Korean J. Food Sci. An. Vol. 35, No. 4, pp. 479~485 (2015)
DOI http://dx.doi.org/10.5851/kosfa.2015.35.4.479
ISSN 1225-8563 eISSN 2234-246X

ARTICLE

Effects of Yogurt Containing Fermented Pepper Juice on the Body Fat and Cholesterol Level in High Fat and High Cholesterol Diet Fed Rat

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Abstract

This experiment investigated whether yogurt containing fermented pepper juice (FPJY) affects cholesterol level in high fat and high cholesterol diet (HFCD) fed rat. Twenty five Sprague-Dawley male rats of 7 wk were divided into 5 groups, and fed following diets for 9 wk; CON (control diet), HFCD (HFCD), PY (HFCD supplemented with 2% of plain yogurt), LFY (HFCD supplemented with 2% of FPJY), and HFY (HFCD supplemented with 5% of FPJY). In the LFY group, hepatic total lipid level decreased significantly compared to the HFCD group ($p<0.05$). Serum HDL cholesterol level tended to increase and hepatic total cholesterol level decreased and were comparable to the CON group ($p>0.05$). In HFY group, body weight and hepatic total lipid level significantly decreased over the HFCD group ($p<0.05$). Serum and hepatic total cholesterol level, kidney, and body fat weights decreased, and were compared to the CON group ($p>0.05$). Liver weight decreased as FPJY content was increased. Results suggested FPJY would inhibit organ hypertrophy and accumulation of body fat, hepatic lipid, and cholesterol in HFCD fed rat.

Keywords: fermented pepper juice, yogurt, high fat and high cholesterol diet, *Bacillus licheniformis*, body fat accumulation

Received February 4, 2015; Revised June 4, 2015; Accepted June 4, 2015

Introduction

High cholesterol in blood can cause cardiovascular disease (Wardlaw *et al*., 2005), piles up and creates plaque at vessel walls, possibly leading to atherosclerosis. The blood vessel can narrow and blood circulation can be restricted so oxygen in the blood cannot reach the heart or brain, causing makes heart attacks (Wardlaw *et al*., 2005; Xiao *et al*., 2003).

Yogurt contains lactic acid bacteria. It is a probiotic food which can cause beneficial effects in the intestinal tract of humans (Odamaki *et al*., 2011; Tang *et al*., 2014). Yogurt is proven to remove cholesterol as well as decrease the serum cholesterol and low density lipoprotein (LDL) cholesterol (Akalin *et al*., 1997; Anderson *et al*., 1999; Kapila *et al*., 2006; Pereira *et al*., 2003). Therefore, yogurt can be a beneficial food for patients with cardiovascular disease.

Meanwhile, Korean pepper, especially chung-yang pepper, is famous for its pungency which results from capsaicin. Capsaicin and its several homologues are categorized as capsaicinoids. Most of the pungency results from capsaicin and dihydrocapsaicin (Iwai and Watanabe, 1999). Capsaicin inhibits body fat accumulation and promotes energy expenditure, pain relief, antibacterial activity, antioxidant activity, anti-stress activity, and inhibition of tumor cell growth (Chatteriee *et al*., 2010; Chen *et al*., 2014; Dou *et al*., 2011; Gerner *et al*., 2008; Iwai and Watanabe, 1999; Luo *et al*., 2011; Reinbach *et al*., 2009; Shin and Moritani *et al*., 2007). Although capsaiscin has various effects, its application in the food industry is limited because of its pungency (Kim *et al*., 2011).

Capsaicinoids can be degraded by several microorganisms (Cho *et al*., 2014; Flagan and Leadbetter, 2006; Kim *et al*., 2011; Lee *et al*., 2008). In a previous experiment, the amount of capsaicin and dihydrocapsaicin decreased as pepper was fermented by *Bacillus licheniformis* (Yeon *et al*., 2013). Fermented pepper inhibited body fat accu-

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mulation in mice fed a high fat diet even if it lost pungency. It might have lost pungency because the food intake between the control diet fed group and the fermented pepper diet fed group showed no significant difference (Yeon *et al*., 2013). On the other hand, a group fed the same concentration of normal pepper diet showed significantly lowered total food intake (Yeon *et al*., 2013).

There is little information about the application of fermented pepper on yogurt. Yogurt is popular probiotic desert which can drink easily. It is important whether fermented pepper act as functional effect even if it is in yogurt. In this experiment, pepper juice was fermented and added to yogurt to identify the effect of degraded capsaicin in yogurt on decreasing cholesterol concentration and body fat accumulation in rats fed a high fat and high cholesterol diet (HFCD).

