

Hypolipidemic Properties of Fermented Capsicum and Its Product

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Abstract

This study was conducted to investigate the effects of fermented capsicum and a capsicum product on lipid metabolism. Fermented capsicum was prepared from red pepper puree for three months. After 90 days of fermentation, capsaicin and dihydrocapsaicin concentrations were reduced from 24.7 and 14.7 g/mL to 15.5 and 6.45 g/mL, respectively. The capsicum product was prepared from the fermented capsicum mixed with prune extract, green tea extract, neroli extract and oligo-saccharide. Thirty-two male Sprague-Dawley rats were assigned to four dietary groups (control, high-fat control (HF-control), high-fat-fermented capsicum (HF-S-1), high-fat-capsicum product (HF-S-2)). Plasma and hepatic lipid profiles were examined after three weeks of experimental diet. Food intakes were significantly lower in the HF-S-1 and HF-S-2 groups compared to the control group ($p < 0.05$). The weight of perirenal fat pads was lowest in animals on the control diet (low-fat) and highest in high-fat control diet. The addition of fermented capsicum to high fat diets, HF-S-1 and HF-S-2 groups, resulted in significantly lower fat pad weights compared with the HF-control group. Both fermented capsicum (HF-S-1) and the capsicum product (HF-S-2) groups had lower plasma TG levels, atherogenic-index, and liver TG levels than the HF-control group ($p < 0.05$). Liver TC levels were significantly lower in the HF-S-2 group than the HF-control group. The results demonstrate a hypolipidemic effect of fermented capsicum and the fermented capsicum product.

Key words: capsicum, fermented capsicum, capsaicin, hypolipidemic effect

INTRODUCTION

Spices are common food adjuncts that impart flavor, aroma or piquancy to foods. Spices are consumed in a variety of combinations depending on taste preferences. Spices or aromatic vegetable products are used in cooked or semi-cooked foods, sauces, dressings and soups, and some of the vegetable spices like onions and capsicum are consumed raw.

Worldwide interest in the various species of the *Capsicum* genus of plants is increasing (1). Capsicums may be either pungent or non-pungent spices such as pimiento, paprika, and chili that are produced from dried fruit and are ground into powders. To achieve a good spice color quality, completely red fruit are processed. Chilies (*Capsicum annum* L.) are extensively consumed throughout the world because of their color, flavor and pungency. Red pepper (Chili) is a popular spice in Korea. Chili peppers contribute to the uniqueness of pungent Korean foods like *kimchi* (fermented pickle) and *kochujang* (fermented red pepper paste).

Capsaicin is a pungent principle of hot red pepper, which is used as an important spice for enhancing the palatability

of food and medicinally as a counterirritant. The chemistry, biochemistry, and pharmacology of capsaicin have been the subject of several reviews (2-5). Recently, the effects of dietary components on lipid metabolism have received much attention. For example, vegetable protein, dietary fiber and the essential oils of garlic and onion appear to lower blood cholesterol (6-8). Recent studies have shown that capsaicin exerts a lipotropic effect, similar to that of choline, in rats (9,10) and decreases total serum, myocardial and aortic cholesterol levels in turkeys (11).

In spite of the medicinal benefits, the strong pungency of red pepper discourages its widespread use. Therefore, reduction of pungency is necessary to broaden the use of red pepper. In this study, red pepper puree was fermented for 3 months to reduce pungency and increase palatability, and then evaluated for hypolipidemic properties in rats.

MATERIALS AND METHODS

Preparation and chemical analysis of fermented capsicum

Capsicum was crushed and finely ground to paste with a homogenizer. The paste was blended with dextrin to 40

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brix, and incubated at 30°C for 90 days. After incubation, the mixture was filtered through a filter press and the filtrate was used as fermented capsicum to test for the hypolipidemic effect. Because slight pungent taste remained in the fermented capsicum, masking of the pungency was necessary for the use of food additives. Therefore, a capsicum product was prepared from 12% of fermented capsicum to which 3% prune (*Prunus domestica*) extract, 1% of green tea extract, 1% neroli (*Citrus aurantium*) extract, and 30% oligo-saccharide were added. The capsicum product was also used to test for the hypolipidemic effect of capsicum.

Capsaicin and dihydrocapsaicin were measured by HPLC using the method of Saria et al. (12). Capsaicin was extracted from a suspension of fermented capsicum which was separated from the fermented broth by vacuum filtration using Whatman No. 44 filter paper in a Buchner funnel. The filtrate was then extracted twice with two volumes of chloroform in separatory funnels. The extracts were dried over sodium sulfate and evaporated to dryness *in vacuo* (130 rpm, 45°C). The residue, suspended in methanol, was applied to HPLC (Beckman, System Gold) employing a 5 m ODS spherical C₁₈ analytical column (4.6 mm width; 250 mm length; Bodman Chemicals). Absorbance profiles for chloroform extracts of the filtrates were 280 nm. Mobile phase was methanol/water (60/40, v/v) at a flow rate of 1.5 mL/min.

