Korean J. Food Sci. Ani. Resour.
Vol. 30, No. 5, pp. 773~779(2010)

ARTICLE

Physicochemical Properties of *Dioscorea opposita* Thunb. Solution Fermented by *Lactobacillus bulgaricus*

Ju Hyun Park, Joungjwa Ahn[1], and Hae-Soo Kwak*
Department of Food Science and Technology, Sejong University, Seoul 143-747, Korea
[1]Department of Herb Industry, Jungwon University, Goesan 367-805, Korea

Abstract

The present study was designed to determine the physicochemical properties of yam (*Dioscorea*) solution fermented by *L. bulgaricu*s, the protective effect of fermented *Dioscorea opposita* Thunb. on stomach lesions, and the possibility of its application to fermented dairy products. When the concentration of yam increased, the pH increased in raw powder samples but not in extracted powder samples. In 6% raw powder containing the sample, the viable cell counts showed a dramatic increase, whereas those in extracted yam powder significantly decreased in all concentrations during 32 h of fermentation. The viscosity of extracted yam powder showed a lower value than that of raw powder, which was maintained throughout the fermentation. The solution of *Dioscorea opposita* Thunb. raw powder showed a significantly higher allantoin content compared with the extracted powder ($p<0.05$). At 2% of added *Dioscorea opposita* Thunb., 0.65 mg/mL was contained in both samples; however, when 6% of *Dioscorea opposita* Thunb. was added, the amount of diosgenin increased up to 1.9 mg/mL. When 200 mg of powder/kg body wt. was injected, gastric lesions were apparently reduced compared with those of control rats. With 100 mg/kg body wt. injected into rats, the protective effect was a little lower than that in 200 mg/kg body wt. Based on these data, the present study indicated that yam powder fermented by *L. bulgaricus* showed a protective effect on gastric lesions in rats; therefore, it may be included as an added ingredient in yogurt manufacture.

Key words: *Dioscorea opposita* Thunb., *L. bulgaricus* fermentation, allantoin, diosgenin, gastric lesion

Introduction

Yam, the tuber part of the genus *Dioscorea* is cultivated world-wide as a food source, especially in tropical areas. In Asia, various yams have been used for many years as a traditional medicine (Shin *et al*., 2006) for the treatment of anorexia, chronic diarrhea, diabetes, and excessive leucorrhea (Pharmacopoeia of the People's Republic of China, 1997). Recently, immune response modifying activities of *Dioscorea* rhizomes have been confirmed, including immunomodulation (Zhao *et al*., 2005) and anti-inflammatory (Kim *et al*., 2004), anti-tumor (Hu and Yao, 2003a, 2003b), and anti-osteoporotic effects (Yin *et al*., 2004). In addition, the anti-oxidative activities of a storage protein of *Dioscorea* tubers have also been extensively investigated (Hou *et al*., 1999; Hou *et al*., 2001). Furthermore, the feeding of *Dioscorea* rhizomes has been found to improve certain metabolic abnormalities, including hyperglycemia (McAnuff *et al*., 2005), obesity, gut function (Shin *et al*., 2006), and lipid metabolism (Jeon *et al*., 2006).

Recently, there has been a global trend toward the use of natural substances in fruits, vegetables, and herbs as antioxidants and anti-cancer agents. Among them, the yam is an important pharmaceutical plant that is widely used in drug industry. The *Dioscorea* rhizome contained much higher level of allantoin than those in potato, sweet potato, that is considered to present inflammation and ulcers in the human body (Fu *et al*., 2006). Based on the wide use and clinical acceptance of allantoin (Garnick, Singh and Winkley, 1998; Yamamoto *et al*., 1998), allantoin has long been known to enhance the efficacy and desirability of cosmetic creams and lotions through its action as a skin protectant. The Merck Index lists the therapeutic applications of allantoin as a topical vulnerary (wound healer) and treatment for skin ulcers (The Merck Index, 2001). The allantoin is demulcent and works to soothe irritative conditions, both internally and externally. Therefore, the allantoin protects tissues in the stomach,

*Corresponding author: Hae-Soo Kwak, Department of Food Science and Technology, Sejong University, Seoul 143-747, Korea. Tel: 82-2-3408-3226, Fax: 82-2-3408-4319, E-mail: kwakhs@sejong.ac.kr

accelerates the healing processes throughout the stomach and bowels, and promotes increased tissue repair throughout the entire gastrointestinal tract.

