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Chungkookjang Improves Blood Circulation

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INSTRUCTION

Food contains not only nutrition substances but also bioactive components which can prevent life-style related diseases. Functional food components can lower sugar and cholesterol levels in the blood, reduce blood pressure. Some functional food can improve intestine function, and allergy sensitivity, stimulate immune function, and mineral absorption. In Korea, Chungkookjang, a fermented soybean food emerges as a functional food to improve gastrointestinal conditions and blood circulation (1). Chungkookjang contains microorganisms, enzymes, and diverse bioactive material by fermentation, which are absent in original soybean.

Also, Chungkookjang can be made without using sodium chloride, which is different from making Doenjang (another Korean fermented soybean product). Koreans take too much salt. This may be related to high occurrence of stomach cancer, high blood pressure, and strokes. Thus, eating Chungkookjang is desirable in this sense.

Bacillus species ferment soybean (1). Bacillus protease possesses fibrinolytic activity in human body (2,3). The fibrinolytic protease dissolves fibrin directly or stimulate tPA production indirectly. During fermentation, Bacillus β-glucosidase cleaves genistin's sugar molecule, converting it to the better-absorbed genistein (4). Genistein in Chungkookjang possesses antioxidant and anti-cancer effects (5). Fermented soyfood may decrease the risk of breast or prostate cancer. Reactive oxygen and free radicals are believed to result in aging, inflammation, and cancer. Chungkookjang contains diverse antioxidant components including amino acids and amino-carbonyl reactive substances (melanoidin) (1). The antioxidants might prevent oxidative injury, preventing life-style related diseases. The antioxidant activity of Chungkookjang was investigated in this study. Some peptides play the role of angiotensin converting enzyme (ACE) inhibitor (6). Chungkookjang contains various oligo-peptides. Whether or not eating Chungkookjang can lower blood pressure was also determined. Chungkookjang rich in antioxidants and anti-hypertensive substances may be helpful in improving blood circulation.

MATERIALS AND METHODS

Chungkookjang fermentation

Bacillus licheniformis B1 was cultured in LB media 37°C for 18 hr. The culture was used as a starter. Soybean was soaked in water for 18 hr, and autoclaved at 120°C for 30 min. The culture was added onto 500 g of autoclaved soybean, to be 1%. Then, it was incubated at 40°C.

Extraction of antioxidants

Powder of steamed soybean and fermented soybean were prepared by freeze-drying. They were suspended in 100% ethanol to be 1% and stored 26°C for 20 hr. It was centrifuged at 4°C, 15,000×g for 30 min. The supernatant was used to determine the antioxidant activity.

Determination of antioxidant activity

Antioxidant activity was determined using DPPH (1,1-diphenyl-2-picrylhydrazyl, Sigma, St. Louis, USA), by

measuring absorbance decrease at 517 nm (4).

Determination of antioxidant activity of fermented soybean in vivo

E. coli DH5 was cultured in LB liquid media for 15 hr. This was inoculated onto GM media (7) Paraquat (0.02 g/mL) was added into the media. Its inhibitory effect on growth was measured. Ethanol extract of fermented soybean powder (0.1 g) was added into 100 mL of the GM liquid media containing paraquat. Growth of strain was measured at OD 600 nm, to determine effect of fermented soybean components on the recovery of suppressed *E. coli* growth.

Determination of blood pressure

Blood pressure was measured using automatic blood pressure checker (Omron R4 model, Omron Corporation, Tokyo, Japan). Blood pressure of volunteers was measured before taking the powder and 2 hr after the single oral administration, three times, and its average was recorded.

RESULTS AND DISCUSSION

Antioxidant activity of fermented soybean

Grains, vegetables, fruits, see food, and medicinal herb contain their antioxidants mainly in the envelopes to protect themselves from toxic UV. Antioxidant activity of soybean was twice as high as that of barley, wormwood, and sea tangle as determined by DPPH method (data not shown). Antioxidant activity of fermented soybean was 1.9 times that of steamed soybean (Fig. 1). The inhibitory effect of the natto fractions on LDL oxidation in vitro and in vivo was demonstrated (8,9).

In soybean, ferulic acid, *p*-coumaric acid, chlorogenic acid, isochlorogenic acid, caffeic acid, syringic acid, vanillic acid, *p*-hydorxybenzoic acid, genistin, daidzin, amino acids are known to be antioxidants (4).

