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FULL-LENGTH RESEARCH ARTICLE

Antioxidant Activities of *Murraya koenigii* (L.) *Spreng* Berry Extract: Application in Refrigerated (4 ± 1 °C) Stored Meat Homogenates

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Abstract The antioxidant activity of extract of berries of Spreng (curry tree), *Murraya koenigii* (L.), was estimated by DPPH free radical scavenging activity method. Total phenolics, total flavonoids, and reducing power were also estimated. The additional study was carried out to evaluate the antioxidant potential of curry berry extract (CBE) in raw chicken meat homogenate during refrigerated (4 ± 1 °C) storage. Total phenolics in CBE were 9.5 ± 0.03 mg TAE/gdw and total flavonoid contents were 11.9 ± 0.66 mg CE/gdw. CBE also showed remarkable DPPH radical scavenging activity (20.9 ± 0.15 %) and reducing power. During refrigerated storage, the TBARs (mg malonaldehyde/Kg), FFA (%), and odor scores at all stages were significantly ($P < 0.01$) more in control than CBE treated groups. Thus, curry tree berries may be a good natural source of antioxidative compounds to prevent oxidative damage of meat and meat products.

Keywords Antioxidant · Meat preservation · Spreng (curry tree) Berry · Meat · Antioxidant · *Murraya koenigii* · TBARs · FFA

Introduction

Lipid oxidation is, in most instances, a free radical chain reaction that can be described in terms of initiation, propagation, and termination processes [8]. It is one of the primary mechanisms of quality deterioration such as development of rancidity and off-flavor, textural degradation, discoloration in meat and meat products during storage, rendering them unfit for human consumption.

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High oxidative stability of muscle-based foods is important when attempting to avoid or delay the development of rancid products or warmed-over flavor [22] and to increase the acceptability of meat and meat products. Increased oxidative stability of the raw meat product is considered beneficial for both the consumer and the processing industry. Thus, various synthetic antioxidants, such as butylated hydroxytoluene (BHT), are being widely used in the meat industry to ensure product preservation as well as to reduce risks to consumers' health from ROS and lipid-oxidation secondary products. However, recent increases in consumers' awareness of health benefits from natural products and concerns about use of synthetic antioxidants because of their potential toxicity [2] and carcinogenicity [6] have led the meat industry to consider substituting natural plant extracts for synthetic preservatives.

Natural extracts from various plant sources, such as herbs, spices, vegetables, fruits, and grains, possess strong antioxidant activities. The antioxidant activities of the plant's natural extracts are mainly attributed to the presence of various phenolic and flavonoid compounds and may be enhanced by their synergism activities. *Murraya*

koenigii (curry tree) is a tropical to sub-tropical tree in the family *Rutaceae*, which is native to India. The leaves of *Murraya koenigii* are aromatic and often used in the Indian cuisine and also used as an herb in Ayurvedic medicine. Curry leaves have been identified as containing a rich profile of simple phenolic acids including tannic, gallic, caffeic, cinnamic, chlorogenic, ferulic, and vanillic acids [17]. Curry leaves can be used as antioxidants in high fat diets as they contain the antioxidants tocopherol, b-carotene, and lutein [12]. However, there is no report of antioxidant activity of *Murraya koenigii* berries (fruit) in vitro system as well as their effect on oxidative stability of lipids in meat model system during refrigerated storage period.

Therefore, the aim of this work was to study the antioxidant activity, reducing power, total phenolics, and total flavonoids of *Murraya koenigii* berries and their importance for meat quality parameters such as water-holding capacity (WHC), cooking losses, color and oxidative stability of chicken breast meat homogenates during refrigerated storage period.

Materials and Methods

Materials

Different ingredients like vegetable oil and chicken breast meat (6 weeks of age) were obtained from local market. The representative samples of curry tree berries were obtained at green–red stage from local gardens of Ludhiana. All reagents and solvents were purchased from Merck and Sigma unless otherwise mentioned. All chemicals used in the experiments were of analytical grade.

Preparation of Aqueous Extract of Curry Berries

Seeds were removed manually from curry tree berries. The seedless berries were dried in hot air oven at a 55 °C for 48 h and powdered using a heavy duty kitchen grinder, and the powder was sieved through 0.6-mm mesh size sieve. About 10 g of each powder was mixed with 200 ml boiled (50 mg/ml) sterilized distilled water and left for 2 h with frequent stirring. This was centrifuged at 5000 rpm for 10 min; the supernatant called as curry berry extract (CBE) was collected in another sterile tube and stored at 4 ± 1 °C and used for further analysis.

