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Effect of Paprika (*Capsicum annuum* L.) on Inhibition of Lipid Oxidation in Lard-Pork Model System During Storage at 4°C

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Abstract This study was conducted to investigate the antioxidant activity of paprika in the lard-pork model system adding ground fresh paprika (3%) and paprika powders (5%). Paprika powders were obtained through 4 drying methods (freeze, vacuum, far infrared-ray, and hot-air). In the lard and meat-fat mixture (containing lard 30%) containing paprika powders, the rate of increase in the peroxide value (POV) and thiobarbituric acid (TBA) value decreased notably during the refrigerated storage (4°C) compared to the control without paprika. Therefore, paprika powders showed potent antioxidant activity and especially the freeze dried paprika powder revealed the most effective activity among them. However, its antioxidant activity was still lower than that of the fresh paprika because the addition of fresh paprika in the lard and meat-fat mixture merely increased the POV and TBA value. In linoleic acid oxidation, the addition of capsanthin 500 ppm to mixed linoleic acid and 10 ppm of $FeCl_3$ (LF) inhibited the formation of peroxides by 15.2% compared to LF, showing its iron scavenging ability. When mixed antioxidants (β-carotene 200 ppm + ascorbic acid 100 ppm, capsanthin 200 ppm + ascorbic acid 100 ppm) were added in LF, synergistic effects were obtained with 57.7 and 60.4% of inhibition of peroxide formation, respectively.

Keywords: paprika, antioxidant, lipid oxidation, iron scavenging activity, lard-pork model system

Introduction

The lipid oxidation in meat products occurs from autoxidation by radical reaction. The lipid oxidation is accelerated by temperature, light, radiation, peroxides, lipoxidase, organic metal compound, and micro metal catalyst (1). Lipid oxidation of meat products causes deterioration in flavor, color, texture, and nutrition. Lipid oxidation products are potentially toxic to humans and animals (2, 3). Oxidized cholesterol and lipid peroxides accelerate tumor generation, whereas malonaldehyde a secondary oxidation product facilitates mutation and the formation of N-nitrosamine (4). In particular, the lipid oxidation of pork progresses at a faster speed than in beef and mutton, because pork contains a large amount of unsaturated fatty acid in comparison with other meat products (5). Pork meat is a rich source of iron (6) that is the major catalyst for oxidative rancidity in meat (7). According to epidemiological evidence, people that prefer red meat to white meat were exposed to a greater risk of colorectal cancer (8). Therefore, it is necessary to scavenge iron from pork products to improve health-related functions of the products.

In the meat processing industry, phenolic compounds, tocopherol, plant derivatives, and chelating agents were added to raw and cooked pork products to inhibit lipid oxidation (9, 10).

Paprika (*Capsicum annuum* L.) is a rich source of carotenoids. Capsanthin and capsorubin that contain one or two keto groups are its main compounds (11). Besides, phenolic compounds, tocopherols, and ascorbic acid are abundantly contained in the flesh pericarp and seeds of paprika (12-14). Paprika's redox potential is low (15) and thus its high antioxidant capability can be expected. It will be possible to use paprika as a natural antioxidant in food because of its safety for humans (16).

In pork sausage that contains fresh paprika (3%) and garlic (1%), paprika was superior to garlic in inhibiting lipid oxidation (17). Similarly, Sanchez-Escalante *et al.* (18) reported that beef patties containing paprika were superior to those with lycopene-rich tomato in inhibiting lipid oxidation. Furthermore, radical scavenging ability of capsanthin was more effective and long lasting than that of β-carotene (19).

This study was to evaluate the antioxidant effectiveness of paprika in the lard-pork model system during storage at 4°C and the iron scavenging ability of capsanthin and its synergism with other antioxidants in the linoleic acid model system. Paprika powders prepared by different drying methods were used to compare their antioxidant activities. Therefore, the paprika which is less commercially valued could be utilized in the form of powder as a natural antioxidant.

