Journal of Food and Nutrition Research, 2016, Vol. 4, No. 12, 789-794
Available online at http://pubs.sciepub.com/jfnr/4/12/4

DOI:10.12691/jfnr-4-12-4

Antioxidative Effect of Dietary Tea Catechins on the Quality of Enulsified Pork Meatballs under Refrigeration

C.K. Yeung, S.C. Huang*

Food Industry Research and Development Institute, P.O. BOX 246, Hsinchu, Taiwan, ROC
*Corresponding author: hsc30@firdi.org.tw

Abstract After meat products are heated and placed in storage, the lipid contained inside undergoes oxidation, which causes the meat to become malodorous and reduces its acceptability in the market. In this study, catechin was added to emulsified pork meatballs in the hope of reducing lipid oxidation. The influence that catechin additions at different concentrations (0, 400 and 800 ppm) have on the pH value, color, texture profiles, microbial testing, sensory analysis, thiobarbituric acid reactive substance value, and antioxidant capacity of the emulsified pork meatball is investigated in this study. The test results on lipid oxidation indicated that after 15 days of refrigerated storage at 4°C, oxidation in the control group was 2.46 mg malondialdehyde equal (MDA eq.)/kg meatball. The 400 ppm and 800 ppm of catechin-treated groups yielded 0.56 and 0.31 mg MDA eq./kg, respectively, which were significantly lower than the control group. The results were similar after 90 days of frozen storage at -20°C, where the 400 ppm and 800 ppm of catechin-treated groups yielded 0.60 and 0.25 mg MDA eq./kg, respectively, which were significantly lower than the oxidation level in the control group (1.39 mg MDA eq./kg). Regarding the antioxidant capacity tests, the meatballs that were treated with 400 ppm and 800 ppm of catechin displayed a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity of 1.31 and 1.97 mg of ascorbic acid/g, respectively, whereas the total antioxidant capacity was 2.63 and 4.53 mg of Trolox/g, respectively. The results of both groups were significantly higher than the DPPH radical scavenging capacity (0.07 mg as ascorbic acid/g) and the total antioxidant capacity (0.36 mg as Trolox/g) of the control group. After 90 days of frozen storage at -20°C, the antioxidant capacity of catechin-treated groups were still higher than the control group. The mentioned results indicate that adding catechin to emulsified pork meatballs inhibits lipid oxidation and can help boost the quality and functionality of these emulsified pork meatball products.

Keywords: *meat product, Kung-Yuan, green tea extract, antioxidant, shelf life*

Cite This Article: C.K. Yeung, and S.C. Huang, "Antioxidative Effect of Dietary Tea Catechins on the Quality of Enulsified Pork Meatballs under Refrigeration." *Journal of Food and Nutrition Research*, vol. 4, no. 12 (2016): 789-794. doi: 10.12691/jfnr-4-12-4.

1. Introduction

The lipid stability and the color of meat products often affect whether consumers accept them. Once the lipid oxidizes, the meat changes in color and becomes malodorous and off-flavor; this deterioration in quality can produce hazardous substances during meat processing [1]. Emulsified pork meatballs (Kung-Yuan) are a traditional type of emulsified processed meat product common in Taiwan. Lipid oxidation occurs easily during the processing, heating, and storage of these meatballs compared to unprocessed meat, and thus lowers their acceptability in the market and shortens their shelf life. Studies have shown that the lipid oxidation of meat can be inhibited or reduced by adding antioxidants [2]. The antioxidants that are used most often are synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tertiary butyl hydroquinone (TBHQ), which work effectively in delaying lipid oxidation [3,4,5]. However, these synthetic antioxidants are suspected carcinogens [6] or health hazards [7], and sometimes consumers reject them [8]. To overcome the disadvantages of synthetic antioxidants, in recent years, natural antioxidants have gradually being the focus of attention as an ideal alternative.

Catechin is a type of flavonoid that consists primarily of epigallocatechin gallate, epigallocatechin, epicatechin gallate, epicatechin [9]. Many recent studies have discovered that catechin possesses strong antioxidant properties [10,11,12]. The antioxidant effect of catechin in lipid-containing food is higher than synthetic BHT, BHA, TBHQ, and even vitamin E [11]. Wanasundara and Shahidi [5] added green tea powder, green tea extract, and pure catechin into mackerel meat and also discovered that catechin had greater antioxidant effects than commercially-sold BHT, BHA, TBHQ or α-tocopherol. Catechin is also more easily accepted by consumers because it is toxicologically safer.

Studies have pointed out that adding catechin in food can inhibit lipid oxidation in lard [13], edible oils, fats [14], vegetable oil [15], fish oil [5] and emulsified lipid systems [16]. It can also lengthen the shelf life of meat products with high lipid content [3]. Related studies have also discovered that adding catechin in cooked beef and chicken [17] or raw red meat, poultry, and fish meat [18] can prevent lipid oxidation. Therefore, catechin can serve as a substitute for synthetic antioxidants, thus providing a selection for meat processing plants during the production of healthy meat products.