Materials and Methods

Preparation of fermented pepper juice

Pepper juice was extracted from chung-yang pepper that was purchased from a local market in Seoul, Korea by a juicer. This sample was mixed with bacillus minimal medium of double concentration (Bacillus minimal medium ×2 : NaCl 1 g/L, Na_2HPO_4 9.52 g/L, KH_2PO_4 6 g/L, NH_4Cl 2 g/L, $MgSO_4$ 0.48 g/L, $CaCl_2$ 0.02 g/L, glucose 3.6 g/L) without glucose (1:1, v/v). After adjustment to pH 7, it was sterilized and 5% of *Bacillus licheniformis* SK1230 was inoculated after cooling at 37°C. It was then incubated at 37°C for 5 d.

Preparation of yogurts

The composition of yogurt mix is expressed in Table 1. Milk, skim milk powder, pectin, sugar, distilled water and fermented pepper juice were mixed using a homogenizer (Homogenizer T25, Janke and Kunkel type, Germany) for 10 min. Then, it was heated at 85°C for 30 min. After cooling at 42°C, the starter was inoculated and the mixture was incubated at 42°C until it reached pH 4.5. This yogurt was stored at 4°C for 24 h and then freeze dried to add to experimental diets. Ten milliliter of distilled water was added to the premix of plain yogurt as a substitute for fermented pepper juice. Starter (Lyofast YAB 450 AB, Sacco srl., Italy) contained *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus acidophilus* and *Bifidobacteriumanimalis* ssp. *lacti*.

Animal experiment

Seven wk old 25 Sprague-Dawley male rats (Central Lab. Animal Inc., Korea) were individually housed in plastic cages and were acclimated for one wk. Then, animals were randomly assigned into five groups (five rats/group); the weights of each group were not significantly different (p> 0.05). The room was maintained at 22 ± 2°C, 50 ± 5% humidity, and a 12 h light/dark cycle during the 9 wk experimental period. The animal experiment was approved by the Institutional Animal Care and Use Committee (IACUC approval no. KU13090).

Experimental groups were as follows; CON (control diet), HFCD (45% kcal high fat and high cholesterol diet; HFCD), PY (HFCD supplemented with plain yogurt diet),

Table 1. The composition of yogurt premix

Ingredients	Fermented pepper juice	Plain
Milk (mL)	850	850
Skim milk powder (g)	40	40
Pectin (g)	2	2
Sugar (g)	15	15
Distilled water (mL)	95	105
Fermented pepper juice (mL)	10	-
Starter[1)] (mg)	20	20

[1)]Starter: *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus acidophilus* and *Bifidobacterium-animalis* ssp. *lacti*. (Lyofast YAB 450 AB, Sacco srl., Codarago, Italy).

Table 2. The ingredients of the experimental diets

Groups / Ingredients (g)	CON[1)]	HFCD[2)]	PY[3)]	LFY[4)]	HFY[5)]
Casein	200.0	233.0	228.4	228.9	222.9
Corn starch	397.5	84.8	73.4	72.4	53.4
Sucrose	100.0	201.4	201.4	201.4	201.4
Dextrose	132.0	116.5	116.5	116.5	116.5
Cellulose	50.0	58.3	58.3	58.3	58.3
Soybean oil	70.0	29.1	25.6	25.8	20.9
Lard	0.0	206.9	206.9	206.9	206.9
Mineral mixture	35.0	52.4	52.4	52.4	52.4
Vitamin mixture	10.0	11.7	11.7	11.7	11.7
L-cystine	3.0	3.0	3.0	3.0	3.0
Choline bitartrate	2.5	2.5	2.5	2.5	2.5
Cholesterol	-	10.0	10.0	10.0	10.0
Cholin acid	-	5.0	5.0	5.0	5.0
Plain yogurt	-	-	20.0	-	-
Fermented pepper yogurt	-	-	-	20.0	50.0
Total (g)	1000.0	1015.0	1015.0	1015.0	1015.0
Total (kcal)	4000.0	4865.5	4865.5	4865.5	4865.5

[1)]CON: control diet fed group.
[2)]HFCD: high fat and high cholesterol diet fed group.
[3)]PY: high fat and high cholesterol diet supplemented with 2% plain yogurt powder.
[4)]LFY: high fat and high cholesterol diet supplemented with 2% fermented pepper juice yogurt powder.
[5)]HFY: high fat and high cholesterol diet supplemented with 5% fermented pepper juice yogurt powder.