Materials and Methods

Preparation of paprika powder Fresh paprika was purchased locally at a farm in Haman, Korea. Its seed part was discarded. The rest of paprika was divided into the four, then sliced 3 mm thick by the food processor (Rondo 2500; Tefal, Burgundy, France) and dried until about 14% of final moisture content by the following drying methods: a freeze drier (Bondiro; Ilshin Lab Co., Ltd., Gyeonggi, Korea), a vacuum drying oven, a far infrared-ray drying oven (SLD-1400S; Cilic, Gyeonggi, Korea), and a hot-air drying oven (FO-600M; Jeio Tech., Daejeon, Korea). The drying conditions of vacuum oven and freeze drier were 40°C at 20 mmHg and -45°C at 50 mmHg, respectively. Far-infrared ray drying (FIRD) and hot-air drying (HD) were carried out at 50°C air temperature and 1.2 m/sec air

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velocities. Finally, the dried paprika was ground by a mill (SQ-107; Ilzinjunggong Co., Gyeonggi, Korea) to pass through 0.85 mm sieve.

Preparation of lard-pork model system The shoulder loin of individual porks were purchased at 24 hr post slaughter from a local supplier and trimmed of external fat. Separated pork fat (lard) or pork meat without fat was ground using food processor. For the lard samples, ground lard was mixed with 3% of ground fresh paprika or 5% of paprika powder by the food processor for 3 min. For the meat-fat mixture samples, ground meat and lard (70 and 30%, respectively) were mixed using the food processor, and then added with the paprika as the same way of the ground lard samples. The prepared ground lard or meat-fat mixture (approximately 30 g each) was introduced in a pouch made of oxygen permeable (52.6 mL/m^2·24 hr) nylon film, and then stored at 4°C for 8 days to measure pH and lipid oxidation every 2 day of storage.

pH measurements The pH of lard and meat-fat mixture was measured after being mixed with 5 g of sample and 20 mL of distilled water and being homogenized for 1 min at 26,000×g using the homogenizer (Ultra-Turrax; Ika Werke, Staufen, Germany) according to Kim *et al.* (20).

Measurement of lipid oxidation Fat was extracted by Folch's method (21) and peroxide value of the samples was determined by the AOCS Official Method (22). Thiobarbituric acid (TBA) value of the samples was determined according to Tarladgis *et al.* (23) and expressed as mg of malonaldehyde per kg of meat. The fatty acid composition of meat-fat mixture samples was analyzed using GC/MS (GC/MSD 5973 series; Hewlett-Packard, Palo Alto, CA, USA) on the storage day 0 and 8. The analytic column was used with HP-INNOWAX (30 × 0.32 i.d. × 0.5 μm), and the linear speed of He, the carrier gas, was adjusted to 1.0 mL/min. The oven temperature was kept at 180°C for 3 min and then was raised to 220°C by the rate of 6°C/1 min, which was kept for 5 min. The conditions of MSD analysis were as follows: capillary direct interface 220°C, ion source 230°C, ionization energy 70 eV, mass range 33-500 automic molecular unit, and electron multiplier voltage 1,500 V. The content of fatty acid was calculated with the relative percentage in each peak area.

Measurement of iron scavenging ability Based on the method of Lee and Cheigh (24), 1 g of linoleic acid was added with 10 ppm of $FeCl_3$. The sample was dissolved in 20 mL of ethanol and then mixed with 25 mL of phosphate buffer solution (0.2 M, pH 7.0). Added antioxidants were dissolved with ethanol or phosphate buffer solution depending on their solubility. Then, 1 mL of it was put into samples containing iron. Next, it was oxidized at 50°C for 48 hr, which was later moved into the separatory funnel. Then small amount of water and 2 g of NaCl was added and extracted 3 times with 25 mL of chloroform. The lower layer was transferred to the Erlenmeyer flask, which was added with 25 mL of acetic acid and 1 mL of saturated KI solution. Then it was shaken for 1 min and left in the dark for 10 min. After that, it was added with 50 mL of distilled water and then was titrated to 0.01 N $Na_2S_2O_3$ using 1% of starch solution as an indicator.

Statistical analysis pH, peroxide value, and TBA value were analyzed with analysis of variance using SPSS statistical program, and the level of significance among samples was tested at $p<0.05$ or $p<0.01$ with Duncan's multiple range test. The results of all data were expressed as the average of triplicate.

Results and Discussion

Change of pH The pH results of the lard and meat-fat mixture that contain fresh paprika or paprika powder are shown in Fig. 1. In both the lard and meat-fat mixture, pH significantly decreased during 8 days of refrigeration period ($p<0.05$). Simply adding paprika, which contains a large amount of organic acid, contributed to the lowering of pH. pH decreased to a greater extent in the meat-fat mixture than in lard because of the action of lactobacilli during storage and dissociation of CO_2 from muscle (25). As paprika powder has a more organic acid content than the fresh paprika containing more than 90% of H_2O, the lard and meat-fat mixture with paprika powder seemed to have a lower pH.