Treatment of Chicken Breast Meat Homogenates with Extracts

Chicken breast meat was minced using a mincer (8-mm plate). For treatment of meat homogenates, 200 mg/ml CBE was made as per the procedure described earlier. In group 1, one kilogram minced meat ground with 20 g salt, 2.5 % (25 ml) ice cold curry berry extract (CBE) + 25 ml distilled water, and 50 ml vegetable oil for 5 min in a kitchen grinder, in group 2 only 5 % (50 ml) CBE was used, whereas in control samples 50 ml extract was replaced with 50 ml distilled water keeping other contents same as that of treated samples. These treated and control meat homogenates were then filled in LDPE and stored at 4 ± 1 °C for further studies.

Total Phenolics

The concentration of total phenolics in the extract was determined by the Folin–Ciocalteus (F–C) assay [5] with slight modifications. Suitable aliquots of extracts were taken in a test tube and the volume was made to 0.5 ml with distilled water followed by the addition of 0.25 ml F–C (1 N) reagent and 1.25 ml sodium carbonate solution (20 %). The tubes were vortexed and the absorbance was recorded at 725 nm after 40 min. The values were reported as mg of tannic acid equivalent (TAE) by reference to tannic acid standard curve and the results were expressed as milligrams of TAE per gram dry weight (gdw) of powder.

Measurement of Reducing Power

The reducing power was quantified by the method described by Jayaprakasha et al. [7]. The 2.5 ml extract was mixed with 2.5 ml phosphate buffer (200 μM, pH 6.6) and incubated with 2.5 ml potassium ferricyanide (1 % w/v) at 50 °C for 20 min. At the end of incubation, 2.5 ml of 10 % trichloroacetic acid solution was added and centrifuged at $9700 \times g$ for 10 min. The supernatant was mixed with 5 ml distilled water and 1 ml ferric chloride (0.1 % w/v) solution. The absorbance was measured at 700 nm. Increase in absorbance of the reaction indicated increase in the reducing power.

Flavonoid Content

The total flavonoid content of the extract was determined according to the calorimetric method described by Zhishen et al. [23], with some modification. Briefly, 0.5 ml extract was mixed with 2 ml of distilled water. Add 0.15 ml of sodium nitrite ($NaNO_2$, 5 % w/v) into each subsequently and allow the reaction mixture to stand for 6 min. Then 0.15 ml aluminum trichloride ($AlCl_3$, 10 %) was added and allowed to stand for 6 min, followed by the addition of 2 ml of sodium hydroxide (NaOH, 4 % w/v) to the reaction mixture. Then distilled water was added to the mixture to bring the final volume up to 5 ml. The reaction mixture was mixed thoroughly and allowed to stand for another

15 min. Then absorbance of pink color that developed was measured at 510 nm using spectrophotometer (UV-1800 PharmaSpec, SHIMADZU, Japan). Distilled water was used as blank.

The final absorbance of each sample was compared with a standard curve plotted from catechin. The total flavonoid content was expressed in mg of catechin equivalent per gram of dried powder (mg CE/gdw).

DPPH Radical Scavenging Activity

The method of Singh et al. [16] was employed to assess the ability of extract to scavenge 1,1-diphenyl 1-2-picrylhydrazyl (DPPH) radicals with slight modification. The 400 µl extract diluted with 1600 µl 0.1 M Tris–HCI buffer (pH 7.4) was mixed with 2 ml of DPPH (500 µM) with vigorous shaking. The reaction mixture was stored in the dark at room temperature for 20 min and the absorbance was measured at 517 nm. The scavenging activity (SA) was calculated by the following equation:

$$\text{Scavenging activity (SA)}\% = [1 - (\text{Absorbance sample}/\text{Absorbance control})] \times 100$$

Determination of WHC and Cooking Loss

Water-holding capacity (WHC) was determined according to Wardlaw et al. [20]. For cooking loss determination, 20 g sample was sealed in a plastic bag and cooked in a water bath at 100 °C for 20 min. Each piece was cooled, removed from the bag, and then weighed. The weights of samples were recorded before and after cooking and the loss was expressed as a percentage.