Another compound, diosgenin, a member of steroidal sapogenin found in several plants including *Solanum* and *Dioscorea* species, has gained in importance recently. Diosgenin has been shown to have not only favorable effects on lipid metabolism but also anti-tumor effects on cancer cells (Liu *et al*., 2005).

Fermented foods constitute a large proportion of the diets. Such foods are important because of their increased nutritional and physiological functions as well as improved flavor and aroma characteristics. Many of the fermented food products result in new and desired products and preservative effects are incidental. Lactic acid bacteria, as probiotic bacteria, have been known to be beneficial to health, including increased food digestibility and bioavailability of vitamin and minerals, control of gastrointestinal infection, reduction of serum cholesterol level, immune stimulation, and reduction of blood pressure (Carr *et al*., 2002; Gilliland, 1990; Gorbach, 1990; Yoo *et al*., 2005). In Asian countries, lactic acid fermented grains, vegetables, and fish for their long-term preservation have been taken for many years (Lee, 1997; Yoon *et al*., 2004). However, until now, lactic acid fermented natural herbs have not been reported. In addition, yam (*Dioscorea*) has been revealed the various functions compared with other genus, little information of its application and research has been shown. Therefore, this study was designed to find the physicochemical properties of fermented yam (*Dioscorea*) solution by *L. bulgaricus* and possibility of its application on fermented dairy products.

Materials and Methods

Materials

Dioscorea opposita Thunb. was used in the present study. Tubers of *Dioscorea opposita* Thunb. were obtained from Research Center of Dongyang Industry (Seoul, Korea) and stored at 4°C until use. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA), and all solvents were chromatographic grade.

Preparation of raw and extracted *Dioscorea opposita* Thunb. powder solution

To prepare the raw *Dioscorea opposita* Thunb. solution powder, tubers of yam was washed, minced and spray-dried. Then two times of distilled water was added into the spray-dried yam sample and heated to 95°C for 120 min to discard the viscous mucilage. After centrifugation, the supernatant was transferred and added with Non-GMO dextrin powder (DE=8) and centrifuged again. Then, the supernatant was filtered and spray-dried for the extracted powder yam.

Preparation of fermented *Dioscorea opposita* Thunb. powder

The fermented *Dioscorea opposita* Thunb. was prepared as follows: *Lactobacillus bulgaricus* was incubated at MRS broth at 37°C for 24 h and centrifuged. The precipitant (1.0×10^{10} CFU/mL) was washed with distilled water for 3 times and used as fermenting microorganism. The raw and extracted powder were pasteurized at 60°C for 30 min and three different concentrations of powder (2, 4, and 6%) were made with distilled water and inoculated by microorganism for fermented *Dioscorea opposita* Thunb. solution at 37°C for 0, 8, 16, 24, and 32 h in 200 rpm shaking incubator (KSI-200L, Seoul, Korea).

Chemical analyses

Chemical composition: Protein, fat, moisture, and ash contents were measured by AOAC method. The pH and titratable acidity (TA): pH and titratable acidity of the samples were measured at room temperature. The TA was determined after mixing the 9 mL sample with same amount of distilled water and titrating with 0.1 N NaOH using a 1% phenolphthalein indicator to an end point of faint pink color. The formula for calculating percentage of lactic acid follows:

$$\text{Lactic acid (\%)} = \text{0.1 N NaOH used (mL)} \times 0.009 \times 100/\text{sample (mL)}$$

Microbial analysis

MRS plate count agar was used for *L. bulgaricus* counting. One millileter of sample diluted with 9 mL of sterile 0.1% peptone and water diluent. Subsequent dilutions of each sample were plated in triplicate and incubated at 37°C for 72 h.

Viscosity

Samples were placed in a Brookfield viscometer (spindle No. 2) and measured from 1 to 5 min with 1 min period with 100 rpm.

Measurement

Allantoin and diosgenin were quantitatively analyzed by HPLC. Allantoin was analyzed on a Waters HPLC

system consisting of a 600 pump and operated using Millennium software, and diosgenin was analyzed on a Agilent 1200 Series HPLC system. The column used was a Phenomenex (USA) Luna 5u CN 100A (250 mm×4.60 mm) column for allantoin, SunFire™ C_{18} 5 μm (4.6 mm ×250 mm) for diosgenin analysis. Samples were placed in a Brookfield viscometer (Model LVDV I+, Version 3.0, Stongton, MA, USA) and pH meter (Orion 900A, Boston, MA, USA) was used for pH measurement.