Animals and diets

Thirty-two male Sprague-Dawley rats (6~8 weeks old) were obtained from Daihan-Biolink Co. (Korea). After an adaptation period, the rats were provided free access to water and standard semi-purified low-fat and high-fat diets (Table 1). Animals were housed under controlled temperature (about 21°C) and humidity (about 60%) conditions. The rats were randomly divided into 4 dietary groups: con-

trol diet, high-fat control diet (HF-control), high-fat with the fermented capsicum (HF-S-1) and high-fat with the capsicum product (HF-S-2)). The last two groups were orally administered either 0.25 mL of the fermented capsicum (containing 15.5 g capsaicin) or 0.25 mL of the capsicum product (containing 0.47 g capsaicin) per 100 g body weight.

Plasma and hepatic lipid analyses

Food consumption and weight-gain were measured every third day. At the end of the experimental period, the rats were anesthetized with Ketamine following a 12 hr fast. Blood was collected from the aorta ventralis into tubes containing EDTA and plasma separated by centrifugation at 1,100 × g for 20 min at 4°C. Plasma levels of total cholesterol (TC), HDL cholesterol, and triacylglycerol (TG) were measured using enzymatic kits. The liver was perfused *in situ* with cold saline (8.5 g NaCl/L), removed, weighed, and stored in plastic bags at -20°C. Liver lipids were extracted by the gravimetric method of Folch et al. (13) and re-dissolved in ethanol. Liver TC and TG were assayed by using the same method as for plasma TC and TG, after treatment with triton X-100.

Statistical analysis

The data were subjected to analysis of variance and expressed as the mean ± SD. Significance of differences were compared by Duncan's multiple range test. Differences were considered significant at p < 0.05.

RESULTS AND DISCUSSION

Changes in capsaicin and dihydrocapsaicin concentrations in the fermented capsicum

The contents of capsaicin and dihydrocapsaicin decreased with the lapse of fermentation time (Fig. 1). At the early stage of fermentation, contents of capsaicin and dihydrocapsaicin were 24.7 and 14.7 g/mL, respectively. The residual capsaicin and dihydrocapsaicin concentrations decreased during the entire fermentation period, and by the 90th day were 15.5 and 6.45 g/mL, respectively. The fermented capsicum was 14.7% carbohydrate, 1.5% lipid, 4.9% protein, and 0.3% ash, and 78.2% moisture content (data was not shown). Capsaicin and dihydrocapsaicin contents were 0.15% and 0.06%, respectively. The pungent taste of capsicum was reduced by fermentation, but the mechanism and component responsible for the reduction in pungency is unknown, and will be the subject of future study.

Effects capsicum diets on food intake, weight gain, and organ weights

Changes in the weights of the body, liver and other internal organs after 3 weeks of administration of the fer-

Table 1. Composition of low-fat and high-fat diets

Nutrient	Low-fat diet (g/100 g diet)	High-fat diet (g/100 g diet)
Casein	19.6	26.2
Corn starch	56.6	27.6
Sucrose	10.0	10.0
Lard	-	22.4
Soybean oil	4.0	4.0
Mineral mixture ¹⁾	3.5	3.5
Vitamin mixture ²⁾	1.0	1.0
Cellulose	5.0	5.0
L-methionine	0.3	0.3

¹⁾Composition of mineral mixture was as follows (g/kg): CaPO₄ · 2H₂O, 145.6; KH₂PO₄, 257.2; NaH₂PO₄, 93.5; NaCl, 46.6; calcium lactate, 350.9; ferric citrate, 31.8; MgSO₄, 71.7; ZnCO₃, 1.1; MnSO₄ · 4H₂O, 1.2; CuSO₄ · 5H₂O, 0.3; KI, 0.1.

²⁾Vitamin mixture: ICN Vit. Mixture (No 904654).