LFY (HFCD supplemented with 2% fermented pepper juice yogurt), and HFY (HFCD supplemented with 5% fermented pepper juice yogurt). The ingredients of feed are represented in Table 2. The feeding amount was restricted to 20.5 g/mouse/1 d and tap water was available *ad libitum*.

The body weight was measured once a week and food intake was weighed at the same time each day using a balance (Adventurer AR2140, OHAUS crop, USA). Food intake was calculated as the difference of the feed ration of the day before and remains of the day. Feed efficiency ratio (FER) was calculated as follows; FER (%) = body weight gain (g) / total food intake (g) × 100.

All animals had an adaptation period of 1 wk and each experimental diet was offered to each group for 9 wk. After fasting of 24 h, dissection was performed. Anesthesia was accomplished by diethyl ether. Blood was gathered from the abdominal vein and the spleen, liver, cecum, kidney, epididymal adipose tissue (EAT), and perirenal adipose tissue (PAT) were weighed.

Serum analysis

After standing at 25°C for 3 h, serum was separated from blood and centrifuged (VS-550, Vision Scientific, Korea) at 3,000 rpm for 20 min at 4°C. Serum aspartate aminotransferase (AST), total cholesterol, high density lipoprotein cholesterol (HDL cholesterol) and glucose were analyzed by Green Cross Corp. (Korea).

Liver analysis

Total lipid content was analyzed by modified Folch's method (Folch *et al*., 1956). The liver was homogenized with 0.85% NaCl, and the residue was washed with Chloroform/methanol (2:1, v/v). After centrifuging at 3,000 rpm for 20 min, the supernatant and floating matter were removed. Residue was transferred to previously weighed tube and concentrated with N_2 gas. Total lipid content was calculated as concentrated tube weight minus previous weight of the tube.

Total cholesterol content was analyzed using a kit (Asan Pharm. Co., Korea). 2 mL of ethanol was added to the concentrated total lipid sample. Then, 20 μL was mixed with 3 mL of enzyme solution. After storage at 37°C for 5 min, absorbance was measured at 500 nm (Optizen 2120 UV, Mecasys Co. Ltd., Korea).

Statistical analysis

All data were expressed as mean ± standard deviation, and analyzed by Tukey's multiple-range test using SAS ver. 8.2 software (SAS Institute, USA). Significance was declared at $p<0.05$. Extremely different value was excluded.

Results and Discussion

Body weights, food intake, and FER during the experimental period are summarized in Table 3. Initial body weight in each group was not significantly different ($p>0.05$). Final body weight in the HFY group significantly decreased comparing to the HFCD group ($p<0.05$). FER in the high fat diet fed groups were significantly higher than that of the CON group ($p<0.05$), but the HFY group showed lower FER as compared to other high fat diet fed groups ($p>0.05$). Total food intake was same except for the HFCD group but it was not significantly lower than the other groups ($p>0.05$). Kim *et al.* (2010) also reported lower food intake in high fat and high cholesterol diet fed groups

Table 3. Body weights and food intake for the 9 wk of experimental period

Item	CON[1)]	HFCD[2)]	PY[3)]	LFY[4)]	HFY[5)]
Iinitial body weight (g) (n=5)	270.6±9.29$^{NS7)}$	270.4±5.86	270.4±5.86	270.3±5.94	270.3±5.91
Final body weight (g) (n=4)	477.5±6.25^{c}	541.5±14.27^{a}	536.5±2.38^{ab}	535.5±8.02^{ab}	518.8±4.35^{b}
Total food intake (g) (n=5)	1433.0±0.00^{NS}	1424.2±13.42	1433.0±0.00	1433.0±0.00	1433.0±0.00
FER[6)] (%) (n=5)	14.7±1.25^{b}	18.7±0.83^{a}	18.7±0.73^{a}	18.5±0.39^{a}	17.9±0.73^{a}

$^{a-c}$Superscripts with different letters indicate significant difference ($p<0.05$).
[1)]CON: control diet fed group.
[2)]HFCD: high fat and high cholesterol diet fed group.
[3)]PY: high fat and high cholesterol diet supplemented with 2% plain yogurt powder.
[4)]LFY: high fat and high cholesterol diet supplemented with 2% fermented pepper juice yogurt powder.
[5)]HFY: high fat and high cholesterol diet supplemented with 5% fermented pepper juice yogurt powder.
[6)]FER: food efficacy ratio.
[7)]NS: not significant.