Animals and sampling

Twenty-five 7-week-old male rats (specific pathogen free) were purchased from G-bio Animal, Inc. (Seoul, Korea). All rats were allowed access to diets and distilled water *ad libitum*. Rats were housed in individual cages at 22.3 ±2ºC, 50±10% humidity, and lights on from 19:00 to 07:00 h.

All rats were fasted for 18 h, and 60% EtOH containing 150 mM HCl was injected orally and induced the stomach lesion. For experimental groups, 100 or 200 mg/kg body weight of raw yam powder or fermented yam powder were treated. After 1 h, HCl-EtOH was injected and rats were sacrified. For control, 0.5% of CMC (carboxymethyl cellulose) was injected.

After sacrifice, stomach with partial duodenum and esophagus was taken and area of gastric lesion was measured. By the formula, the inhibition of gastric lesion was calculated as follows:

$$\text{Inhibition (\%)} = \text{gastric lesion area of experimental group (mm}^2\text{)} - \text{gastric lesion of control (mm}^2\text{)} \times 100 / \text{gastric lesion area of experimental group (mm}^2\text{)}$$

Statistical analysis

All statistical analyses were performed using SAS version 9.0 (SAS Institute Inc., Cary, NC). An ANOVA was performed using the general linear models procedure to determine significant differences among the samples. Means were compared by using Fisher's least significant difference procedure. Significance was defined at the 5% level.

Results and Discussion

Chemical composition

The composition of raw and extracted yam (*Dioscorea opposita* Thunb.) was presented in Table 1. The content of crude protein in raw yam was six times higher than that in extracted. However, crude fat was almost negligible in both powders. The content of ash in raw powder was significantly higher than that in extracted ($p<0.05$). But moisture content was significantly lower in raw powder than extracted ($p<0.05$). The crude carbohydrates were 71.2 and 68.2% in raw and extracted powder, respectively. As a functional component, the amount of allantoin showed that the content of raw powder was 0.69% and extracted powder was 0.44%. The other functional component was diosgenin which contained 3.19% in raw powder and 3.17% in extracted powder.

Table 1. Chemical composition of *Dioscorea opposita* Thunb. powder and extracted powder[1)]

Composition (%)	Raw powder	Extracted powder
Crude protein	14.33±0.77[a]	2.34±0.77[b]
Crude fat	0.01±0.01[a]	0.01±0.01[a]
Ash	3.25±0.49[a]	1.13±0.40[b]
Moisture	3.14±0.15[b]	4.29±0.30[a]
Crude carbohydrate	71.17±5.00[a]	68.20±3.55[a]
Allantoin	0.69±0.08[a]	0.44±0.07[b]
Diosgenin	3.19±0.25[a]	3.17±0.21[a]

[1)]Values within the same row with different superscripts are significantly different at $p<0.05$.

Yoon *et al*. (2008) reported about the content of allantoin that *Dioscorea batatas* Decne. (long yam) had 0.44-0.45%, *Dioscorea japonica* Thunb. 0.62-0.64%, and *Dioscorea opposita* Thunb. 0.69-0.71%. This data agreed with our result in allantoin of *Dioscorea opposita* Thunb. and showed other *Dioscoreas* have significantly lower in concentrations. Chang *et al*. (2005) studied about the content of diosgenin that *Dioscorea japonica* Thunb. had lower amount of diosgenin (2.61%) than that of *Dioscorea opposita* Thunb. (3.32%) which was close to our result. Finally, we observed that *Dioscorea opposita* Thunb. was superior to the other *Dioscoreas*.

Physicochemical properties of fermented powder of *Dioscorea opposita* Thunb.

pH and titratable acidity (TA)

Figs. 1 and 2 showed the changes in pH and titratable acidity of the present study, respectively. Before the fermentation of raw or extracted *Dioscorea opposita* Thunb. powders, pH was about 6.0 for raw powder and 5.3 for extracted powder (Fig. 1). When the *L. bulgaricus* was inoculated, pHs of all different concentration groups (2, 4, and 6% of raw or extracted powder) decreased profoundly to 5.3 and 4.4 for raw and extracted powder containing groups, respectively.