There is no report why fermented soybean shows increasing antioxidant activity. Genistin in soybean is converted into aglycon genistein by β-glucosidase during fermentation (4). Also antioxidant activity of 8-hydroxy-

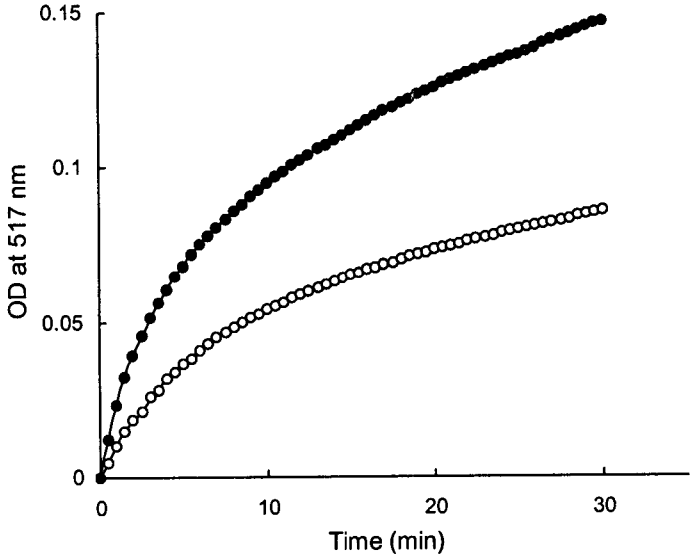

Fig. 1. Increase of antioxidant activity. Steamed soybean (○) and fermented soybean (●) were extracted with 100% ethanol. Extracted material was mixed with DPPH solution, and each antioxidant activity was determined by measuring decrease of OD at 517 nm.

genistein further metabolized during fermentation was twenty times as high as that of genistein (4). The enzymes produced during fermentation in soybean include xylanase, pectinase, cellulase and protease (1). These diverse enzymes might convert inactive antioxidants into active ones. Melanoidin, another antioxidant (10), produced during fermentation might contribute to increasing antioxidant activity in Chungkookjang (1).

The absorbance spectra of ethanol extract of soybean and fermented soybean were measured to detect any difference between the two. In a range between 250 and 300 nm, the absorbance values of fermented soybean were higher than those of steamed soybean (Fig. 2). The higher level of fermented soybean in the range might be related to higher antioxidant activity of fermented soybean. Phenol absorbance is evident at 285 nm. The amino acids containing phenolic compounds might increase during fermentation.

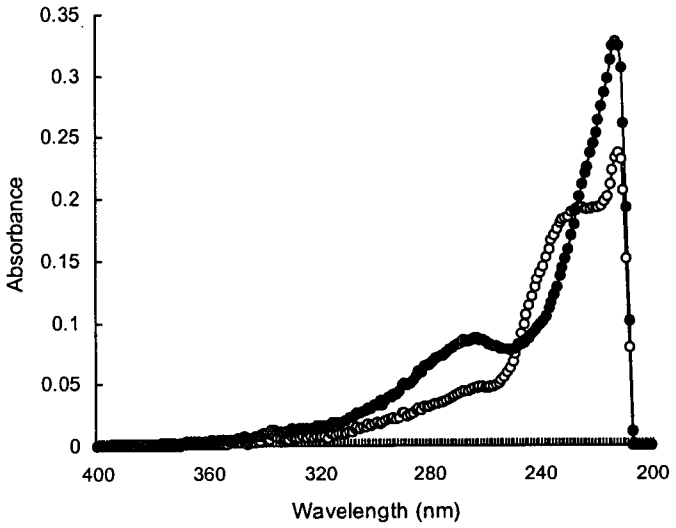

Fig. 2. UV-VIS spectra of extract from soybean and fermented soybean. Substances extracted with ethanol from steamed soybean (○) and fermented soybean (●) were scanned by the spectrophotometer in a range between 200 and 500 nm.

Usage of fermented soybean in vivo

Paraquat is highly toxic to organisms (7). Once paraquat is metabolized in *E. coli*, it produces superoxide (7). In the normal media, induced Mn-SOD (superoxide dismutase) can detoxify superoxide within 1 hr (7). In the GM minimal media, Mn-SOD is barely produced (7). *E. coli* could not grow in the minimal media containing paraquat for 12 hr (Fig. 3). If *E. coli* growth can be recovered by adding fermented soybean, then the recovery is due to antioxidant activity of fermented soybean to catalize the dismutation of intracellular superoxide.