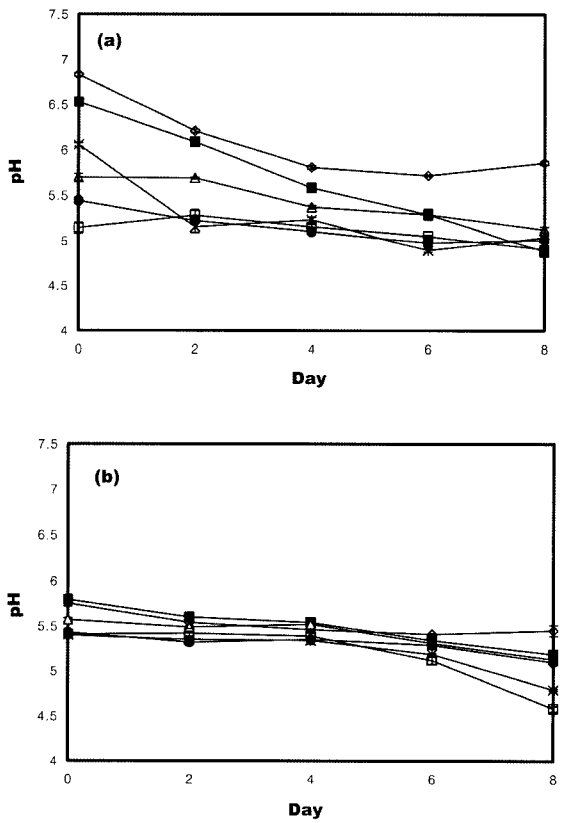

Fig. 1. Changes in pH of ground lard (a) and meat-fat mixture (b) treated with paprika powders using various drying methods during storage at 4°C. ◇ control, ■ FP 3%, △ FDP 5%, □ VDP 5%, * FIDP 5%, ● HDP 5% (FP, fresh paprika; FDP, freeze-dried paprika; VDP, vacuum-dried paprika; FIDP, far-infrared ray dried paprika; HDP, hot-air dried paprika). Values are means±SD, n=3.

Effect of paprika on lipid oxidation of the lard-pork model system The changes in the peroxide value for the ground lard and meat-fat mixture during refrigerated storage are shown in Fig. 2a and 2b, respectively. In the control ground lard sample without paprika, the peroxide formation increased sharply up to 14 meq/kg on day 8. Highly significant differences ($p<0.01$) in the peroxide value were evident between the control ground lard and those treated with the fresh paprika or paprika powders. In the control ground meat-fat mixture without paprika, the peroxide formation was indicated from day 2 along with the various samples with paprika powders and increased during the rest of storage with less marked differences from the samples with paprika than those in lard (Fig. 2b). The peroxide value did not significantly increase both in the lard and in the meat-fat mixture containing FP ($p<0.01$). At the low levels of peroxide formation in the lard and meat-fat mixture in decreasing order are profiles of samples with far-infrared ray dried paprika (FIDP), hot-air dried paprika (HDP), vacuum-dried paprika (VDP), freeze-dried paprika (FDP), and fresh paprika (FP). It appears that the addition of paprika powders inhibited the peroxide formation more effectively in lard than in the meat-fat mixture.

The changes in the TBA value for the ground lard and meat-fat mixture are shown in Fig. 3a and 3b, respectively. The TBA value measured the amount of malonaldehyde in carbonyl compounds, secondary oxidation products. The results of the TBA value showed similar trends to the peroxide value. The control ground lard sample without paprika showed significant increase of malonaldehyde compared to those with paprika ($p<0.05$). The TBA value merely increased, both in the lard and in the meat-fat mixture containing FP just like the peroxide value. All the TBA values for the meat-fat mixture kept being lower than those for the lard samples due to a less amount of fat, but the antioxidant effect of the paprika powders against lipid oxidation showed the same trend as the lard. The lard and the meat-fat mixture with 3% of FP had the best stability