This study chose to add catechin, a natural antioxidant, to emulsified pork meatballs to reduce lipid oxidation effects and enhance the preservability and nutritional value of meat products. The effects of different catechin concentrations (0, 400, and 800 ppm) on the physical characteristics of emulsified pork meatballs were evaluated, as well as the ability to reduce lipid oxidation and provide antioxidant effects.

2. Materials and Methods

2.1. Preparation of Meatballs

The meatballs were manufactured according to a traditional formula for Taiwan-style meatballs (Kung - Yuan): 85% Fresh pork ham (Cha I Shan Foods Co., Ltd, Taiwan) and 15% pork back fat (Cha I Shan Foods Co., Ltd, Taiwan) were mixed with 1.8% salt (99.5%, Taiyen Biotech Co., Ltd, Taiwan), 3.0% sugar (99.5%, Taiwan Sugar Co., Taiwan), 0.3% spices (Tomax Enterprise Co., Ltd, Taiwan), and 2% corn starch (LINCO Enterprise Co., Ltd, Taiwan). The tissues were ground with a meat chopper fitted with a plate of 20 mm diameter holes. The ground meat was packaged in plastic bags, 1.0 kg each, and stored at -18°C. Before use, the meats were maintained 20 h at 0°C and then they were mixed with the condiments and commercial catechin(green tea extract 50% Epigallocatechin gallate (MasterAsia Marketing Co., Taipei, Taiwan). The sample was manufactured without catechin was considered as control. After meatballs were boiled at 85°C until an internal meat temperature of 72°C, it were cooled and packaged in plastic bags (NY/LLDPE laminated film). All sample(0, 400, and 800 ppm) were measured at 3 day intervals stored for up to 15 days at 4°C and on days 0, 30 and 90 of storage at -20°C.

2.2. Physicochemical Properties of Meatballs

Hunter-*L*, *a*, *b* color values of the meatballs were measured by color difference meter (Tokyo Denshoku Co., Model TC-1800 MK II, Japan). The mean of ten measurements was taken for each Hunter-*L*, *a*, *b* values.

The pH value of the meatballs was measured after homogenization (Interscience Co., Model BagMixer® 400P, France) with distilled water at a ration of 1:10 using a pH meter (Denver Instrument, USA).

2.3. Texture Profile Analyses

Frozen meatballs were placed in a cooler (2°C) for approximately 12h. Once thawed, meatballs were heated at 80°C water bath for 5 min and then cooled to room temperature. For TPA measurement, the meatballs were cut by two sides to get a 20 mm depth strip. The texture profile analyses (TPA) indices of meatballs were determined using a texture analyzer (Model TA-XT2 Texture Analysis, England). The conditions of texture analyzer were modified by [19]. Pre-test speed: 3.0 mm/s; test speed: 1.0 mm/s; post-test speed: 3.0 mm / s; distance: 10.0 mm; time: 5.0s; trigger type: auto; and trigger force, 10 g.

2.4. Sensory Analysis

The sensory analysis of meatballs was evaluated by 30 untrained assessors selected according to their habits. Samples were labeled with 3-digit random numbers and served in random order to assessor in individual booths. Assessors were instructed to cleanse their palates with water between samples. A hedonic test was carried out using 9 point scales (9 = like extremely and 1 = dislike extremely) in which the assessors evaluated different attributes: appearance, taste, texture, flavor, overall acceptability [20].

2.5. Microbial Status of Meatballs

The total plate count of meatballs were determined using 3 M Petrifilm^{TM} (3M Co., USA) Aerobic Count Plate (AOAC OMA: 990.12) [21]. Plates were incubated at 37°C for 48 hour. Results were expressed as $\log_{10}$ CFU/g meatballs.

Coliform and *E. coli* of meatballs were determined using 3 M Peterifilm^{TM} (3M Co., USA) *E. coli* and Coliform Count Plate (AOAC OMA: 991.14) [22]. Plate were incubated at 37°C for 24 hour. Results were expressed as $\log_{10}$ CFU / g meatballs.

2.6. Antioxidant Activity

The antioxidants of the meatballs were extracted after homogenization (Interscience Co., Model BagMixer® 400P, France) with methanol at a ration of 1:10. The mixture was centrifuged at 1000 x g for 10 min. Free radical scavenging effect was estimated according to the method of [23] with some modification. The upper layer (0.1 mL) was added into the distilled water (0.4 mL) and 0.2 mM DPPH radical (0.5 mL). The mixture was shaken and left to stand for 20 min at room temperature and measured at 517 nm with a spectrophotometer. Total antioxidant capacity was estimated according to the method of [24].