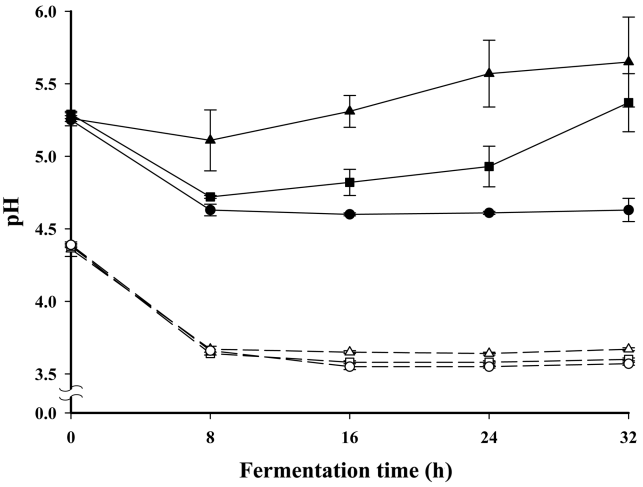

Fig. 1. Changes of pH in different concentrations of raw or extracted powder of *Dioscorea opposita* Thunb. fermented with *Lactobacillus bulgaricus* at 37°C for 32 h. –▲–: Raw powder 2%, –■–: Raw powder 4%, –●–: Raw powder 6%, ––△––: Extracted Powder 2%, ––□––: Extracted powder 4%, ––○––: Extracted powder 6%.

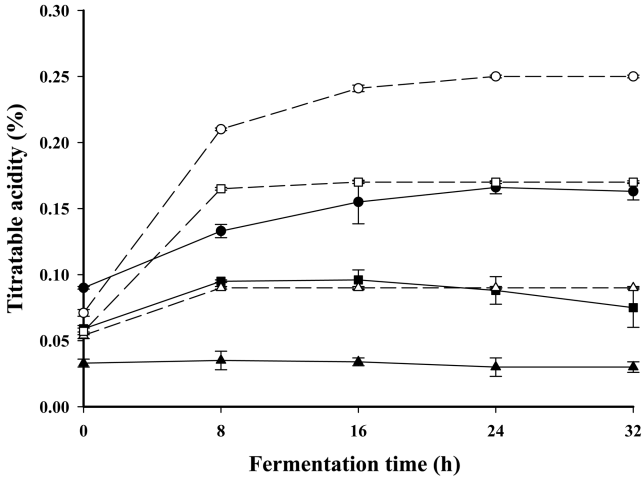

Fig. 2. Changes of titratable acidity in different concentrations of raw or extracted powder of *Dioscorea opposite* Thunb. fermented with *Lactobacillus bulgaricus* at 37°C for 32 h. –▲–: Raw powder 2%, –■–: Raw powder 4%, –●–: Raw powder 6%, ––△––: Extracted Powder 2%, ––□––: Extracted powder 4%, ––○––: Extracted powder 6%.

After 8 h fermentation, both of two powder groups showed a significant decrease in pH and maintained thereafter up to 32 h fermentation period, except for 4 and 6% raw powder containing groups. When the concentration of yam increased, the pH increased in raw powder samples but not in extracted powder samples.

Fig. 2 shows the changes of TA (%) values for the raw and extracted powder with different concentrations during 32 h fermentation. Unexpectedly, the TA values were significantly different within the extracted samples ($p<0.05$), which were not different in pH measurement. These results may be attributed to the hydrolysis of the carbohydrate and subsequent conversion of the sugars to acid by the fermenting microbes.

Shin *et al*. (2006) studied the fermentation of *Dioscorea batatas* Decne. in solution 5 and 10% fermented by the combination culture of *L. acidophilus*, *S. thermophilus* and *B. bifidum* at 37°C for 20 h. They observed 4.33 in pH and 0.24% TA which was similar trend to our result. This result indicated that the fermentation of *Dioscorea* by lactic acid bacteria slightly decrease pH.

Counts of *Lactobacillus bulgaricus* during fermentation

Change in *L. bulgaricus* counts during fermentation are presented in Table 2. At the early stage of inoculation, microbial counts of raw powder samples were in the range of 1.32 to 1.40×10^8 CFU/mL, and showed the increasing trend thereafter up to 32 h. Especially, 6% raw powder containing sample showed an increase for 32 h fermentation. In contrast, the viable cell counts of *L. bulgaricus* in extracted yam powder showed the significant decrease in all three concentration samples during 32 h fermentation period. In 2% extracted powder containing sample, 2.03×10^6 cell counts was found at 32 h fermenta-

