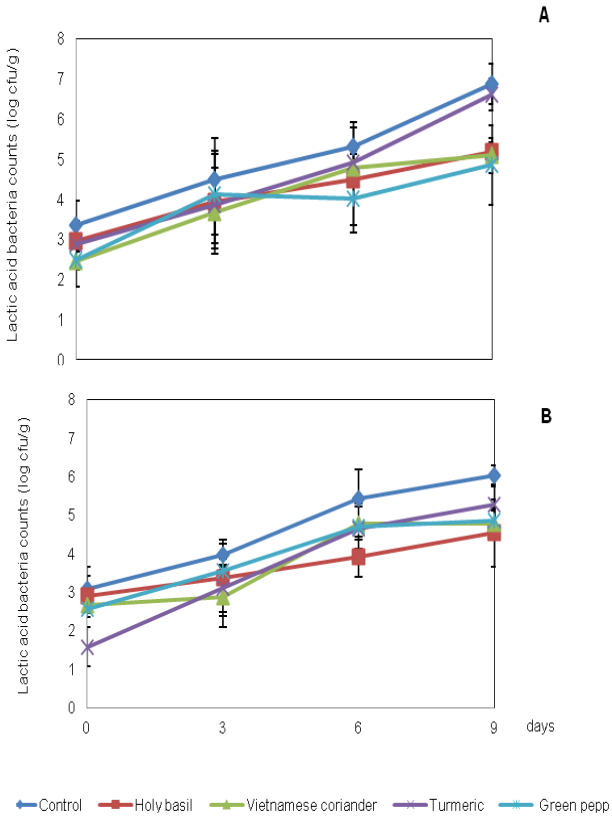

Figure 2. Lactic acid bacteria counts (LAB; log cfu/g) of pork meatballs added herb and spice extracts and stored at 4°C for 9 days; n=4; A = aerobically packaged and B = vacuum packaged

changes in hexanal content were similar to changes in TBARS value. TBARS values and hexanal contents correlated well over the storage period, with a correlation coefficient of 0.95 ($p<0.05$).

Antimicrobial activities of selected herb and spice extracts in pork meatballs

It was also found that all four selected culinary herb and spice extract had ability to inhibit microbial growth in the meatballs and higher inhibition was found in vacuum pack condition. Initial total viable counts (TVC) of all pork meatballs sampled at day 0 were less than 4 log cfu/g sample. Figure 1 shows the microbial profiles of meatball samples during storage at 4°C in both aerobic and vacuum packages. Similar trend of microbial growth was found in both packaging conditions. The control meatballs had higher counts than those with herb and spice extracts at all time of storage. Among four selected culinary herbs and spices used in this study, holy basil and green peppercorn extract gave less antimicrobial efficacy. According to Thai FDA of which the set standard for the marginal acceptable microbial counts of ≤ 5.0 log cfu/g sample, the control meatballs packaged in both conditions could be kept less than 6 days while aerobically packaged meatballs with turmeric, Vietnamese coriander, holy basil and green peppercorn extract could be kept for about 6, 6, 9 and 9 days, respectively. However, it was obvious that under vacuum condition the meatballs added with holy basil, Vietnamese coriander and green peppercorn had similar shelf life of about 9 days while turmeric extract added meatballs could have the shelf life only up to 6 days. Microbial growth retardation due to these selected herb and spice extracts in meat product was consistent with other natural extracts studied to a greater or lesser degree. Many studies have reported a high correlation between antimicrobial efficacy and the level of phenolic components present in certain herb and spice extracts (Salawu *et al*., 2011; Nitiema *et al*., 2012; Alves *et al.*, 2013). However, turmeric and Vietnamese coriander extracts had exhibited total phenolic compounds and total flavonoid content higher than green peppercorn and holy basil extracts, but exhibited relatively lower antibacterial activity than green peppercorn and holy basil extracts. This finding was in agreement with Kim *et al.* (2013) who reported that the ethanol extracts of butterbur and crown daisy exhibited poor antioxidant properties but their antibacterial activity was relatively higher