Instrumental Color

Color measurement was conducted on the surface of samples from day 0 to 8 at 2 day intervals using a miniscan XE plus (Hunter Associated Labs, Inc, Reston, VA, USA) that had been calibrated against black and white reference tiles ($X = 78.6$, $Y = 83.4$, and $Z = 89.0$). Values from 4 random locations from the duplicate sample surface were taken.

Lipid Oxidation

To evaluate the extent of lipid oxidation, thiobarbituric acid reactive substances (TBARs) were determined from day 0 to 8 by the extraction method described by Witte et al. [21] with slight modification. Four gm sample was homogenized with 20 % trichloro acetic acid solution (20 ml) and the slurry was centrifuged at 3,000×*g* (MP 400R Eltek Ltd., India) for 10 min; 2 ml of supernatant was mixed with equal volume of freshly prepared (0.1 %) thiobarbituric acid in glass test tubes and heated in water bath at 100 °C for 30 min followed by cooling under tap water. The absorbance of the mixture was measured at 532 nm using UV–VIS spectrophotometer (UV-1800 PharmaSpec, SHIMADZU, Japan) and the TBARs values were calculated using a TBA standard curve and expressed in mg malonaldehyde/kg. The methods described by Koniecko [9] were followed for the determination of free fatty acids (FFA) in refrigerated meat homogenates.

Sensory Evaluation

Subjective odor evaluations were conducted by a six member panel. Odor scores were determined by opening a sample bag, sniffing the samples, and recording a score. Scores of 1–5 were used according to the following descriptors: (1) fresh meat odor; (2) no odor; (3) slight off-odor development but still acceptable; (4) definite off-odor indicative of spoiled meat; (5) very strong off-odor associated with spoiled meat. Scores of 1–3 were considered indicative of acceptable meat; whereas, scores of 4 or 5 represented unacceptable and spoiled meat.

Statistical Analysis

All measurements including extract formation were done in triplicate with three measurements in each unless otherwise mentioned, and results obtained were subjected to the analysis of variance (ANOVA) and to Duncan's multiple range procedure to determine the significant differences among treatments ($P < 0.05$).

Results and Discussion

Total Phenolics, Flavonoid Contents, Reducing Power, and DPPH Radical Scavenging Activity

Total mean values of phenolic contents and other properties of CBE are presented in Table 1. Results showed that CBE was an excellent source of phenolics and flavonoid compounds. The total phenolic contents and total flavonoid contents analyzed in CBE were 9.5 ± 0.03 mg TAE/gdw and 11.9 ± 0.66 mg CE/gdw respectively. Reducing power, estimated as absorbance of the reaction, indicated that CBE may serve as a good reducing agent. DPPH free radical SA of CBE (20.9 ± 0.15 %) was also comparable to that of other plant extracts reported earlier.

The enrichment of phenolic compounds within plant extracts is correlated with their enhanced antioxidant activity [10] and radical-scavenging activity of the

Table 1 Total phenolics (TP), total flavonoids (TF), reducing power (RP_{OD700}), and DPPH radical scavenging activity (SA) of CBE and other chemical preparations

	CBE	BHT	AA	TA
TP mg TAE/ gdw	9.5 ± 0.03	–	–	–
TF mg CE/ gdw	11.9 ± 0.66	–	–	–
RP_{OD700}	0.91 ± 0.12	1.1 ± 0.13	1.6 ± 0.09	1.9 ± 0.19
DPPH radical SA %	20.9 ± 0.15	78.2 ± 1.7	65.1 ± 1.9	76.43 ± 1.39

All values are expressed as mean ± standard deviation of triplicate determinations ($n = 9$)

CBE curry berry extract; *BHT* butylated hydroxytoluene; *AA* ascorbic acid; *TA* tannic acid; *TAE* tannic acid equivalent; *CE* catechin equivalent; *gdw* gm dry weight; *OD* optical density

compounds is due to their hydrogen-donating ability. Radical-scavengers are believed to interrupt the free radical chain of oxidation and to donate hydrogen from the phenolic hydroxyl groups, thereby forming relatively stable end product, which does not initiate or propagate further oxidation of lipid. Thus, radical-scavengers are free radical inhibitors and primary antioxidants. Water extract (100 µg/ml) of curry leaves showed [11] DPPH radical scavenging activity of 41 %. Known antioxidants such as BHA (72 µg), a-tocopherol (85.5 µg), Curcumin (73.5 µg), b-carotene (107.33 µg) showed 86, 82, 76, and 61 % DPPH radical scavenging activity, respectively. In previous works, extracts from green tea and grape seeds also showed higher DPPH radical-scavenging activity [13]. In the present investigation, the results indicated that CBE is a powerful free radical scavenger compared to known antioxidants.