Table 2. Changes of viable cell count in different concentrations of *Dioscorea opposita* Thunb. powder and extracted powder fermented by *Lactobacillus bulgaricus* at 37°C for 32 h (CFU/mL)

Dioscorea opposita Thunb.	Concentration (%)	Fermentation time (h)				
		0	8	16	24	32
Raw powder	2	1.40×10^8	1.51×10^8	1.62×10^8	1.53×10^8	1.45×10^8
	4	1.32×10^8	1.70×10^8	2.05×10^8	1.95×10^8	1.90×10^8
	6	1.35×10^8	2.35×10^8	3.83×10^8	3.37×10^8	3.35×10^8
Extracted powder	2	1.25×10^8	7.56×10^7	9.23×10^6	2.75×10^6	2.03×10^6
	4	1.55×10^8	1.57×10^8	1.24×10^8	1.61×10^7	7.24×10^6
	6	2.17×10^8	2.25×10^8	2.30×10^8	6.18×10^7	2.34×10^7

tion, which were 100 times less than viable cell counts in 6% raw powder containing sample. Based on these data, raw yam powder was more effective for fermentation by *L. bulgaricus* than extracted powder. Shin *et al*. (2006) reported the fermentation of *Dioscorea batatas* Decne. in solution 5 and 10% fermented by the combination of *L. acidophilus*, *S. thermophilus* and *B. bifidum* for at 37°C 20 h was counted in 7.02 log CFU/mL which was lower than that in our study.

Viscosity

The change of viscosity was presented in Fig. 3. There was a decrease in the raw yam powder containing sample with 2, 4, and 6% and a similar viscosity was found at the end of fermentation. Especially, the viscosity of 6% raw yam powder containing sample was the highest value among samples and decreased after 8 h fermentation and continuously decreased thereafter. In contrast, the samples containing extracted yam powder did not show any change from the beginning to the end of fermentation period. These results were expected because there was a heating process to remove the viscous materials in extracted yam powder preparation. The present study indicated if there were the viscous materials in the samples, fermentation may decrease the viscosity.

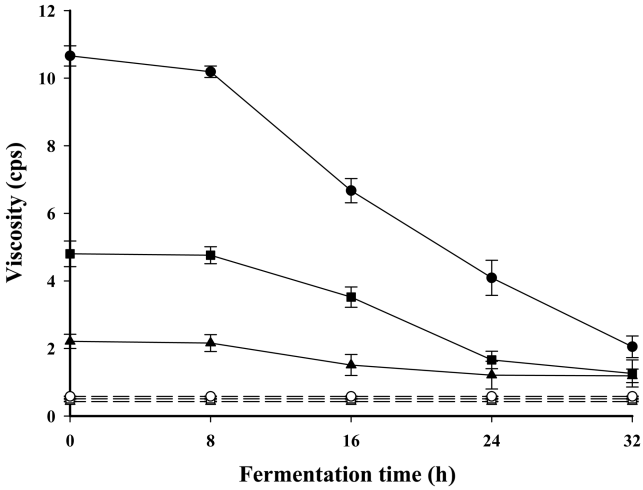

Fig. 3. Changes of viscosity in different concentrations of raw or extracted powder of *Dioscorea opposita* Thunb. fermented with *Lactobacillus bulgaricus* at 37°C for 32 h. –▲–: Raw powder 2%, –■–: Raw powder 4%, –●–: Raw powder 6%, ––△––: Extracted Powder 2%, ––□––: Extracted powder 4%, ––○––: Extracted powder 6%.

Allantoin and diosgenin contents

The changes of allantoin and diosgenin contents during fermentation are presented in Tables 3 and 4. In allantoin content, there was a significant difference between raw and extracted powder of *Dioscorea opposita* Thunb. ($p<$

Table 3. Concentrations of allantoin in different concentrations of *Dioscorea opposita* Thunb. powder and extracted powder fermented by *Lactobacillus bulgaricus* at 37°C for 32 h[1)] (mg/mL)

Dioscorea opposita Thunb.	Concentration (%)	Fermentation time (h)				
		0	8	16	24	32
Raw powder	2	0.14±0.01[a]	0.14±0.01[a]	0.13±0.01[a]	0.14±0.01[a]	0.13±0.01[a]
	4	0.26±0.03[a]	0.28±0.01[a]	0.27±0.01[a]	0.28±0.02[a]	0.27±0.01[a]
	6	0.41±0.01[a]	0.41±0.01[a]	0.42±0.01[a]	0.41±0.01[a]	0.41±0.01[a]
Extracted powder	2	0.09±0.01[a]	0.09±0.02[a]	0.09±0.02[a]	0.09±0.02[a]	0.09±0.01[a]
	4	0.17±0.01[a]	0.19±0.01[a]	0.19±0.02[a]	0.18±0.01[a]	0.19±0.02[a]
	6	0.30±0.02[a]	0.31±0.01[a]	0.30±0.06[a]	0.29±0.02[a]	0.31±0.02[a]

[1)]Values within the same row with different superscripts are significantly different at $p<0.05$.