against *Bacillus subtilis*. It could be due to non-phenolic antimicrobial in the extracts of butterbur and crown daisy. In addition, Al-Shahwany (2014) reported antibacterial activity of *Piper nigrum* extract was due to alkaloid compounds. The possible modes of action for phenolic as antimicrobial agents have been reported. The effect of phenolic compounds may be due to their ability to alter microbial cell permeability, permitting loss of macromolecules from the cell. They could also interfere with membrane function such as electron transport, nutrient uptake, protein, nucleic acid synthesis and enzyme activity, and interact with membrane proteins, causing deformation in structure and functionality (Cox *et al*., 2000; Dorman *et al*., 2000; Trombetta *et al*., 2005; Bajpai *et al*., 2008). Shan *et al*. (2007) reported that in vitro antibacterial activities of a total of 46 extracts from dietary spices and medicinal herbs investigated by agar-well diffusion method against five foodborne bacteria (*Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Escherichia coli,* and *Salmonella anatum*) and found that many herb and spice extracts contained high levels of phenolics and exhibited antibacterial activity against foodborne pathogens.

Lactic acid bacteria counts (LAB) as facultative anaerobic bacteria can grow under high concentrations of CO_2 and is the important competitors of other spoilage related microbial groups under vacuum or modified atmosphere packaging conditions (Tsigarida *et al*., 2000). They are associated to the spoilage of refrigerated raw meat and they can also dominant throughout storage in reduced O_2 availability (Labadie, 1999; Lambert *et al.*, 1991). Similarly, *Lactobacillus* is the major component of the microbiota in chilled pork for vacuum packaged (Blixt *et al*., 2002). More species of lactobacilli can be found during the storage under vacuum at 4°C (Pavelková *et al*., 2013). Many spices and herbs are considered as alternative means of delaying the onset of spoilage or preventing the growth of foodborne pathogens since their essential oils possess antimicrobial activity (Nychas *et al*., 2000).

Lactic acid bacteria counts (LAB) of all meatballs, aerobically and vacuum packaged, are shown in Figure 2. The initial total LAB contents in both packaging conditions were less than 4 log cfu/g sample. However, throughout the storage period total LAB reduction of the meatballs due to the effect of selected culinary herb and spice extracts was higher in vacuum packaging than in aerobic packaging condition. At the end of storage time, the meatballs added holy basil, Vietnamese coriander and green peppercorn extracts had lower LAB than did control meatballs of about 2 log and 1.5 log cycles in aerobic and vacuum package condition, respectively, while those with turmeric extract had similar counts to control meatballs of about 6.61-6.88 log cfu/g and 5.28-6.04 log cfu/g, respectively.

Conclusions

From antioxidant activities in terms of FRAP, DPPH and TBARS values used for preliminary screening ethanolic extracts of 22 Thai culinary herbs and spices commonly used for cooking, four herb and spice extracts were selected consisting of Vietnamese coriander, turmeric, green peppercorn and holy basil in the order of higher to lower antioxidant activity in spite of their total phenolic compounds and total flavonoid contents were not in the same order as their antioxidant activities.

Among the four culinary herb and spice extracts added in the pork meatballs, holy basil extract had the highest efficacy to retard oxidation in pork meatballs while turmeric extract was found to have the least efficacy. For antimicrobial activity of all four selected culinary herb and spice extracts in pork meatballs packaged in both aerobic and vacuum conditions, it was observed as well that holy basil extract exhibited the highest inhibition of microbial growth in the meatballs. According the marginal acceptable microbial counts, holy basil and green peppercorn extracts could be used to extend the meatball shelf life up to 9 days while only 6 day shelf life was observed for Vietnamese coriander and turmeric extracts and less than 6 days for control meatballs.

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