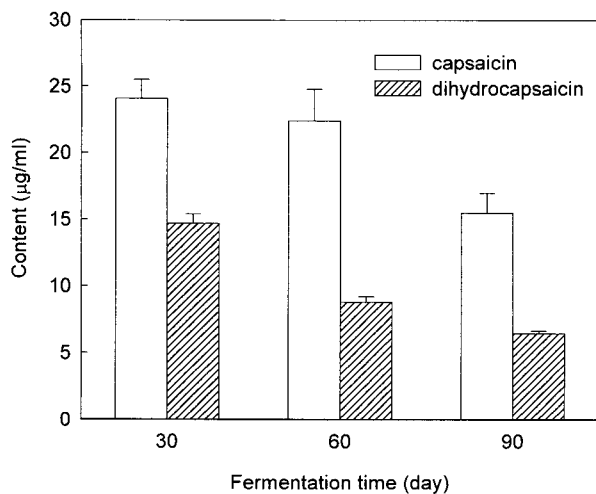


Fig. 1. Changes in capsaicin and dihydrocapsaicin content during the fermentation of capsicum.

mented capsicum and the capsicum product are shown in Tables 2 and 3. Food intakes were not significantly different among the high-fat diet control (HF-control) and high-fat diet groups with orally administered fermented cap-

Table 2. Net weight, food intake, and food efficiency ratio in rats fed experimental diets

Group	Net weight gain (g/day)	Food intake (g/day)	Food efficiency ratio ¹⁾
Control	5.44 ± 0.87 ^{a,b2)}	46.9 ± 9.5 ^b	0.12 ± 0.0052 ^a
HF-control	6.03 ± 0.59 ^b	36.5 ± 6.7 ^{a,b}	0.17 ± 0.0147 ^b
HF-S-1	4.15 ± 1.35 ^a	28.4 ± 7.4 ^a	0.14 ± 0.0103 ^a
HF-S-2	5.73 ± 1.08 ^{a,b}	30.5 ± 5.8 ^a	0.19 ± 0.0003 ^a

¹⁾Food efficiency = (body wt gain/food intake).

²⁾Means with different superscript letters within a column are significantly different at $p < 0.05$ by Duncan's multiple range test.

Table 3. Weights of liver, spleen, kidney, and perirenal fat

Group	Liver	Spleen	Kidney	Perirenal fat pad
Control	3.57 ± 0.32 ^{1)NS2)}	0.24 ± 0.03 ^{NS}	0.84 ± 0.04 ^{NS}	1.29 ± 0.06 ^{a3)}
HF-control	3.43 ± 0.23	0.25 ± 0.04	0.89 ± 0.33	1.78 ± 0.15 ^c
HF-S-1	3.13 ± 0.25	0.27 ± 0.03	0.76 ± 0.06	1.48 ± 0.08 ^b
HF-S-2	3.33 ± 0.61	0.24 ± 0.01	0.75 ± 0.01	1.41 ± 0.13 ^{a,b}

¹⁾Values are mean ± SD (n=8).

²⁾NS: not significant ($p < 0.05$).

³⁾Means with different superscript letters within a column are significantly different at $p < 0.05$ by Duncan's multiple range test.

Table 4. Concentrations of total cholesterol, HDL-cholesterol, VLDL-LDL cholesterol, and atherogenic-index in plasma (mg/dL)

Group	TG	Cholesterol			Atherogenic-index ³⁾
		Total	HDL	VLDL-LDL	
Control	37.1 ± 17.6 ^{1)a2)}	90.9 ± 1.8 ^{a,b}	26.7 ± 3.9 ^b	64.2 ± 8.3 ^b	2.4 ± 0.4 ^a
HF-control	60.9 ± 13.2 ^b	111.8 ± 22.1 ^b	21.6 ± 0.5 ^a	90.2 ± 2.5 ^a	4.2 ± 0.9 ^c
HF-S-1	33.9 ± 12.2 ^a	87.3 ± 9.8 ^{a,b}	19.7 ± 1.3 ^a	67.6 ± 7.5 ^b	3.4 ± 0.2 ^b
HF-S-2	37.5 ± 5.4 ^a	85.1 ± 6.7 ^a	23.6 ± 1.6 ^{a,b}	61.5 ± 7.8 ^b	2.6 ± 0.04 ^{a,b}

¹⁾Values are mean ± SD (n=8).

²⁾Means with different superscript letters within a column are significantly different at $p < 0.05$ by Duncan's multiple range test.

³⁾Atherogenic-index: (Total cholesterol - HDL) / HDL.

sicum (HF-S-1) and capsicum product (HF-S-2). Body weight gains were lower in the rats fed fermented capsicum (HF-S-1) than the other groups.