Without paraquat treatment, *E. coli* grew to OD 1.10 after 12 hr culture in the GM minimal media (Fig. 3). Paraquat treatment inhibited *E. coli* growth to OD 0.23 (Fig. 3). By adding 0.1 mL of the ethanol extract of fermented soybean, *E. coli* growth was recovered to OD 0.37 (Fig. 3). By adding 1 mL of the ethanol extract of fermented soybean, *E. coli* growth was recovered to OD 0.53 (Fig. 3). Thus, recovery of *E. coli* growth was dependent upon concerntration of fermented soybean. This suggests that some components in fermented soybean enter *E. coli* cells, act as SOD mimics, and protect them against the lethality of paraquat.

SOD is known to be effective at reducing superoxide (7). However, it can not enter the cell. Amino acids and peptides coupled with cupper exhibit high SOD-like activity (11). It seems that some amino acids in fermented soybean can be taken into *E. coli* and shows SOD-like activity. It will be possible that antioxidant material derived from fermented soybean can be absorbed into human body, as it was used in *E. coli*.

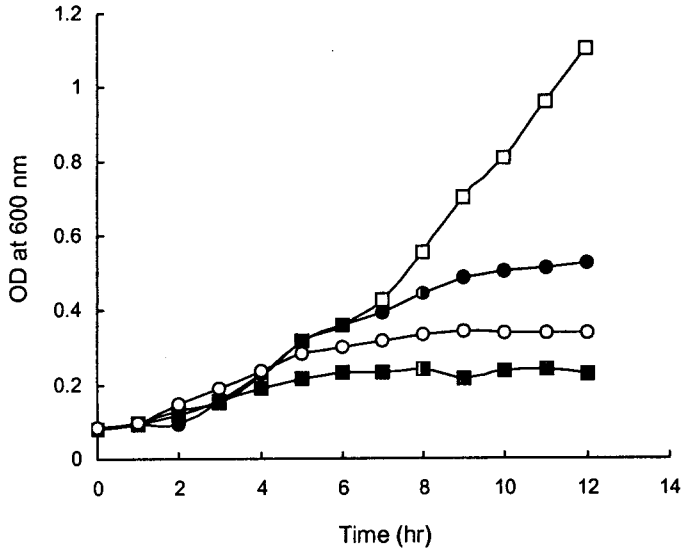

Fig. 3. Effect of fermented soybean extract on the growth of *E. coli* suppressed by paraquat. *E. coli* growth in the GM media (□), in the GM media containing paraquat (■), in the GM media containing paraquat plus 0.1 mL of ethanol extract of fermented soybean (○) or 1 mL (●) was measured by determining OD values at 600 nm, respectively.

Blood-pressure reduction effect

The participants were given 20 g of fermented soybean and their blood pressures 2 hr after the administration were measured. Among normal group of hypertension, significant change of blood pressure was not observed (data not shown). Average of volunteer's systolic blood pressure among the group of hypertension fell from 151 mmHg to 137 after taking it. Diastolic blood pressure dropped from 118 mmHg to 115. Considering drop of average blood pressure(14 mmHg) in 2 hr, Chungkookjang powder seems to be effective in lowering blood pressure. More volunteers in a large scale are needed to determine anti-hypertensive effect of Chungkookjang. Once the effect is proved, Chungkookjang can be developed as a functional food to lower blood pressure.

Angiotensin converting enzyme (ACE) converts angiotensin I into II which is involved in increase of blood pressure (6,12). ACE also inactivates bradykinin which is involved in blood pressure reduction, resulting in increase of blood pressure (6). ACE inhibitors were found in the protein digests of seed (13) and sardine (6) protein. In fermented soybean, Ile-Phe-Leu, Trp-leu, His-His-Leu were reported to be ACE inhibitors (14-16). Some peptides in our Chungkookjang sample might play roles of ACE inhibitors. Purification of inhibitory peptides in Chungkookjang and its characterization is under study.

CONCLUSION

Ethanol extract of Chungkookjang demonstrated increasing antioxidant activity when compared to that of autoclaved soybean. The inhibitory effect of paraquat was reversed by the ethanol extract of Chungkookjang. Chungkookjang powder was shown to have hypotensive effect.

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