Table 4. Weights of organs and body fats in CON, HFCD, PY, LFY, and HFY group after 9 wk of experiment

Item	CON[1)]	HFCD[2)]	PY[3)]	LFY[4)]	HFY[5)]
Liver (g) (n=5)	10.4±0.32[b]	25.1±1.95[a]	25.0±1.70[a]	23.6±2.22[a]	22.8±1.17[a]
Spleen (g) (n=5)	0.7±0.03[c]	1.0±0.19[ab]	1.1±0.05[a]	1.0±0.13[ab]	0.9±0.14[b]
Kidney (g) (n=5)	2.5±0.09[b]	2.7±0.12[a]	2.7±0.04[ab]	2.8±0.16[a]	2.6±0.18[ab]
Cecum (g) (n=5)	3.1±0.49[NS8)]	3.7±0.31	3.4±0.36	3.3±0.23	3.5±0.60
PAT[6)] (g) (n=4)	11.9±3.29[b]	16.7±0.65[a]	18.0±1.71[a]	18.8±1.33[a]	16.0±1.21[ab]
EAT[7)] (g) (n=4)	9.5±1.07[b]	14.8±2.15[a]	14.7±1.81[a]	14.7±0.98[a]	12.7±3.09[ab]

[a-c]Superscripts with different letters indicate significant difference ($p<0.05$).
[1)]CON: control diet fed group.
[2)]HFCD: high fat and high cholesterol diet fed group.
[3)]PY: high fat and high cholesterol diet supplemented with 2% plain yogurt powder.
[4)]LFY: high fat and high cholesterol diet supplemented with 2% fermented pepper juice yogurt powder.
[5)]HFY: high fat and high cholesterol diet supplemented with 5% fermented pepper juice yogurt powder.
[6)]PAT: perirenal adipose tissue.
[7)]EAT: epididymal adipose tissue.
[8)]NS: not significant.

compared to the CON group.

Weights of liver, spleen, kidney, cecum, PAT and EAT in animals are shown in Table 4. Weights of liver in high fat diet fed groups were significantly higher than CON group ($p<0.05$) possibly because of the existence of the depot fat in liver. There were no significant differences in liver weights in high-fat diet groups, which showed decreasing tendency as fermented pepper juice concentrate in yogurt was increased ($p>0.05$). Spleen weights in high fat diet fed groups were significantly higher than in the CON group ($p<0.05$), whereas the weight of the cecum was not significantly different weight among the 5 groups ($p>0.05$). Kidney weights in the HFCD and LFY groups were significantly higher than the CON group ($p<0.05$). Weights of PAT and EAT in the HFCD, PY, and LFY groups were significantly higher than the CON group ($p<0.05$), but HFY group was not ($p>0.05$). This might bring about the significantly lower body weight in HFY group than HFCD group ($p<0.05$). Lipid such as high body fat and cholesterols is absorbed to liver and it can cause fatty liver, moreover other hepatic diseases, which are associated with obesity (Tessari *et al*., 2009). HFY group showed significantly lower body fat ($p<0.05$), therefore fermented pepper might lower occur of hepatic diseases.

Amounts of serum glucose, AST, total cholesterol, and HDL cholesterol are expressed in Table 5. Akiyama et al

Table 5. Level of serum glucose, aspartate aminotransferase, total cholesterol, HDL cholesterol in CON, HFCD, PY, LFY, and HFY group after 9 wk of experiment

Item	CON[1)]	HFCD[2)]	PY[3)]	LFY[4)]	HFY[5)]
GLU[6)] (mg/dL) (n=5)	78.4±11.01[NS10)]	63.8±13.48	81.6±16.40	72.0±5.48	83.8±19.07
AST[7)] (U/L) (n=4)	329.3±16.56[b]	644.0±80.94[a]	606.8±125.23[a]	704.5±46.62[a]	572.0±132.77[a]
TC[8)] (mg/dL) (n=4)	70.8±2.87[b]	106.5±18.12[a]	113.3±11.09[a]	111.5±9.98[a]	88.0±16.99[ab]
HDL-C[9)] (mg/dL) (n=4)	68.0±9.20[a]	51.0±4.24[b]	61.0±1.15[ab]	62.0±7.26[ab]	54.75±4.57[b]

[a,b]Superscripts with different letters indicate significant difference ($p<0.05$).
[1)]CON: control diet fed group.
[2)]HFCD: high fat and high cholesterol diet fed group.
[3)]PY: high fat and high cholesterol diet supplemented with 2% plain yogurt powder.
[4)]LFY: high fat and high cholesterol diet supplemented with 2% fermented pepper juice yogurt powder.
[5)]HFY: high fat and high cholesterol diet supplemented with 5% fermented pepper juice yogurt powder.
[6)]GLU: glucose.
[7)]AST: aspartate aminotransferase.
[8)]TC: total cholesterol.
[9)]HDL-C: high density lipoprotein cholesterol.
[10)]NS: not significant.