2.7. Measurement of Lipid Oxidation

Lipid oxidation was measured by the 2-thiobarbituric acid distillation method of [25] and results were expressed as 2-thiobarbituric acid reactive substances (TBARS) in mg as malondialdehyde equal (MDA eq.)/kg meatball.

2.8. Statistical Analysis

Data were analyzed using SPSS 12.0 for one-way ANOVA. Duncan's new multiple range test was used to

resolve the difference among treatment means. A value of $p < 0.05$ was used to indicate significant difference.

3. Results and Discussion

3.1. Color and pH Value of Catechin-added Meatballs

Lipid oxidation gives products that may change the color, aroma, flavor, texture and even the nutritive value of the food. The effects of adding catechin to meatballs color and pH value during the storage period are shown in Table 1. Regarding color, the *L* value of the 800 ppm catechin-added group (with catechin added on day 0) increased from 69.39 to 72.73, whereas the *a* value decreased from 3.20 to 2.08. [23] discovered that adding 0.1% freeze-dried green tea extract in pork pies could increase the *L* value and lower the *a* value, which were similar to the values found in this study. Tang et al. [26] found discolouration in beef and chicken meat caused by the 400 ppm of catechin addition. This study's results showed that the addition of catechin to meatballs during their 4°C storage period increased its *L* value. The 400 ppm-treated group exhibited an *L* value increase from 71.30 to 75.60 and *a* value reduced from 2.12 to 1.69. The 800 ppm-treated group displayed an *L* value increase from 72.73 to 75.43; the changes in the *a* value were smaller and ranged between 2.08 to 2.32. The storage at -20°C had less significant effects on the *L* value, because after 90 days of storage at -20°C, the *L* value of the control group was 67.44, whereas the *L* value of the 400 ppm and 800 ppm-treated groups were 69.45 and 72.10, respectively. Regarding the *a* value, it increased in the control group, whereas it did not significantly change in the 400 ppm and 800 ppm-treated groups. [23] reported that the patties with added green tea extract powder had delay the change of color in during storage.

Regarding the difference in the pH value, the meatballs to which 400 ppm and 800 ppm of catechin was added displayed pH values of 6.29 and 6.22, respectively, which were both lower than the value of the control group (6.34). After storage at 4°C, the pH value of all groups declined as the storage time increased. The pH value of catechin-treated groups exhibited a lower level of decline, with the 800 ppm-treated group showing a 6.07 pH value after 15 days of storage, compared to 6.03 of the control group. No significant difference was found between the control and catechin-treated groups after 90 days of storage at -20°C, with pH values ranging between 6.34 and 6.37.

3.2. Texture Profile Analysis and Sensory Analysis

The effects of adding different concentrations of catechin (0, 400, and 800 ppm) to the texture profile of meatballs are shown in Table 2. Adding 400 ppm of catechin did not significantly affect the hardness, springiness, cohesiveness, gumminess, and chewiness of meatballs. However, when the catechin amount increased to 800 ppm, the gumminess and chewiness of the meatballs elevated from 741.5 to 811.8 and from 615.3 to 678.7, respectively.

Table 1. Changes of Color and pH Value in Meatballs Added with Catechin during Storage at Refrigerated (4°C) and Frozen (-20°C) (Mean ± SD, n = 10)