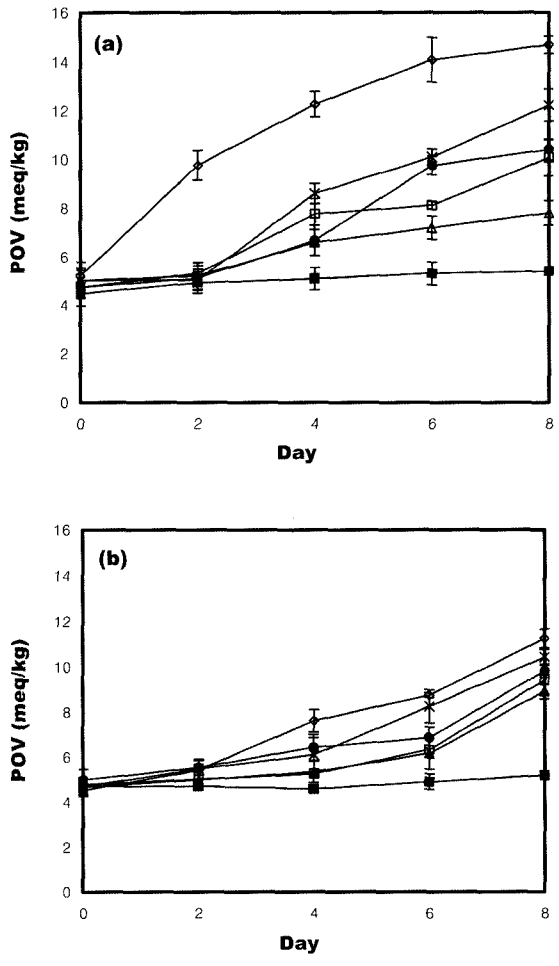

Fig. 2. Changes in peroxide value (POV) of ground lard (a) and meat-fat mixture (b) treated with paprika powders using various drying methods during storage at 4°C. ◇ control, ■ FP 3%, △ FDP 5%, □ VDP 5%, * FIDP 5%, ● HDP 5% (FP, fresh paprika; FDP, freeze-dried paprika; VDP, vacuum-dried paprika; FIDP, far-infrared ray dried paprika; HDP, hot-air dried paprika). Values are means±SD, n=3.

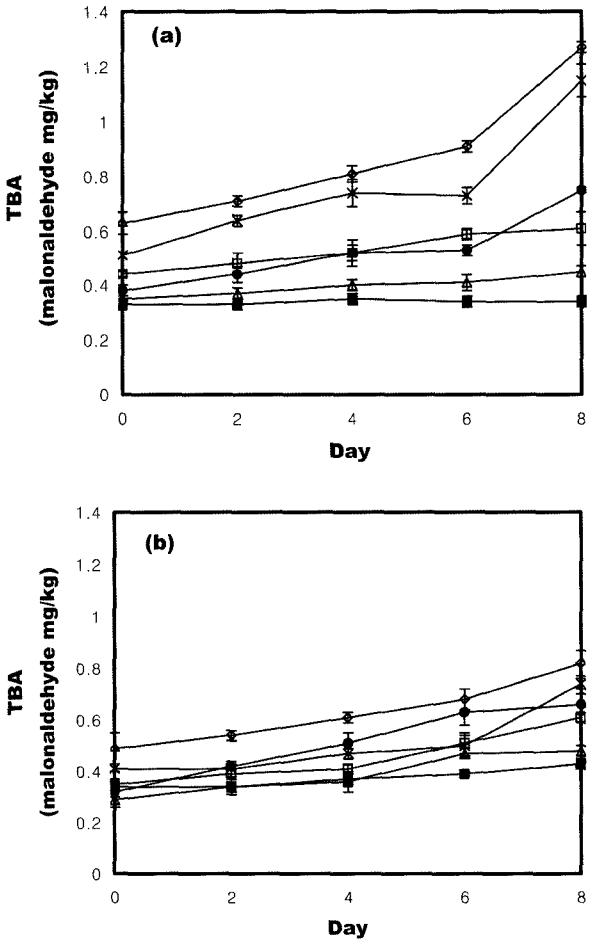

Fig. 3. Changes in thiobarbituric acid (TBA) value of ground lard (a) and meat-fat mixture (b) treated with paprika powders using various drying methods during storage at 4°C. ◇ control, ■ FP 3%, △ FDP 5%, □ VDP 5%, * FIDP 5%, ● HDP 5% (FP, fresh paprika; FDP, freeze-dried paprika; VDP, vacuum-dried paprika; FIDP, far-infrared ray dried paprika; HDP, hot-air dried paprika). Values are means±SD, n=3.

