Changes in FA Composition and Antioxidative Activity of Pigment Extracts from Korean Red Pepper Powder (*Capsicum annuum* L.) Due to Processing Conditions

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ABSTRACT: The FA compositions by parts of Korean red pepper (*Capsicum annuum* L.) were analyzed, and the changes in FA composition and antioxidative activity in pigment extracts due to processing were investigated. The main FA in the pericarp and seeds were palmitic, oleic, and linoleic acids. Linoleic acid in the seeds was 73.9% of the total FA, or about 1.7 times as much as in the pericarp. Linolenic acid was scarcely present in the seeds. The percentages of unsaturated FA in the pericarp and seeds were 73.3 and 83.2, respectively. The ratios among FA varied according to the processing conditions, but the FA compositions themselves remained approximately the same. The antioxidative activity of pigment extracts in the photooxidation of linoleic acid was measured by monitoring oxygen consumption and conjugated diene formation. Changes in drying methods resulted in significant differences in antioxidative activity, but changes in storage conditions had little effect. Conjugated diene formation and oxygen consumption were positively correlated. An improved drying method could allow the antioxidative activity of red pepper pigments to be conserved.

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KEY WORDS: Conjugated diene, drying, fatty acid, oxygen consumption, red pepper, storage.

Ripe fruits of red pepper (*Capsicum annuum* L.) are widely consumed as vegetables and are used as food colorants because they are a good source of the red carotenoids capsanthin and capsorubin. Capsanthin accounts for 30–60% of total carotenoids in fully ripe fruits (1). It contains 11 conjugated double bonds, a conjugated keto group, and a cyclopentane ring, and has stronger antioxidative effects than β-carotene (2). These structural characteristics give rise to singlet oxygen-quenching ability (3) and prevent colon carcinogenesis (4).

The crude FA contents in Korean red pepper powder are about 17.4%, and have been attributed to processing quality deterioration due to rancidity (5,6). FA esterified with capsanthin were mainly lauric, myristic, and palmitic acids rather than oleic and linoleic acids (7). The effects of processing on the FA composition of peppers have hardly been studied, in spite of the possibility of chain reactions such as oxidation involving the pigments. In addition, changes in FA may be affected by the antioxidative activity of capsanthin esterified with FA.

In this study, the FA composition of Korean red pepper was analyzed, and the changes in FA composition under different processing conditions were studied. Second, the changes in the singlet oxygen-quenching ability during processing in red pepper pigment extracts were investigated.

EXPERIMENTAL PROCEDURES

Materials. One hundred kilograms of red pepper (*Capsicum annuum* L., cv. Da-Bok) cultivated in a plastic greenhouse in the Munsan area (Kyungki-do, Korea), was harvested on Febuary 15, 2001. In this study, the drying method and storage temperature and period were varied. In drying method A, each red pepper (50 kg total) was washed, its stem was removed, and it was then cut into three parts and dried at 70°C for 6 h. In drying method B, the method used commercially in Korea, the whole red pepper pods (50 kg) were washed, dried at 80°C for 5 h, followed by further drying at 60°C for 18 h, and then the stems of red pepper were removed. The red peppers dried by methods A and B were separated into pods and seeds. For manufacture of powders, 90% pods and 10% seeds were mixed, powdered, and separated according to particle size by using an 18-mesh sieve and a Ro-Tap sieve shaker (Cheonggesa CG-213, Seoul, Korea). The red pepper powders were sealed in packing material (polyethylene/nylon) and then stored at 0 or 20°C for different time periods (6 mon). The FAME esters and linoleic acid, used as standards, were purchased from Sigma Chemical Co. (St. Louis, MO).

Moisture contents. Moisture content was determined by AOAC method 966.02 (8).

FA analysis. About 2 g red pepper was mixed with 20 mL diethyl ether and extracted for 2 h in a shaking water bath. The residue was evaporated to dryness *in vacuo* at 40°C. For methylation, the residue was dissolved in 5 mL of 0.5 N NaOH/MeOH, reacted in 250°C hot sand for 5 min, and 5 mL BF_3 was added as a catalyst. The above solution was extracted with 5 mL hexane for 2 min and then 15 mL saturated NaCl solution was added. After phase separation, the hexane phase

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was used for the analysis of the FA by GC. GC was performed using a Hewlett-Packard (HP) 6890 system equipped with a FID and an HP-FFAP column (0.32 mm i.d.× 25 m × 0.52 µm film thickness). The oven temperature was programmed to hold at 180°C for 1 min, followed by an increase at 3°C/min to 220°C, where it held for 9 min. The injector and detector temperatures were set to 230 and 250°C, respectively. Helium was used as the carrier gas, and 1 µL was injected.