	Catechin addition (ppm)	Initial	Storage at 4°C					Storage at -20°C	
		0 day	3 days	6 days	9 days	12 days	15 days	30 days	90 days
L	0	69.4 ± 1.0^{c}	71.8 ± 0.6^{b}	72.5 ± 0.8^{b}	71.7 ± 0.7^{c}	71.0 ± 0.4^{b}	72.0 ± 0.7^{b}	68.4 ± 0.5^{b}	67.4 ± 1.1^{c}
	400	71.3 ± 0.8^{b}	74.5 ± 0.7^{a}	75.0 ± 0.8^{a}	74.2 ± 0.5^{b}	74.3 ± 0.9^{a}	75.6 ± 0.5^{a}	71.8 ± 1.9^{a}	69.5 ± 0.7^{b}
	800	72.7 ± 0.9^{a}	74.2 ± 0.7^{a}	74.9 ± 0.4^{a}	75.4 ± 0.9^{a}	74.9 ± 1.1^{a}	75.4 ± 0.8^{a}	72.3 ± 0.5^{a}	72.1 ± 1.1^{a}
a	0	3.2 ± 0.3^{a}	3.7 ± 0.1^{a}	3.3 ± 0.3^{a}	3.4 ± 0.2^{a}	3.6 ± 0.4^{a}	3.4 ± 0.3^{a}	3.5 ± 0.1^{a}	3.8 ± 0.5^{a}
	400	2.1 ± 0.2^{b}	2.1 ± 0.2^{b}	2.1 ± 0.2^{b}	1.7 ± 0.2^{b}	1.9 ± 0.2^{c}	1.7 ± 0.2^{c}	2.2 ± 0.3^{b}	2.1 ± 0.3^{b}
	800	2.1 ± 0.2^{b}	2.2 ± 0.2^{b}	2.3 ± 0.1^{b}	2.2 ± 0.3^{c}	2.3 ± 0.3^{b}	2.2 ± 0.3^{b}	2.3 ± 0.2^{b}	2.2 ± 0.3^{b}
b	0	9.2 ± 0.5^{a}	10.0 ± 0.4^{a}	10.3 ± 0.4^{a}	9.8 ± 0.5^{a}	10.6 ± 0.5^{a}	10.5 ± 0.9^{a}	9.8 ± 0.7^{a}	10.2 ± 0.8^{a}
	400	8.3 ± 0.6^{b}	9.0 ± 0.4^{b}	9.2 ± 0.4^{c}	9.3 ± 0.4^{b}	10.1 ± 0.4^{b}	9.8 ± 0.5^{b}	8.9 ± 0.5^{b}	8.5 ± 0.7^{b}
	800	9.3 ± 0.2^{a}	9.8 ± 0.4^{a}	9.9 ± 0.3^{b}	10.0 ± 0.6^{a}	10.1 ± 0.5^{b}	10.1 ± 0.4^{ab}	10.1 ± 0.5^{a}	9.7 ± 0.8^{a}
pH	0	6.3 ± 0.0^{a}	6.3 ± 0.0^{a}	6.3 ± 0.0^{a}	6.1 ± 0.0^{a}	6.0 ± 0.0^{a}	6.0 ± 0.0^{a}	6.4 ± 0.0^{a}	6.4 ± 0.0^{a}
	400	6.3 ± 0.0^{a}	6.3 ± 0.0^{a}	6.2 ± 0.0^{a}	6.2 ± 0.0^{a}	6.2 ± 0.0^{a}	6.1 ± 0.0^{a}	6.4 ± 0.0^{a}	6.4 ± 0.0^{a}
	800	6.2 ± 0.0^{a}	6.3 ± 0.0^{a}	6.3 ± 0.0^{a}	6.2 ± 0.0^{a}	6.1 ± 0.0^{a}	6.1 ± 0.0^{a}	6.4 ± 0.0^{a}	6.4 ± 0.0^{b}

Means with different superscript letters within the same column are significantly different at $p < 0.05$.

Table 2. The Texture Properties (TPA) of Meatballs Added with Catechin (Mean ± SD, n = 10)

Catechin addition (ppm)	Hardness (g)	Springiness	Cohesiveness	Gumminess (g)	Chewiness (g)
0	1118.6 ± 68.2^{a}	0.83 ± 0.01^{ab}	0.66 ± 0.02^{b}	741.5 ± 42.9^{b}	615.3 ± 39.2^{b}
400	1129.8 ± 25.2^{a}	0.82 ± 0.01^{b}	0.67 ± 0.03^{ab}	758.0 ± 49.6^{ab}	624.0 ± 37.3^{b}
800	1166.4 ± 86.8^{a}	0.84 ± 0.01^{a}	0.70 ± 0.01^{a}	811.8 ± 57.4^{a}	678.7 ± 48.7^{a}

Means with different superscript letters within the same column are significantly different at $p < 0.05$.

Sensory analysis was then performed on catechin-added meatballs to determine its effect on texture and flavor. Results are shown in Table 3. Adding 400 ppm and 800 ppm of catechin did not display significant differences on each of the sensory properties when compared to the control group ($p > 0.05$) because all items received scores of 7. [23] conducted evaluation tests involving odor, taste, and tenderness on pork pies with 0.1% added freeze-dried green tea extract powder, and found that the resulting scores did not display significant differences compared to the control group. [26] discovered that adding 200 ppm and 400 ppm of catechin in minced beef gravy and minced chicken gravy did not significantly affect the flavor, taste, and tenderness of the meat. Therefore, adding catechin did not significantly influence the sensory properties of meatballs.