Table 1. Changes in fatty acid composition of meat-fat mixture treated with paprika powders using various drying methods during storage at 4°C[1)]

(%)

Fatty acid composition	Control		FP		FDP		VDP		FIDP		HDP	
	0	8	0	8	0	8	0	8	0	8	0	8 (day)
Myristic acid	1.97±0.01	2.06±0.04	1.82±0.10	1.92±0.02	1.63±0.03	1.53±0.01	1.62±0.02	1.67±0.02	1.56±0.02	1.73±0.03	1.60±0.01	1.88±0.03
Palmitic acid	23.92±0.03	25.25±0.01	24.31±0.00	24.32±0.00	22.15±0.00	22.44±0.08	20.92±0.07	24.17±0.01	22.78±0.07	24.24±0.15	22.11±0.03	23.95±0.03
Palmitoleic acid	3.23±0.01	1.32±0.15	3.12±0.01	2.84±0.01	2.82±0.01	2.77±0.07	3.02±0.01	2.66±0.11	2.77±0.07	2.62±0.38	2.78±0.01	3.10±0.04
Stearic acid	13.76±0.06	13.63±0.02	13.56±0.02	12.43±0.12	14.98±0.02	13.18±0.01	12.61±0.00	13.39±0.52	12.51±0.02	12.62±0.06	13.74±0.17	14.29±0.01
Oleic acid	40.02±0.01	39.76±0.02	39.65±0.03	41.45±0.01	39.18±0.01	41.34±0.02	41.32±0.01	40.50±0.01	41.44±0.02	40.30±0.00	41.37±0.01	39.08±0.01
Linoleic acid	13.69±0.03	12.97±0.02	14.22±0.01	14.16±0.01	14.31±0.01	14.85±0.00	14.88±0.06	14.28±0.03	14.84±0.08	14.42±0.33	14.81±0.09	13.87±0.01
Linolenic acid	0.79±0.03	0.72±0.01	0.82±0.02	0.72±0.00	0.97±0.00	0.91±0.01	0.93±0.08	0.95±0.04	0.91±0.01	0.94±0.01	1.15±0.03	1.02±0.01

[1)]FP, fresh paprika; FDP, freeze-dried paprika; VDP, vacuum-dried paprika; FIDP, far-infrared ray dried paprika; HDP, hot-air dried paprika. Values are means±SD, n=3.

against lipid oxidation. In our previous work (26), FP contained much higher amounts in total carotenoid, capsanthin, ascorbic acid, and total polyphenol than dried paprika powders (FDP, VDP, FIDP, and HDP). The retentions of those antioxidant compounds were different among the paprika powders. It was found that a higher retention of antioxidant compounds was obtained by freeze drying operating at low temperature in the absence of air, than the other drying methods. Therefore, FP and FDP containing more amounts of antioxidant compounds could act as effective natural antioxidants inhibiting lipid oxidation in the lard-pork model system. When paprika powder of 0.1, 0.5, and 2%, respectively, was added to extend a shelf-life of the pork sausage, the rate of increase in the TBA value was reduced as the amount of the paprika powder was increased (27). Matsufuji *et al.* (19) reported that the capsanthin of paprika had a radical scavenging ability by measuring the free radical oxidation of methyl linoleate. Likewise, our results suggested that formations of peroxides and malonaldehydes were inhibited by adding paprika, acting as a radical scavenger in the lard-pork model system.

Change of fatty acid compositions Table 1 showed the change of fatty acid compositions of the ground meat-fat mixture during the refrigerated storage. The control without paprika and samples with VDP, FIDP, and HDP showed a decrease of oleic and linoleic acid and an increase of myristic and palmitic acid during storage, while the ground meat-fat mixture with FP and FDP showed little change in their contents. It was reported that unsaturated fatty acids (UFA), oleic and linoleic acid in goat (28) and chicken muscle (29) decreased and saturated fatty acids (palmitic and stearic acid) increased after storage, indicating severe oxidation, which is in close agreement with our results. In our results, both FP and FDP were very effective in preventing the loss of UFA such as oleic acid, linoleic acid, and linolenic acid relating with a low increase rate in peroxide and the TBA value. This result was similar with that of Shin *et al.* (3) who reported that UFA was little or never destructed in unheated ground pork that was mixed with 0.1% of grape fruit seed extract, 0.5% of carnosine, and 0.2% of rosemary.