Measurement of antioxidative activity. This experiment was carried out using a method modified from Hirayama *et al.* (3). For pigment extraction, 0.5 g dried red pepper was extracted with 50 mL hexane using a shaking water bath for 2 h, and the extracts were diluted to 10 times with hexane. In a 2-mL glass vial, 0.25 mL of 0.2 mM methylene blue and 0.33 mL of 0.5 mM linoleic acid were mixed with or without pigment solutions (0.25 mL), and the final volume was adjusted to 1 mL with hexane/ethanol (1:1, vol/vol). The vial was tightly closed with a screw cap, which had a septum. The mixture was illuminated at 4,000 lux at 30°C for 2 h in a photochamber. After completion of the reaction, 100 µL of headspace gas was removed with a microsyringe and the gas composition analyzed by GC. GC was performed using a GC-14A (Shimadzu, Japan) equipped with a TCD detector and CTR-I column (Alltech Co., Deerfield, IL). The oven temperature was programmed to remain at 35°C, and the injector and detector temperatures were both set to 60°C. The reaction mixture (300 µL) was diluted to 9 mL with ethanol, and the absorbance at 235 nm was measured with a UV-vis spectrophotometer (V-550, Jasco, Japan) in order to estimate the formation of conjuated dienes.

Statistical analysis. All data were based on dry weight, and statistical analysis was performed by ANOVA and Duncan's multiple range test ($\alpha = 0.05$). The correlation analysis was carried out using Pearson correlation coefficients ($\alpha = 0.05$).

RESULTS AND DISCUSSION

FA composition and the effect of processing. The pericarp and seeds of the red pepper had very different FA compositions (Table 1). The main FA in the pericarp and the seeds were palmitic (16:0), oleic (18:1), and linoleic (18:2) acids. The percentage of 18:2 in the seeds was 73.9%, which was about 1.7 times as much as that in the pericarp. On the other hand, linolenic acid (18:3), was 10.4% in the pericarp but was scarcely present in the seeds (0.4%). The percentages of unsaturated FA in the pericarp and the seeds were 73.3 and 83.2%, respectively. The ratio of saturated FA (SFA) to UFA was higher in the pericarp (0.37) than in the seeds (0.20). The FA composition of the red pepper powder was related to the mixing ratio between the pericarp and the seeds. In Korea, commercial red pepper powder contains 10–15% seeds. However, Perez-Galvez *et al.* (9) found that paprika contained 30–40% seeds and the FA distribution in paprika is very similar to that found in the seeds, i.e., having a high linoleic acid content. In this case, the SFA/UFA ratio of pericarp was 0.54, which was higher than the SFA/UFA ratio (0.37) observed in our study. That of the powder, however, was 0.21, which was less than the ratio (0.30) observed in our study. This difference in SFA/UFA is believed to be related to the stability of the powdered product.

The FA composition also varies between different cultivars and harvesting areas. Breithaupt and Schwack (10) analyzed the FA composition of red pepper from Germany, and except for linoleic acid (18:2), myristic (14:0), palmitoleic

TABLE 1
FA Composition of the Pericarp, Seeds, and Powder in Red Pepper, and Relationships Among Different FA Fractions