3.3. Microbial Status of Catechin-added Meatballs

Table 4 shows the total plate count, Coliforms, and *E. coli* of catechin-added meatballs. At day 0, the total plate count in all groups ranged from 2.38 to 2.45 log10 CFU/g meatball; no significant difference was displayed among the groups ($p > 0.05$). This indicated adding catechin did not affect the sanitary quality of meatballs. After storage at 4°C, the total plate count of all groups increased along with storage time. After 3 days of storage, the total plate count of both catechin-treated groups was lower than the control group. Maqsood et al. [27] discovered that adding 200 ppm of catechin in camel meat could retard microbial growth. however, after 9 days of storage, the total plate count of all groups increased rapidly. By the 15 days of storage, the 400 ppm and 800 ppm-treated groups displayed a total plate count of 5.51 and 6.52 log10 CFU/g meatball, respectively, and a Coliforms of 2.29 and 1.15 log10 CFU/g meatball, respectively. Both of these tested items were higher than that of the control group (total plate count 4.95 log10 CFU/g meatball, Coliforms < 1 log10 CFU/g meatball). Regarding the storage at -20°C, after 90 days of storage, the meatballs with 400 and 800 ppm of added catechin displayed no increase in the total plate count, because the results were 2.46 and 2.62 log10 CFU/g meatball respectively, compared to a lower value of 3.40 log10 CFU/g meatball of the control group. The Coliforms and *E. coli* test results for all three groups were smaller than 1 log10 CFU/g meatball.

3.4. Antioxidant Activity of Catechin-added Meatballs

In recent years many studies have indicated that catechin possesses excellent 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical scavenging effects [10,11,12]. In addition, its antioxidant effects are superior to those of commercially used synthetic antioxidants. Shahidi and Alexander [12] discovered that catechin delivered better antioxidant effects compared to the same dose of α-tocopherol and BHT.

Table 3. The Sensory Evaluation of Meatballs Added with Catechin (Mean ± SD, n = 30)

Catechin addition (ppm)	Appearance	Taste	Texture	Flavour	Overall
0	7.0 ± 0.9^{a}	7.2 ± 0.9^{a}	7.1 ± 0.7^{a}	7.3 ± 0.8^{a}	7.1 ± 0.8^{a}
400	7.1 ± 1.2^{a}	7.2 ± 1.2^{a}	7.3 ± 1.1^{a}	7.1 ± 1.0^{a}	7.3 ± 1.2^{a}
800	7.1 ± 1.3^{a}	7.1 ± 0.8^{a}	7.0 ± 1.8^{a}	7.1 ± 0.9^{a}	7.1 ± 1.2^{a}

Means with different superscript letters within the same column are significantly different at $p < 0.05$.

Table 4. The Total Plate Count, Coliforms and *E. coli* of Meatballs Added with Catechin during Storage at Refrigerated (4°C) and Frozen (-20°C) (Mean ± SD, n = 3)

	Catechin addition (ppm)	Plate count (LogCFU / g)							
		Initial	Storage at 4°C					Storage at -20°C	
		0 day	3 days	6 days	9 days	12 days	15 days	30 days	90 days
Total plate count	0	2.5 ± 0.1^{a}	3.7 ± 0.1^{a}	3.6 ± 0.1^{a}	3.6 ± 0.1^{a}	4.3 ± 0.1^{b}	5.0 ± 0.0^{c}	3.1 ± 0.0^{a}	3.4 ± 0.1^{a}
	400	2.4 ± 0.2^{a}	2.5 ± 0.1^{b}	3.5 ± 0.2^{a}	3.7 ± 0.4^{a}	4.8 ± 0.1^{b}	5.5 ± 0.2^{b}	3.0 ± 0.1^{a}	2.6 ± 0.3^{b}
	800	2.4 ± 0.0^{a}	2.7 ± 0.2^{b}	3.7 ± 0.2^{a}	3.6 ± 0.4^{a}	5.6 ± 0.1^{a}	6.5 ± 0.0^{a}	2.5 ± 0.1^{b}	2.5 ± 0.5^{b}
Coliforms	0	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
	400	< 1	< 1	< 1	< 1	< 1	2.3 ± 0.3^{a}	< 1	< 1
	800	< 1	< 1	< 1	< 1	2.2 ± 0.1	1.2 ± 0.2^{b}	< 1	< 1
E. coli	0	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
	400	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
	800	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1

Means with different superscript letters within the same column are significantly different at $p < 0.05$.

Table 5. The Scavenging $DPPH^{+}$ Free Radical Ability of Meatballs Added with Catechin during Storage at Refrigerated (4°C) and Frozen (-20°C) (Mean ± SD, n = 3)

Catechin addition (ppm)	Scavenging $DPPH^{+}$ free radical ability (mg as ascorbic acid / g)							
	Initial	Storage at 4°C					Storage at -20°C	
	0 day	3 days	6 days	9 days	12 days	15 days	30 days	90 days
0	0.08 ± 0.00^{c}	0.09 ± 0.01^{c}	0.07 ± 0.00^{c}	0.07 ± 0.01^{c}	0.09 ± 0.01^{c}	0.07 ± 0.00^{c}	0.10 ± 0.01^{c}	0.11 ± 0.00^{c}
400	1.01 ± 0.03^{b}	0.89 ± 0.01^{b}	0.95 ± 0.04^{b}	0.95 ± 0.02^{b}	1.20 ± 0.02^{b}	1.31 ± 0.02^{b}	1.46 ± 0.03^{b}	1.37 ± 0.07^{b}
800	2.13 ± 0.03^{a}	2.59 ± 0.01^{a}	2.39 ± 0.05^{a}	2.35 ± 0.12^{a}	1.96 ± 0.08^{a}	1.97 ± 0.12^{a}	2.62 ± 0.02^{a}	2.69 ± 0.06^{a}

Means with different superscript letters within the same column are significantly different at $p < 0.05$.