(1996) reported that feeding a high fat diet to rat increases serum glucose concentration even after a 24 h fasting for 4 wk. However, in this experiment, serum glucose content showed no significant difference ($p>0.05$). This difference might be resulted from different strain and feeding duration (Wirth-Dzięciołowska *et al*., 2009). AST exist in almost all organ such as heart, liver, skeletal muscle, and kidney and it would be released to blood, if the organs are damaged (Rappaport, 1993). In this research, AST content in serum was measured, and the high fat diet fed groups were significantly higher than CON group ($p<0.05$), maybe because of the damage from depot fat in the liver. Total cholesterol concentration in the HFY group was decreased compared to other high-fat diet groups, and it was not significantly different from the CON group ($p>0.05$). According to reports of Tani et al (Tani *et al*., 2004) and Srinivasan and Satyanarayana (Srinivasan and Satyanarayana, 1987), total cholesterol level in capsaicin supplemented diet fed group significantly decreased ($p<0.05$), and cholesterol excretion through fecal showed increasing tendency than control group (Tani *et al*., 2004). In this study, fermented product such as cleavage of capsaicin might act as a role of capsaicin in report of Tani *et al*. It means fast lipid metabolism in HFY group than HFCD group. HDL cholesterol amount in the HFCD group was the lowest and showed significantly lower amounts compared to that of the CON group ($p<0.05$). On the other hand, the PY and LFY groups showed insignificant differences with the CON group ($p>0.05$).

Amounts of total lipid and total cholesterol in liver are represented in Table 6. The HFCD group showed the highest amount of hepatic total lipid among the 5 groups, significantly ($p<0.05$). Level of total lipid in the PY, LFY, and HFY groups were decreased significantly compared to the HFCD group ($p<0.05$). The PY and LFY groups showed no significant differences with the CON group ($p>0.05$). In total cholesterol, the HFCD and PY groups were significantly higher than the CON group ($p<0.05$) and the LFY and HFY groups showed decreased levels compared to the HFCD group and had no significant differences with the CON group ($p>0.05$). This result means that fermented pepper powder might inhibit the formation of cholesterol in liver.

Generally, absorbed capsaicin is metabolized to vanillylamine, and subsequently vanillin, vanillyl alcohol, and vanillic acid (Kawada and Iwai, 1985). These bind with transient receptor potential vanilloid 1 (TRPV1) and it secretes catecholamine and stimulates the sympathetic nerve (Iwai and Watanabe, 1999). It results in lipolysis, body heat by energy metabolism, and inhibition of body fat accumulation (Iwai and Watanabe, 1999; Iwasaki *et al*., 2011).

Fermentation products, such as cleavages of degraded capsaicin, in fermented pepper juice would be transferred and added to yogurt. When it is absorbed, this also would stimulate TRPV1, as capsaicin metabolites, but without pungency.

In this study, fermented pepper juice supplemented yogurt fed group showed anti-obesity, and improvement in lipid metabolism. Therefore, the final fermentation product made from fermented pepper juice might act as an inhibitor of both hypertrophy in organ and accumulation of body fat. It might effect on accumulation of hepatic lipid and high cholesterol in high fat and high cholesterol diet fed rats. Further study would be needed about the fermentation product of pepper and the identification of mechanism.

Acknowledgements

This research was supported by Technology Development Program for iPET (Korea Institute in Food, Agricul-

Table 6. Hepatic total lipid and total cholesterol amounts in CON, HFCD, PY, LFY and HFY group after 9 wk of experiment

Item	CON[1)]	HFCD[2)]	PY[3)]	LFY[4)]	HFY[5)]
TL[6)] (g) (n=3)	95.5±0.47[c]	124.5±2.29[a]	94.9±5.33[c]	100.4±3.88[c]	110.9±1.14[b]
TC[7)] (mg/dL) (n=3)	43.1±9.11[b]	137.5±35.96[a]	122.0±45.46[a]	110.2±8.55[ab]	106.5±27.16[ab]

[a-c]Superscripts with different letters indicate significant difference ($p<0.05$).
[1)]CON: control diet fed group.
[2)]HFCD: high fat and high cholesterol diet fed group.
[3)]PY: high fat and high cholesterol diet supplemented with 2% plain yogurt powder.
[4)]LFY: high fat and high cholesterol diet supplemented with 2% fermented pepper juice yogurt powder.
[5)]HFY: high fat and high cholesterol diet supplemented with 5% fermented pepper juice yogurt powder.
[6)]TL: total lipid.
[7)]TC: total cholesterol.

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