WHC and Cooking Loss

The effects of CBE on meat homogenate WHC and cooking losses are depicted in Fig. 1. The significant ($P < 0.05$) results were obtained for WHC between different groups, but nonsignificant ($P < 0.05$) results were observed for cooking losses. The WHC observed more, i.e., 26.0 ± 0.66 % in 50 ml CBE group followed by 25 ml CBE group (23.0 ± 0.54 %) and least WHC was obtained in control group (21.4 ± 1.21 %). WHC is more when net free charges on protein molecules are more. These free charges may bind water molecules loosely or tightly. In addition, when exposing more charged sites where water can be bound, the electrostatic repulsion increases the space between the thin and thick filaments. Increasing the size of the space between the filaments increases the amount of water that can be retained by the muscle.

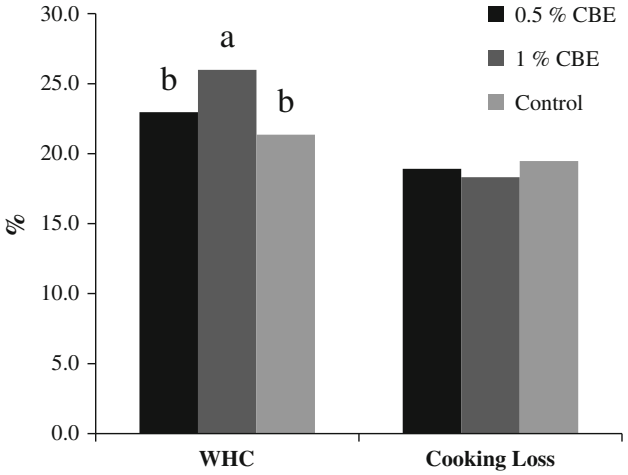

Fig. 1 WHC and cooking losses of treated and control raw meat homogenates ($n = 9$). WHC-water holding capacity; CBE-curry berry extract; Control-No CBE. *Bars* with different data labels differed significantly ($P < 0.05$) at 95 % confidence level

Therefore, anything that influences the spacing between the thick and thin filaments or the ability of the proteins to bind water can affect water holding properties of the meat. In the present investigation, more WHC observed in CBE-treated meat homogenates which may be due to the effect of CBE constituents on meat proteins' net charge and increased exposed site due to increased electrostatic repulsion between meat proteins and filaments.

Instrumental Color

The discoloration of meat and meat products is an important process, which is determined by the relative concentration of the three redox forms of myoglobin (deoxymyoglobin, oxymyoglobin, and metmyoglobin). Loss of the desirable cherry-red appearance with subsequent replacement by reddish browns and browns is a natural process affected by a variety of intrinsic and extrinsic factors. Because consumers often use meat color as a basis for product selection or rejection, the loss in economic value that accompanies meat and meat product discoloration can be considerable. The instrumental color values (mean ± SD) from present investigation are presented in Table 2. The *L* and *b* value at day 0 was nonsignificant, while treatment with CBE significantly ($P < 0.01$) increased the a values at this stage. At day 2, *L* values increased in all groups from day 0, and differed significantly ($P < 0.01$) among each other. More *L* values were obtained in control groups than treated samples. Similarly, at day 2, *a* and *b* values were also increased from day 0. At this point, *a* values differed significantly ($P < 0.05$) among groups and were observed more in the treated groups than control, while *b* values were

Table 2 Colour (*L*, *a*, *b*) values and odor score of chicken meat homogenates with various treatments stored at 4 ± 1 °C