Table 6. The Total Antioxidant Capacity of Meatballs Added with Catechin during Storage at Refrigerated (4℃) and Frozen (-20℃) (Mean ± SD, n = 3)

Catechin addition (ppm)	Scavenging $ABTS^+$ free radical ability (mg as Trolox / g)							
	Initial	Storage at 4°C(days)					Storage at -20°C(days)	
	0 day	3 days	6 days	9 days	12 days	15 days	30 days	90 days
0	0.52 ± 0.00^c	0.41 ± 0.01^c	0.34 ± 0.00^c	0.35 ± 0.01^c	0.36 ± 0.01^c	0.36 ± 0.00^c	0.47 ± 0.04^c	0.36 ± 0.01^c
400	3.65 ± 0.16^b	2.34 ± 0.06^b	2.12 ± 0.10^b	2.15 ± 0.04^b	2.75 ± 0.12^b	2.63 ± 0.04^b	3.24 ± 0.12^b	3.58 ± 0.27^b
800	5.86 ± 0.20^a	5.69 ± 0.39^a	4.52 ± 0.09^a	4.52 ± 0.16^a	4.23 ± 0.22^a	4.53 ± 0.08^a	5.73 ± 0.27^a	5.21 ± 0.08^a

Means with different superscript letters within the same column are significantly different at $p < 0.05$.

The DPPH radical scavenging capacity of catechin-added meatballs during their storage period is shown in Table 5. and the total antioxidant capacity is shown in Table 6. The DPPH radical scavenging capacity of meatballs with 400 ppm and 800 ppm of added catechin were between 0.89 to approximately 1.31 and between 1.96 to approximately 2.59 mg as ascorbic acid/g, respectively, during the storage period, with values of 1.31 and 1.97 mg as ascorbic acid/g, respectively, at 15 days of storage at 4°C. The total antioxidant capacity declined during the storage period, with values being 2.63 and 4.53 mg of Trolox/g, respectively, after 15 days of storage at 4°C. However, both values were still significantly higher than the control group (0.36 mg as Trolox/g). After 90 days of storage at -20°C, the DPPH radical scavenging capacity of meatballs with 400 ppm and 800 ppm of added catechin were 3.58 and 5.21 mg of Trolox/g, respectively. Both of these were significantly higher than the control group and also higher than the scavenging capacity after 15 days of storage at 4°C. [23] discovered that after adding 0.1% freeze-dried green tea extract powder to both raw and cooked pork pies, the radical scavenging capacity of pork pies was higher than the control group. [28] indicated that tea extract contained antioxidants such as catechin; when added to meat paste and fish paste, it could produce antioxidant effects which were superior to those of α-tocopherol.

3.5. Lipid Oxidative Stability of Catechin-added Meatballs

Figure 1 shows the effects of adding catechin to meatballs on the lipid oxidation levels during storage. Meatballs with 400 and 800 ppm added catechin displayed a TBARS value as 0.16 and 0.07 mg MDA eq./kg. respectively on day 0, which were significantly lower than the control group (0.51 mg MDA eq./kg). The likely reason is that catechin starts exerting antioxidant effects on the meat and prevents lipid oxidation during meat processing. After 15 days of storage at 4°C, the TBARS value of the control group was 2.46 mg MDA were 0.56 and 0.31 mg MDA eq./kg, which were both significantly lower than those of the control group. The storage at -20°C also displayed similar effects, because after 90 days of storage, the TBARS value was lower in the catechin-treated groups when compared to the control group, with the 400 ppm and 800 ppm-treated groups displaying a TBARS value as 0.60 and 0.25 mg MDA eq./kg, respectively, and the control group yielding a TBARS value as 1.39 mg MDA eq./kg. Therefore, it can be concluded that catechin significantly prevents lipid oxidation in meatballs. [29] discovered that after adding 0.25% of tea polyphenols in pork pies, the TBARS value was lower compared to the control group, regardless of whether the pork pies were stored directly in a refrigerator, or frozen before refrigerator storage. Jo et al. [23] also obtained similar results, pointing out that green tea extract powder prevented both raw and cooked meat from oxidation and rancidity during 15 days of storage at 4°C. Mitsumoto et al. [26] also reported that adding 200 ppm and 400 ppm of catechin in minced beef gravy and minced chicken gravy could effectively reduce lipid oxidation, and that catechin's effects surpassed those of vitamin C. The same result (using 200 ppm catechin) was found in camel meat [27]. Similarly, McCarthy et al. [29] also found that adding 0-1% of tea catechin in frozen pork could reduce lipid oxidation at held under chilled (4°C) for 10 days.