		Pericarp	Seed	Powder[a]
Saturated FA (SFA)				
	14:0	1.6 ± 0.11[b]	0.1 ± 0.01	0.8 ± 0.02
	16:0	20.5 ± 0.18	13.6 ± 0.02	17.9 ± 0.68
	18:0	3.3 ± 0.02	1.9 ± 0.03	2.7 ± 0.17
	20:0	0.7 ± 0.01	0.3 ± 0.01	0.5 ± 0.01
	22:0	0.5 ± 0.01	0.4 ± 0.01	0.5 ± 0.01
	24:0	0.2 ± 0.01	0.4 ± 0.01	0.3 ± 0.02
Unsaturated FA (UFA)				
	16:1	0.8 ± 0.01	0.2 ± 0.03	0.5 ± 0.01
	18:1	18.7 ± 0.52	8.6 ± 0.02	15.0 ± 0.04
	18:2	43.1 ± 1.07	73.9 ± 0.03	56.7 ± 0.99
	18:3	10.4 ± 0.42	0.4 ± 0.01	4.9 ± 0.08
	20:1	0.2 ± 0.01	0.1 ± 0.01	
Total SFA		26.8	16.8	22.9
Total UFA		73.3	83.2	77.1
MUFA		19.8	8.9	15.5
PUFA		53.5	74.3	61.6
Relation	SFA/UFA[c]	0.37	0.20	0.30
	MUFA/PUFA[d]	0.37	0.12	0.25

[a]Powder was made by mixing 90% pericarp and 10% seeds.
[b]Values are means of three replicate samples ± SD.
[c]Ratio of total SFA to total UFA.
[d]Ratio of monounsaturated FA (MUFA) to PUFA.

(16:1), stearic (18:0), and linolenic (18:3) acids were higher than those found in this study, whereas linoleic acid (18:2) was lower. Perez-Galvez *et al.* (9) reported the FA compositions of two kinds of red pepper cultivars produced in Spain. In particular, the composition of linolenic acid (18:3) in the seeds was higher, as much as 23.4 and 20.9%, in these two cultivars. This is very different from the report of Breithaupt and Schwack (10) as well as our results. This difference in the proportion of linolenic acid may be related to the product quality in the oxidizing process, if we consider that pericarp was mixed with a fixed quantity of seeds to produce the powder and that linolenic acid would be oxidized selectively by lipoxygenase.

Changes in FA composition under different processing conditions were studied. Drying method B, used commercially in Korea, has serious problems in terms of color and nutrients, and drying method A was designed to improve the quality obtained. The changes in FA composition with different drying methods and storage conditions, however, were not significantly different ($P > 0.05$) (Table 2). The changes in the FA ratios according to processing conditions were significantly different (Duncan's multiple range test, $\alpha < 0.05$); however, the FA composition hardly changed during drying and storage.

Changes in antioxidative activity associated with adding pigment extracts from red pepper powders under different processing conditions. Lipids containing methylene-interrupted dienes show a shift in their double-bond position during oxidation that is due to isomerization and formation of conjugation. The resulting conjugated dienes exhibit intense absorption at 235 nm, and conjugated diene formation and PV of edible oils correlate well during their oxidation (11). Hirayama *et al.* (3) found that the singlet oxygen-quenching ability of various naturally occurring carotenoids could be examined by measuring the toluidine blue-sensitized photooxidation of linoleic acid, and so to measure this quenching, the oxidation of linoleic acid was followed by measuring UV at 235 nm and by oxygen consumption.

Table 3 shows the inhibitory effects with respect to linoleic acid of pigment extracts under various processing conditions, which were measured by monitoring oxygen consumption and conjugated diene formation at 235 nm. The pigment extracts obtained from peppers dried by method A exhibited a greater inhibitory effect than those obtained by method B with respect to the photooxidation of linoleic acid ($P < 0.01$), In red pepper powder dried by method A and stored at 0°C, conjugated diene formation was inhibited 30.5% due to the addition of pigment extracts. Definite differences were observed in oxygen consumption. Oxygen consumption was found to be less than 20% when adding pigment extracts resulting from drying method A, whereas it was greater than 50% when adding pigment extracts resulting from drying method B, after 2 mon of storage, regardless of storage temperature. However, the effects of storage temperature and storage periods were not significantly different ($P > 0.05$), although conjugated diene formation was slightly increased with increasing storage temperature and length of storage. Conjugated diene formation and oxygen consumption were correlated positively (Pearson correlation coefficients = 0.8656), and the method used was very effective in measuring antioxidative

TABLE 2
Percentage Composition and Relationships Among Different FA Fractions of Red Pepper Powder in Different Processing Conditions[a]