Table 4. Concentrations of diosgenin in different concentrations of *Dioscorea opposita* Thunb. powder and extracted powder fermented by *Lactobacillus bulgaricus* at 37°C for 32 h[1)] (mg/mL)

Dioscorea opposita Thunb.	Concentration (%)	Fermentation time (h)				
		0	8	16	24	32
Raw powder	2	0.65±0.01[a]	0.64±0.01[a]	0.62±0.07[a]	0.64±0.03[a]	0.63±0.02[a]
	4	1.24±0.07[a]	1.30±0.07[a]	1.32±0.18[a]	1.27±0.03[a]	1.33±0.12[a]
	6	1.94±0.10[a]	1.86±0.03[a]	1.92±0.03[a]	1.89±0.16[a]	1.92±0.06[a]
Extracted powder	2	0.65±0.02[a]	0.65±0.01[a]	0.61±0.07[a]	0.65±0.03[a]	0.62±0.04[a]
	4	1.25±0.11[a]	1.26±0.09[a]	1.32±0.18[a]	1.27±0.04[a]	1.30±0.12[a]
	6	1.86±0.02[a]	1.86±0.03[a]	1.89±0.01[a]	1.92±0.11[a]	1.92±0.15[a]

[1)]Values within the same row with different superscripts are significantly different at $p<0.05$.

0.05), and raw powder showed the higher content of allantoin compared with the extracted powder. However, no difference was occurred by the different concentration added into samples in both raw and extracted powder samples. In addition, fermentation itself did not affect the allantoin content of yam powder.

In diosgenin content, the concentration of *Dioscorea opposita* Thunb. influenced on the diosgenin content of samples on both raw and extracted powders. No difference was found in the content of diosgenin during the fermentation period in both raw and extracted powder samples regardless of *Dioscorea opposita* Thunb. concentration added. Based on these results, the allantoin content was influenced by the extraction process of *Dioscorea opposita* Thunb., while the diosgenin content was dependent on their concentrations. Since both of the functional components have not been widely experimented after the fermentation of *Dioscorea*, it needs more study in various kinds of *Dioscorea*.

Area of stomach lesion

Based on the results, we decided to choose the 6% fermented powder of raw *Dioscorea opposita* Thunb. for examining the protective effect in gastric lesion in rats as shown in Table 5. When 200 mg/kg body wt. was injected, the gastric lesion was reduced apparently, compared with that in control rats. The protective effect was little lower with 100 mg/kg body wt. injected rats compared with those with 200 mg/kg body wt.

Several studies have reported that yam possessed estrogenic effect, angiotensin-converting enzyme inhibitory activity, and antioxidative effect (Fsu *et al*., 2002, Hsu *et al*., 2006). In other study (Hsu *et al*. 2006), Chinese yam supplement facilitated the growth of *Bifidobacterium* and *Lactobacillus* in mice small intestine. These results support that the intake of either Chinese yam or Japanese yam could effectively change intestinal microflora variation.

It has been documented that the antioxidant activities of plant foods are associated with their content of polyphenols and/or flavonoids (Yeh and Yen, 2005). The yam used in this study contained polyphenols and flavonoids, and the improved injury observed in yam-supplemented rats could be partially ascribed to these antioxidant compounds presented in yams.

Table 5. Effects of *Dioscorea opposita* Thunb. powder fermented by *Lactobacillus bulgaricus* on HCl-EtOH induced gastric lesions in rats[1)]

Dioscorea opposita Thunb.	Dose (mg/kg, p.o.)	Gastric lesions (mm^2)	Inhibition ratio (%)
Control[2)]	-	94.0^{a} ±31.2	-
Raw powder	100	57.0^{b}±20.2	39.3
	200	33.3^{bc}±20.8	64.6
Raw powder fermented by *L. bulgaricus*	100	56.1^{b} ±23.2	40.3
	200	32.2^{bc}±20.2	66.7

[1)]Values within the same column with different superscripts are significantly different at $p<0.05$.
[2)]0.5% carboxylmethyl cellulose.