Iron scavenging ability of capsanthin As paprika is a complex system, it is difficult to compare the inhibition effect of lipid oxidation by each antioxidant compound in it. Hence, the representative antioxidant compounds in the paprika including capsanthin, ascorbic acid, and β-carotene were added in the linoleic acid model system, and their iron scavenging abilities and antioxidant effects were compared. In addition, when they were mixed, their synergistic effects on the antioxidant activity were examined (Table 2). The peroxide value of mixed linoleic acid and 10 ppm of $FeCl_3$ (LF) was much higher than the single linoleic acid, which indicated the lipid oxidation was accelerated by $FeCl_3$. When LF added with 500 ppm of β-carotene and 500 ppm of ascorbic acid, respectively, the POV increased to 76.07 and 73.80 meq/kg compared with LF (60.83 meq/kg) without them. In the study of Love and Pearson (30), the TBA value increased from 0.35 to 1.35 mg/kg when the cooked meat treated with 1 ppm of $FeSO_4$ was stored for 72 hr at 4°C. The TBA value increased up to 2.35 mg/kg after being mixed with 1 ppm of $FeSO_4$ and 5 ppm of ascorbic acid. This was agreed with our results as ascorbic acid catalyzed prooxidant of Fe^{2+}. However, LF treated with mixed β-carotene 200 ppm and ascorbic acid 100 ppm showed 57.7% of inhibition of peroxide formation. It is important to note that each of β-carotene and ascorbic acid did not show iron scavenging ability, but instead accelerated the formation of peroxides, while the use of their mixed antioxidants showed an excellent iron scavenging ability. The addition of capsanthin (500 ppm) and tocopherol (500 ppm) into LF inhibited the formation of peroxides by 15.2 and 23.2%, respectively, confirming their iron scavenging ability. LF treated with capsanthin 200 ppm and ascorbic acid 100 ppm showed 60.4% of inhibition. This means that the use of mixed antioxidants is effective obtaining a substantially better iron scavenging ability. When α-tocopherol, epicatechin, and zinc were added to the phospholipids containing 25 μM Fe^{2+}, respectively, the inhibition of Fe^{2+}-induced 2-thiobarbituric-reactive substances (TBARS) formation was 42, 69, and 37% in the study of Zago and Oteiza (31). The combined actions of the 3 compounds completely prevented lipid oxidation (96%). This is agreed with our results suggesting the synergistic effect of mixed antioxidants. In fact, paprika exists with complex antioxidant compounds

Table 2. Peroxide value (POV) of linoleic acid treated with different antioxidants after 48 hr of storage at 50°C

Treatments	POV (meq/kg)	Inhibition %[1)]
Linoleic acid	42.73±0.06[2)]	29.76
Linoleic acid + $FeCl_3$	60.83±0.21	0
Linoleic acid + $FeCl_3$ + β-carotene 500 ppm	76.07±0.15	-25.05
Linoleic acid + $FeCl_3$ + ascorbic acid 500 ppm	73.80±0.17	-21.32
Linoleic acid + $FeCl_3$ + capsanthin 500 ppm	51.57±0.06	15.22
Linoleic acid + $FeCl_3$ + α-tocopherol 500 ppm	46.73±0.12	23.18
Linoleic acid + $FeCl_3$ + β-carotene 200 ppm + ascorbic acid 100 ppm	25.73±0.32	57.70
Linoleic acid + $FeCl_3$ + capsanthin 200 ppm + ascorbic acid 100 ppm	24.07±0.06	60.43

[1)] Inhibition % = [1 − {POV (treatment)/POV of linoleic acid +$FeCl_3$ }] × 100
[2)] Values are means±SD, n=3.

and thus could be expected to have good antioxidant effects on the prevention of lipid oxidation in red meat with an iron scavenging ability.

In conclusion, the rate of increase in lipid oxidation in the uncooked lard-pork model system decreased with the addition of 4 types of paprika powders, showing their potent antioxidant activity. FDP was the most effective among them. However, its antioxidant activity was still lower than that of FP. The increase of peroxide and TBA value related with the loss of unsaturated fatty acid in the meat-fat mixture during storage. In linoleic acid oxidation, it was confirmed that the capsanthin in paprika had an iron scavenging ability and synergistic effect when mixed with other antioxidants. It is suggested that the antioxidant effect of paprika will be maximized as the retention of antioxidant compounds in it increases depending on the drying method. Further research might be conducted in the future to find an inhibition of lipid oxidation in cooked meat with the addition of paprika during the storage.

Acknowledgments

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