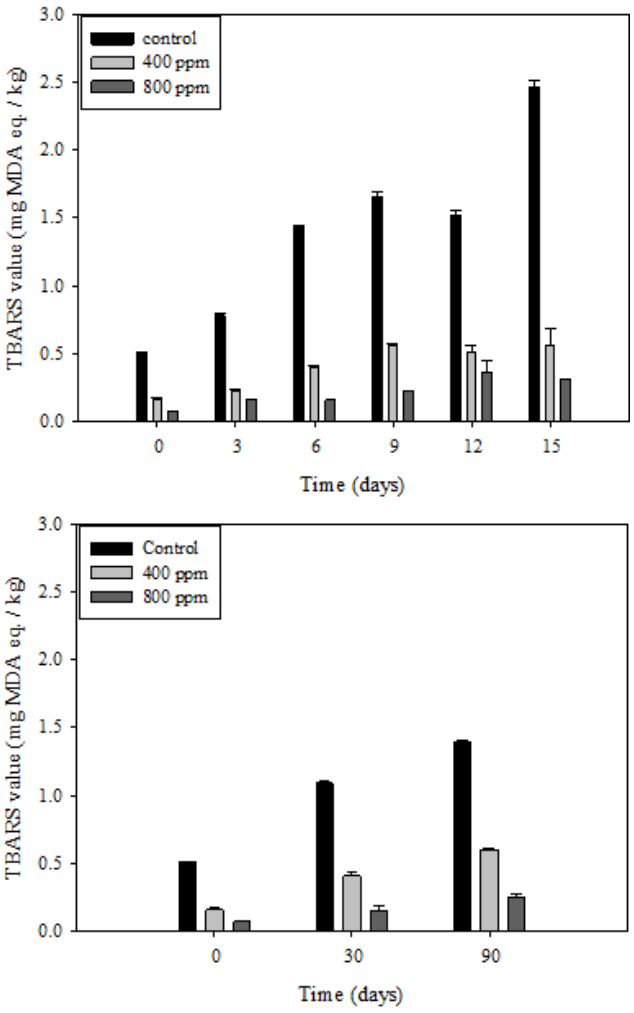

Figure 1. Effect of catechin on lipid oxidation (TBARS value) in meatballs during (a) refrigerated storage at 4°C and (b)frozen storage at -20°C. —: no addition, —:added 400ppm catechin, —: added 800ppm catechin. (Mean ± SD, n = 3)

In conclusion, after storage at low temperature, catechin added to emulsified pork meatballs still possessed excellent antioxidant capabilities. This is likely to be the reason for catechin to prevent lipid oxidation in meatballs stored at low temperature. In addition, catechin, as an additive, effectively lengthens the shelf life of meat products with high fat content [3].

Acknowledgments

The authors would like to thank the Ministry of Economic Affairs of Taiwan for the financial support of this research (99-EC-17-A-03-04-0332).

References

[1] Morrissey, P.A., Sheehy, P.J.A., Galvin A.K., Kerry J.P. and Buckley, D.J., "Lipid stability in meat and meat products," *Meat Science*, 49(1).73-86. 1998.

[2] Gray, J.I., Gomaa, E.A. and Buckley, D.J, "Oxidative quality and shelf-life of meat," *Meat Science*, 43. 111-123. 1996.

[3] Buck, D.F., Edwards, M.K, "Anti-oxidants to prolong shelf life," *Food Science and Technology International*, 2.29-33. 1997.

[4] Duan, S., Weng, X.C., Dong, X.W., Lin, Y.P., Li, H.P. and Jin, J.R, "Antioxidant properties of butylated hydroxytoluene refluxed in ferric chloride solution," *Food Chemistry*, 61.101-105. 1998.

[5] Wanasundara, U.N. and Shahidi, F.C, "Antioxidant and pro-oxidant activity of green tea extract in marine oils," *Food Chemistry*, 63. 335-342. 1998.

[6] Branen, A.C, "Toxicology and biochemistry of butylated hydroxyanisole and butylated and butylated hydroxytoluene," *Journal of the American Oil Chemists' Society*, 52.59-63. 1975.

[7] Hettiarachchy, N.S., Glenn, K.C., Gnanaesbandam, R. and Johnson, M.G, "Natural antioxidant extracts from fenugreek (Trigonella foenumgraecum) for ground beef patties," *Journal of Food Science*, 61.516-519. 1996.

[8] Marshall, W.E, "Health foods, organic foods, natural foods," *Food Technology*, 28.50-56. 1994.