There were no differences among groups in liver, spleen and kidney weights (Table 3). Rats on the HF-control diet had significantly higher perirenal fat pad weights than the control, HF-S-1, and HF-S-2 groups. Therefore, in the HF-S-1 and HF-S-2 groups, the oral administration of fermented capsicum and capsicum product inhibited the accumulation of fat around the kidney organs in rats fed a high fat diet. Kawada et al. reported that capsaicin supplementation (0.014% of the diet containing 30% lard) tended to reduce perirenal adipose tissue weight (14). Therefore, fermented capsicum and capsicum product suppresses fat accumulation resulting from a high fat diet in rats.

Effect on plasma and hepatic lipids

The effects of fermented capsicum on plasma and liver lipids are shown in Tables 4 and 5. Plasma TGs were significantly higher in rats fed a high fat diet without capsicum, but rats fed a high fat diet with fermented capsicum or the capsicum product had plasma TG concentrations that were nearly identical to those on the low fat control diet. This hypertriglyceridemic effect of capsicum is consistent with the results reported by Sambaiah and Satyanarayana (15) in rats fed ad libitum a diet containing 10% fructose and 0.015% capsaicin for 6 weeks.

The two groups with orally administered fermented capsicum (HF-S-1 and HF-S-2) had lower plasma total cholesterol (TC) concentrations than those of the HF-control group. In a study using turkeys fed a high cholesterol diet for 23 days, capsaicin decreased serum TC (11), but in this study there was no effect of capsicum on plasma TC

Table 5. Concentrations of TG and total cholesterol in liver (mg/g)

Group	TG	Total cholesterol
Control	29.8 ± 4.3 ^{1)a,b2)}	78.3 ± 3.2 ^b
HF-control	37.5 ± 0.5 ^c	97.2 ± 1.5 ^c
HF-S-1	33.2 ± 3.4 ^b	93.0 ± 4.5 ^c
HF-S-2	23.8 ± 0.5 ^a	71.4 ± 0.5 ^a

¹⁾Values are mean ± SD (n=8).

²⁾Means with different superscript letters within a column are significantly different at $p < 0.05$ by Duncan's multiple range test.

(HF-S-1 and HF-S-2 groups). The discrepant results may be due to differences in cholesterol intake or in animal species.

The atherogenic index was significantly lower in the capsicum groups (HF-S-1 and HF-S-2) as compared to the HF-control group; the HF-S-2 group had a similar atherogenic-index to that of controls. The atherogenic index value is an indicator of cardiovascular risk. Several studies have demonstrated the protective effect of HDL-cholesterol against atherosclerosis and cardiovascular disease while high levels of LDL-cholesterol constitute a risk factor. The addition capsicum in the HF-S-1 and HF-S-2 groups tended to decrease atherogenic index values (Table 4), which may be beneficial in the prevention and treatment of cardiovascular disease. The fermented capsicum and capsicum product supplementation resulted in a significant decrease in plasma TG and the risk of atherosclerosis.

Liver TG concentrations were significantly higher in the HF-control group, but were significantly lower in the HF-S-2 diet and significantly lower still in the HF-S-1 group, which were not different from the control group (Table 5). Liver total cholesterol was only lower in the HF-S-2 group, among the high fat diet groups. The fact that liver TC was significantly reduced in rats administered the capsicum product was most likely because the rate of its degradation to bile acids was slower than its rate of synthesis.

These results indicate that the administration of fermented capsicum and capsicum product had a profound influence on the metabolism of lipids in rats fed a high-fat diet. A significant decrease in cholesterol and triacylglycerol levels in plasma or liver was observed in rats administered the fermented capsicum and capsicum product. Sambaiah and Satyanarayana reported that the supplement of capsaicin markedly inhibited hepatic lipogenesis, which was a consequence of the inhibition of acetylCoA carboxylase (a rate-limiting enzyme of fatty acid synthesis) and glucose-6-phosphate dehydrogenase activities in rats (15).

Consequently, these results indicate that consumption of capsaicin facilitates lipid metabolism in rats fed a high fat diet. The stimulation of lipid metabolism by capsaicin is supported by the lower perirenal fat pad weights and

plasma TG concentrations. The pungency of capsicum was reduced by fermentation. Although the capsaicin content was reduced, the hypolipidemic effect of the fermented capsicum was evident from the above results. Therefore, an unknown capsicum constituent or capsaicin metabolite may be responsible for the hypolipidemic effect. The hypolipidemic effect of the fermented capsicum might be a result of the lower weight gain which, in turn, is probably due to a lower food intake compared to the controls. The capsicum product, a mixture of the fermented capsicum and other ingredients (prune extract, green tea extract, and neroli), also had a hypolipidemic effect, and the other ingredients may have had a synergistic effect with the fermented capsicum on liver lipids.

Additives and fermentation may reduce the pungency and increase the palatability of capsicum without compromising its functional value.

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