Conclusion

The present study was designed to find the physicochemical properties of fermented yam (*Dioscorea*) by *L. bulgaricus*, the protective effect of fermented *Dioscorea opposita* Thunb. on stomach lesion and the possibility of its application on fermented dairy products. Several beneficial properties of yams have recently been reported in the literature and it has been found that feeding with *Dioscorea* species can improve gut function. In recent, Jeon *et al*. (2006) indicated that a 40% of ethanol extract of yam flour inhibited the secretion of gastric acid and showed the improvement of gut functions as observed by gastrointestinal transit and by lactose-promoting bacteria in feces. In the present study, when 200 mg of fermented yam powder/kg body wt. was injected, the gastric lesion was reduced apparently, compared with that in control rats. In addition, the protective effect was dose-dependent. Based on these data, the yam powder fermented by *L. bulgaricus* showed the protective effect on gastric lesion in rats, therefore, it may be applied for the manufacture of yogurt using fermented yam powder in further study.

Acknowledgement

This study was supported by a grant of the Brain Korea 21 Project in Seoul, Republic of Korea.

References

1. Carr, F. J., Chill, D., and Maida, N. (2002) The lactic acid bacteria: A literature survey. *Crit. Rev. Microobiol.* **28**, 281-370.
2. Chang, K. J., Park, B. J., Paek, J. H., Kim, S. L., and Park, C. H. (2005) Tuber enlargement and chemical components of yams (*Dioscorea opposita* Thunb.). *Korean J. Plant Res*. **18**, 161-168.
3. Fu, Y. C., Ferng, L. H. A., and Huang P. Y. (2006) Quantitative analysis of allantoin and allantoic acid in yam tuber,