[9] Osada, K., Takahashi, M., Hoshina, S., Nakamura, M., Nakamura, S. and Sugano, M, "Tea catechins inhibit cholesterol oxidation accompanying oxidation of low density lipoprotein in vitro," *Comparative Biochemistry and Physiology Part C*, 128. 153-164. 2001.

[10] Terao, J., Piskula, M. and Yao, Q, "Protective effect of epicatechin, epicatechin gallate, and quercetin on lipid peroxidation in phospholipid bilayers," *Archives of Biochemistry and Biophysics*, 308. 278-284. 1994.

[11] Wiseman, S.A., Balentine, D.A. and Frei, B, "Antioxidants in tea," *Critical Reviews in Food Science and Nutrition*, 37(8). 705-718. 1997.

[12] Shahidi, F. and Alexander, D.M, "Green tea catechins as inhibitors of oxidation of meat lipids," *Journal of Food Lipids*, 5. 125-133. 1998.

[13] Hara, Y, *Prophylactic function of tea polyphenols*, In: Food Phytochemicals for Cancer Prevention II, Washington, DC., USA, 34-50. 1994.

[14] Wang, S.M. and Zhao, J.F, "Antioxidant activities of tea polyphenol on edible oil," *Western Cereal and Oil Technology*. 22. 44-46. 1997.

[15] Cao, D., Qiu, A. and Jin, Z, "Tea antioxidant and its by-products utilization," *J. Wuxi Inst. Light Ind*, 13.218-225. 1994.

[16] Huang, S.W. and Frankel, E.N, "Antioxidant activity of tea catechin in different lipid systems," *Journal of Agricultural and Food Chemistry*, 45. 3033-3038. 1997.

[17] Tang, S., Kerry, J.P., Sheehan, D. and Buckley, D.J, "A comparative study of tea catechins and α-tocopherol as antioxidants in cooked beef and chicken meat," *European Food Research and Technology*, 213. 286-289. 2001.

[18] Tang, S., Sheehan, D., Buckley, D.J., Morrissey, P.A. and Kerry, J.P, "Anti-oxidant activity of added tea catechins on lipid oxidation of raw minced red meat, poultry and fish muscle," *International Journal of Food Science & Technology*, 36. 685-692. 2001.

[19] Huang S.C., Shiau, C. Y., Liu, T.E., Chu, C. L. and Hwang, D.F. "Effects of rice bran on sensory and physico-chemical properties of emulsified pork meatballs," Meat Science, 70. 613-619. 2005.

[20] Meilgaard, M., Civille G.V., and Carr. B.T, *Sensory evaluation techniques*, 2nd Ed, CRC Press, USA, 1991.

[21] AOAC, *AOAC Official Method 990.12 for Aerobic Plate Count in Foods, Official methods of analysis*, 14th Ed, DC: Association of Official Analytical Chemists, Wshington, DC., 1995.

[22] AOAC, *AOAC Official Method 991.14 for Coliform and Escherichia coli Counts in Foods, Official methods of analysis*, 14th Ed, DC: Association of Official Analytical Chemists, Washington, DC., 1995.

[23] Jo, C., Son J.H., Son C.B. and Byun M.W, "Functional properties of raw and cooked pork patties with added irradiated, freeze-dried green tea leaf extract powder during storage at 4°C," *Meat Science*, 64.13-17. 2003.

[24] Roberta, R., Nicoletta, P., Proteggente, A., Ananth, P., Yang, M. and Rice-Evans, C, "Antioxidant activity applying an improved abts radical cation decolorization assay" *Free Radical Biology and Medicine*, 26.1231-1237. 1999.

[25] Buege, J.A. and Aust, S.D, "Microsomal lipid peroxidation," *Methods in Enzymology*, 52.302-310. 1978.

[26] Mitsumoto, M., Michael, N.O., Joe, P.K. and Buckley, D.J, "Addition of tea catechins and vitamin C on sensory evaluation, colour and lipid stability during chilled storage in cooked or raw beef and chicken patties," *Meat Science*, 69.773-779. 2005.

[27] Maqsood, S., Abushelaibi, A. Manheem, K. and Rashedi, A.A. "Lipid oxidation, protein degradation, microbial and sensorial quality of camel meat as influenced by phenolic compounds," *Food Science and Technology*, 63. 953-959. 2015.

[28] Shuze T., Sheehan, D., Buckley, D.J., Korrissey, P.A. and Kerry, P.J, "Antioxidant activity of added tea catechins on lipid oxidation of raw minced red meat, poultry and fish muscle," *International Journal of Food Science & Technology*, 36(6). 685-692. 2001.

[29] McCarthy, T.L., Kerry, J.P., Kerry, J.F., Lynch, P.B. and Buckley, D.J, "Assessment of the antioxidant potential of natural of natural food and plant extracts in fresh and previously frozen pork patties," *Meat Science*, 57.177-184. 2001.