Storage temperature		0°C						20°C					
Drying methods		A[b]			B[c]			A			B		
Storage periods (mon)		2	4	6	2	4	6	2	4	6	2	4	6
SFA													
	14:0	1.0b	1.1a	0.7c	0.9b	1.0a	0.6c	1.0b	1.2a	0.9b	1.0b	1.2a	0.9b
	16:0	17.2a,b	17.5a	16.8b	16.8c	17.3a	17.0b	17.1c	17.6a	17.2b	16.9c	17.4a	17.1b
	18:0	2.6b	2.8a	2.7a,b	2.5b	2.6a	2.7a	2.6c	2.8a	2.7b	2.5b	2.7a	2.8a
	20:0	0.5a	0.5a	0.4b	0.4b	0.5a	0.4b	0.5c	0.5a	0.5b	0.5a	0.5b	0.5b
	22:0	0.4b	0.4a	0.4c	0.3c	0.4a	0.4b	0.4b	0.4a	0.4a	0.3b	0.4a	0.4a
	24:0		0.2a	0.2a		0.2a	0.2a		0.2a	0.2a		0.2a	0.2a
UFA													
	16:1	0.5a	0.6a	0.5a	0.5c	0.5a	0.5b	0.5c	0.6a	0.5b	0.5a	0.5a	0.5a
	18:1	14.9a	13.9b	14.3a,b	14.0a	13.1b	13.9a	14.8a	13.9b	14.7a	14.0a	13.2b	14.0a
	18:2	57.1a	57.1a	57.8a	59.2a	58.8b	58.4c	57.4a	56.5b	56.8b	58.9a	58.4b	58.1c
	18:3	5.8c	5.8b	6.2a	5.3c	5.4b	5.7a	5.7b	5.7b	6.1a	5.4a	5.3b	5.3b
	20:1	0.1b	0.2a	0.1b	0.1b	0.1a	0.1b	0.1c	0.2a	0.1b	0.1a,b	0.2a	0.1b
Total SFA		21.7	22.4	22.0	20.9	22.0	21.4	21.4	22.7	21.9	21.1	22.4	22.4
Total UFA		78.4	77.6	78.0	79.1	78.0	78.6	78.6	77.3	78.1	78.9	77.6	77.6
	MUFA	15.5	14.6	15.4	14.6	13.7	14.6	15.4	15.1	15.4	14.7	13.9	14.6
	PUFA	62.8	63.0	62.6	64.6	64.2	64.0	63.1	62.3	62.8	64.3	63.7	63.0
Relation	SFA/UFA[d]	0.28	0.29	0.28	0.26	0.28	0.27	0.27	0.29	0.28	0.27	0.29	0.29
	MUFA/PUFA[e]	0.25	0.23	0.25	0.23	0.21	0.23	0.24	0.24	0.24	0.23	0.22	0.23

[a]Values in the same row for processing conditions, i.e., drying methods and storage temperatures, that are not followed by the same roman letter are significantly different ($P < 0.05$). For abbreviations see Table 1.
[b]Red peppers from which seeds were removed were cut into three parts and dried at 70°C for 6 h.
[c]Whole red peppers were dried at 80°C for 5 h, followed by 60°C for 18 h.
[d]Ratios of total SFA/total UFA. For abbreviations see Table 1.
[e]Ratio of MUFA/PUFA.

TABLE 3
Change of Antioxidative Activity Resulting from Adding Red Pepper Pigment Extracts in Different Processing Conditions

Storage temperature	Drying method**,[a]	Storage periods (mon)	Conjugated diene formation A235	Conjugated diene formation %	O_2 consumption (%)
		Blank	0.896	100	100
0°C	A[b]	2	0.274	30.54	19.50
		4	0.286	31.90	14.83
		6	0.315	35.18	30.48
	B[c]	2	0.317	35.35	51.35
		4	0.341	38.00	42.02
		6	0.428	47.76	54.65
20°C	A	2	0.326	36.33	11.26
		4	0.291	32.42	26.64
		6	0.357	39.81	29.66
	B	2	0.410	45.75	54.63
		4	0.486	54.26	63.16
		6	0.513	57.19	73.43

[a]**Indicates significant difference between drying methods A and B ($P < 0.01$).
[b]Red peppers cut into three parts were dried at 70°C for 6 h.
[c]Whole red peppers were dried at 80°C for 5 h, followed by 60°C for 18 h.

activity. In conclusion, an improved drying method could allow the antioxidative activity of red pepper pigments to be conserved.

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