Colour value	Treatment	Storage days				
		Day 0	Day 2	Day 4	Day 6	Day 8
L	0.5 % CBE	54.1 ± 4.36	56.0 ± 2.19^{b}	54.5 ± 1.79^{a}	44.0 ± 0.98^{b}	43.5 ± 1.20^{b}
	1 % CBE	50.6 ± 4.64	56.4 ± 1.49^{b}	48.8 ± 1.73^{b}	47.0 ± 2.59^{a}	41.4 ± 1.51^{c}
	Control	56.3 ± 8.63	60.5 ± 1.15^{a}	56.2 ± 1.98^{a}	49.1 ± 3.82^{a}	49.0 ± 1.61^{a}
a	0.5 % CBE	7.8 ± 0.41^{a}	11.6 ± 1.10^{a}	10.6 ± 0.69^{b}	13.9 ± 1.11^{a}	11.5 ± 0.42^{b}
	1 % CBE	7.2 ± 0.57^{a}	10.6 ± 0.45^{b}	11.8 ± 0.82^{a}	11.4 ± 1.31^{b}	14.0 ± 1.34^{a}
	Control	6.3 ± 0.69^{b}	10.4 ± 0.69^{b}	10.6 ± 0.93^{b}	11.5 ± 2.44^{b}	10.6 ± 0.24^{c}
b	0.5 % CBE	16.5 ± 0.98^{a}	22.9 ± 1.39	21.4 ± 0.42^{b}	19.6 ± 0.55	18.9 ± 0.46^{b}
	1 % CBE	15.9 ± 0.78^{ab}	21.9 ± 1.02	22.8 ± 0.81^{a}	20.3 ± 1.10	19.4 ± 0.54^{a}
	Control	15.1 ± 1.86^{b}	22.5 ± 0.72	22.2 ± 1.21^{ab}	20.0 ± 0.70	18.8 ± 0.32^{b}
Odor score	0.5 % CBE	1.3 ± 0.27	1.8 ± 0.27^{b}	2.3 ± 0.27^{b}	2.8 ± 0.26^{b}	3.6 ± 0.38^{b}
	1 % CBE	1.1 ± 0.20	1.7 ± 0.26^{b}	1.8 ± 0.26^{c}	2.7 ± 0.26^{b}	3.2 ± 0.41^{b}
	Control	1.2 ± 0.26	2.2 ± 0.26^{a}	2.8 ± 0.26^{a}	3.8 ± 0.26^{a}	4.6 ± 0.49^{a}

All values are expressed as mean ± standard deviation; $n = 8$ for color values and $n = 18$ for odor scores

CBE curry berry extract; *Control* no CBE

Values bearing *different superscript* in a column differ significantly ($P < 0.05$)

nonsignificant. After day 2, *L* values decreased continuously up to day 8 and differed significantly at all analyzed days. At day 8, *L* values were observed more ($P < 0.01$) in control groups than the treated groups, means the treated groups showed more darkness values compared to the control groups. After day 2, *a* values differed significantly at all the analyzed stages, while *b* values differed significantly only at day 4 and 8. After day 2, values for *a* showed no definitive pattern, but *b* values were decreased continuously from day 4 to 8 of storage. From these findings it is very obvious that treatment with CBE increased the darkness and redness values of meat homogenates kept at refrigerated temperature.

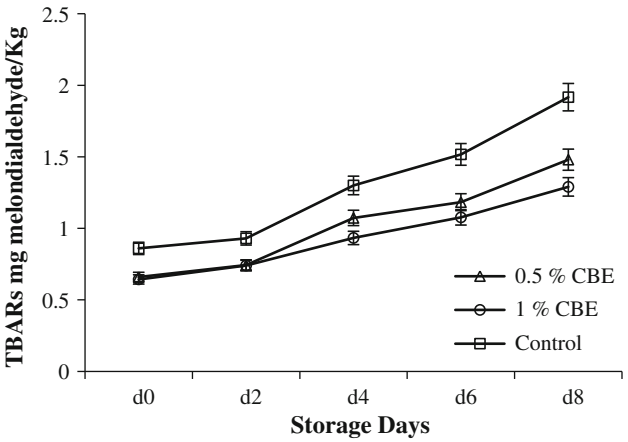

Fig. 2 TBARs' (mg Malonaldehyde/kg) values of treated and control meat homogenates during refrigerated (4 ± 1 °C) storage ($n = 9$). CBE-curry berry extract