mucilage skin and bulbil of the *Dioscorea* species. *Food Chem.* **94**, 541-549.
4. Fsu, F. L., Lin, Y. H., Lee, M. H., Lin, C. L., and Hou, W. C. (2002) Both dioscorin, the tuber storage protein of yam (*Dioscorea alata* cv. Tainong No. 1) and its peptic hydrolysates exhibited angiotensin converting enzyme inhibitory activities. *J. Agric. Food Chem*. **50**, 6109-6113.
5. Garnick, J. J., Singh, B., and Winkley, G. (1998) Effectiveness of a medicament containing silicon dioxide, aloe, and allantoin on aphthous stomatitis. *Oral Survey Oral Med. Oral Pathol. Endodon.* **86**, 550-556.
6. Gilliland, S. E. (1990) Health and nutritional benefits from lactic acid bacteria. *FEMS Microbiol. Rev.* **7**, 175-188.
7. Gorbach, S. L. (1990) Lactic acid bacteria and human health. *Ann. Med*. **22**, 37-41.
8. Hou, W. C., Chen, H. J., and Lin, Y. H. (1999) Dioscorins, the major tuber storage proteins of yam (*Dioscorea batatas* Decne), with dehydroascorbate reductase and monodehydroascorbate reductase activities. *Plant Sci.* **149**, 151-156.
9. Hou, W. C., Lee, M. H., Chen, H. J., Liang, W. L., Han, C. H., Liu, Y. W., and Lin, Y. H. (2001) Antioxidant activities of dioscorin, the storage protein of yam (*Dioscorea batatas* Decne) tuber. *J. Agric. Food Chem.* **49**, 4956-4960.
10. Hsu, C. C., Huang, Y. C., Yin, M. C., and Lin, S. J. (2006) Effct of yam (*Dioscorea alata* compared to *Dioscorea japonica*) on gastrointestinal function and antioxidant activity in mice. *J. Food Sci.* **71**, 513-516.
11. Hu, K. and Yao, X. (2003a) The cytotoxicity of methyl protodioscin against human cancer cell lines *in vitro*. *Cancer Invest.* **21**, 389-393.
12. Hu, K. and Yao, X. (2003b) The cytotoxicity of methyl protoneogracillin (NSC-698793) and gracillin (NSC-698787), two steroidal saponins from the rhizomes of *Dioscorea collettii* var. *hypoglauca*, against human cancer cells *in vitro*. *Phytother. Res.* **17**, 620-626.
13. Jeon, J. R., Lee, J. S., Lee, C. H., Kim, J. Y., Kim, S. D., and Nam, D. H. (2006) Effect of ethanol extract of dried Chinese yam (*Dioscorea batatas*) flour containing dioscin on gastrointestinal function in rat model. *Arch. Pharm. Res.* **29**, 348-353.
14. Kim, M. J., Kim, H. N., Kang, K. S., Baek, N. I., Kim, D. K., Kim, Y. S., Jeon, B. H., and Kim, S. H. (2004) Methanol extract of *Dioscorea batatas* inhibits proinflammatory cytokines and mediators in the synviocytes of rheumatoid arthritis. *Int. Immunopharmacol.* **4**, 1489-1497.
15. Lee, C. H. (1997) Lactic acid fermented foods and their benefits in Asia. *Food Control* **8**, 259-269.
16. Liu, M. J., Wang, Z., Ju, Y., Wong, R. N., and Wu, Q. Y. (2005) Diosgenin induces cell cycle arrest and apoptosis in human leukemia K562 cells with the disruption of Ca^{2+} homeostasis. *Cancer Chemother. Pharmacol.* **55**, 79-90.
17. McAnuff, M. A., Hardingm, W. W., Omoruyi, F. O., Jacobs, H., Morrison, E. Y., and Asemota, H. N. (2005) Hypoglycemic effects of steroidal sapogenins isolated from Jamaican bitter yam, *Dioscorea polygonoids*. *Food Chem. Toxicol.* **43**, 1667-1672.
18. Merck index (2001) An encyclopedia of chemicals, drugs, and biologicals. 13th ed, Smith, A., Heckelman, P., and Oneil, M. J. (eds.). Merck & Co., Inc., NJ.
19. Osuji, G. O., and Ory, R. L. (1987) Regulation of allantoin and allantoic acid degradation in the yam and sweet potato. *J. Agric. Food Chem.* **35**, 219-223.
20. Pharmacopoeia of the People's Republic of China (1997) Chemical Industry Press, Peijing, Vol. 1.
21. Shin, K. O. Jeon, J. R., Lee, J. S., Kim, J. Y., Lee, C. H., Kim, S. CD., Yu, Y, and Nam, D. H. (2006) Lactic acid fermentation of Chinese Yam (*Dioscorea batatas* Decne) flour and its pharmaceutical effect on gastrointestinal function in rat model. *Biotechol. Bioprocess Eng.* **11**, 240-244.
22. Yamamoto, R., Seki, M., Ouchi, K., Koyano, S., Nakazawa, Y., Nagatani, Y., and Sato, H. (1998) A rapid determination of allantoin by high-performance liquid chromatography using tris(hydroxyl-methyl)aminomethane–HCL buffer as a mobile phase. *J. Pharmaceu. Soc. Jpn.* **118**, 310-316.
23. Yeh, C. T. and Yen, G. C. (2005) Effct of vegetables on human phenolsulfotransferases in relation to their antioxidant activity and total phenolics. *Free Radic. Res.* **39**, 893-904.
24. Yin, J., Tezuka, Y., Kouda, K., Tran, Q. L., Miyahara, T., Chen, Y., and Kadota, S. (2004) Antiosteoporotic activity of the water extract of *Dioscorea spongiosa*. *Biol. Pharm. Bull.* **27**, 583-586.
25. Yoo, E. J., Lim, H. S., Park, K. O., and Choi, M. R. (2005) Cytotoxic antioxidative, and ACE inhibiting activities of Dolsan leaf mustard juice (DLMJ) treated with lactic acid bacteria. *Biotechnol. Bioprocess Eng.* **10**, 60-66.
26. Yoon, K. D., Yang, M. H., Chin, Y. W., Park, J. H., and Kim, J. (2008) Determination of allantoin in *Dioscorea Rhizoma* by high performance liquid chromatography using cyano columns. *Natural Product Sci.* **14**, 254-259.
27. Yoon, K. Y., Woodams, E. E., and Hang, Y. D. (2004) Probiotication of tomato juice by lactic acid bacteria. *J. Microbiol.* **42**, 315-318.
28. Zhao, G., Kan, J., Li, Z., and Chen, Z. (2005) Structural features and immunological activity of a polysaccharide from *Dioscorea opposita* Thunb. roots. *Carbohydr. Polym.* **61**, 125-131.

(Received 2010.7.2/Revised 2010.10.11/Accepted 2010.10.11)