Lipid Oxidation

In order to determine if the extracts were capable of reducing in vitro oxidative stress, the traditional lipid peroxidation assay that determines the production of malondialdehyde and related lipid peroxides in meat homogenates was carried out along with percent FFA determination. TBA value is routinely used as an index of lipid oxidation in meat products [14]. In this investigation, TBARs values were significantly ($P < 0.01$) higher in control groups than the two CBE-treated groups during whole storage period (Fig. 2). However, for the initial storage period, the TBARs' values were nonsignificant ($P < 0.05$) between two treated samples. However, after an initial period, the TBARs' values were also differed significantly ($P < 0.01$) between two CBE-treated samples. TBARs' inhibition (%) as compared to control was more in 5 %-treated samples which suggest dose-dependent lipid antioxidative effects of CBE in chicken meat homogenates. The percent FFA contents of meat homogenates during 8 days of refrigerated storage are depicted in Fig. 3. It is obvious from Fig. 3 that percent FFA contents were nonsignificant at day 0, but were significantly more ($P < 0.01$) in the control group than both treated groups. There was a continuously increasing trend in FFA content in all groups. Previous workers also reported the increasing trend of the FFA content of buffalo meat [15] and goat meat [19] during 9 days of refrigerated storage. Recently, researchers [3] also reported an increase of FFA during refrigerated storage of goat meat patties. In the present investigation, less FFA in 5 % CBE-treated group than

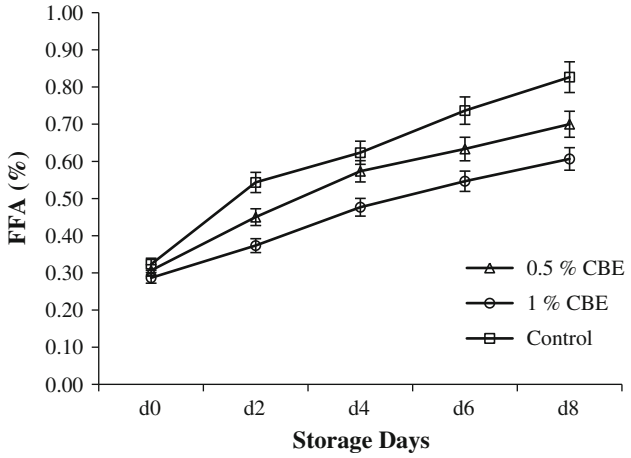

Fig. 3 Free fatty acid % (FFA) in treated meat homogenates as compared to control during refrigerated (4 ± 1 °C) storage ($n = 9$). CBE-curry berry extract

2.5 %-treated group at all stages again indicated dose-dependent inhibition of the lipid oxidation process.

In a previous study [1], lipid oxidation was inhibited effectively in both raw and cooked samples treated with curry leaf powder (CLP). According to their study, CLP showed greater antioxidative effects in raw chicken meat. Similar results were obtained by Das et al. [4], where addition of curry leaf powder into minced goat meat at a concentration of 0.2 % resulted in a significant ($P < 0.05$) reduction of TBARs and FFA values compared to the control. Tachibana et al. [18] reported that carbazole alkaloids of CLP have potent antioxidant activity. This enhanced antioxidant activity of CLP extract was due to higher phenolic content [11]. There were no earlier reports of antioxidative effects of CBE in meat and meat products. In the present investigation, high antioxidant effect of CBE might also be due to higher phenolic contents (Table 1) which were capable of scavenge free radicals in a meat model system.

Sensory Evaluation

Subjective odor score (Table 2) differed significantly ($P < 0.05$) from day 2 onward. In the control group, unacceptable scores were obtained in between day 4 and 6. However, in the treated groups, samples were acceptable for more than 6 days indicating a positive effect of CBE treatment on inhibition of spoilage process. This was because CBE inhibited oxidation of lipid and other nutrients and so it prevents the formation of unsafe volatile compounds. CBE may also possess some antimicrobial compounds that may inhibit the bacterial growth either by bacteriostatic or bactericidal effects and may delay the spoilage process in treated samples. In the previous experiment [4], curry leaf powder also showed an improved odor score than the control sample.

Conclusion

On the basis of the above results, it could be concluded that CBE possesses significant amount of total phenolics and total flavonoids. These phenolics and flavonoids might be the reason for higher free radical scavenging activity and reducing power of CBE. Additional research was conducted to measure antioxidative effect of CBE in meat homogenates and it was found that CBE showed oxidative stabilizing effects by the inhibition of lipid oxidation process which was revealed by low TBARs, FFA and low odor score. This positive effect was dose dependent. CBE also showed a positive effect on some functional properties of meat, like WHC. Thus, curry tree berries may be a good natural source of antioxidative compounds to prevent oxidative damage in meat and meat products.

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