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Nutraceutical Properties of *Dioscorea opposita* Thunb. (Yam) Fermented by *Lactobacillus bulgaricus* and *Streptococcus thermophilus*

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Abstract

This study was performed to determine by the ability of the mixed culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* to ferment *Dioscorea opposita* Thunb. (yam) and to evaluate the nutraceutical value of fermented yam. The titratable acidity (TA) value increased from 2 to 6% with increased concentrations in both raw yam and extracted lactic acid bacteria (LAB) fermented yam (LFY). The viable cell counts and the allantoin and diosgenin contents were higher in raw LFY at large concentrations (6%) than in extracted LFY samples at all fermentation periods up to 32 h. Based on these data, it confirmed that raw yam fermented by the combination culture of *L. bulgaricus* and *S. thermophilus* for various fermentation periods favors the symbiotic growth of LAB and results in higher nutraceutical content.

Keywords: *Dioscorea opposita* Thunb., allantoin, diosgenin, nutraceutical

Introduction

Health promoting functional foods with incorporation of lactic acid bacteria (LAB), nutraceuticals, and other functional components are the major research focus in food industry (Klaenhammer and Kullen, 1999; Yoon *et al*., 2004). This trend has favored the consumption of functional foods enriched with physiologically active components such as prebiotics, probiotics (Betoret *et al*., 2003) and synbiotics. Lactic acid fermentation processes therefore are of special importance for functional foods (Andersson *et al*.,1990; Gilliland, 1990; Gorbach, 1990; Lee, 1997) since the synbiotic (probiotic & prebiotic) properties could be obtained from an adequate combination of lactic acid bacteria (Schrezenmeir and de Vrese, 2001) and yam polysaccharides. This combination might improve the survival of bacteria crossing the upper part of the gastrointestinal tract, thereby improving their effects in the large bowel.

Yam (*Dioscorea*) also riches in starch, allantoin, diosgenin and other phytochemicals proved to be good substrate for prebiotics and health beneficial (Hsu *et al*., 2006; Wu *et al*., 2005). The *Dioscorea opposita* rhizome contained much higher level of allantoin (Fu *et al*., 2006) which accelerates the healing processes throughout the stomach and bowels, protects tissues in the stomach and increases tissue repair throughout the entire gastrointestinal tract. Diosgenin has been shown to have not only favourable effects on lipid metabolism but also anti-tumor effects on cancer cells (Liu *et al*., 2005; Shishodis and Aggarwal, 2006). Although yam has been revealed for various health benefits compared with other genus, little information of its nutraceutical value during fermentation by various cultures and its symbiotic role in fermentation is still limited. Therefore, this study was designed to evaluate the nutraceutical value of *Dioscorea opposita* Thunb. (yam) fermented by combined culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.

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Materials and Methods

1. Materials

Dioscorea opposita Thunb. was used in the present study. Tubers of *Dioscorea opposita* Thunb. were obtained from the Research Center of Dongyang Industry (Seoul, Korea) and stored

at 4℃ until use. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA), and all solvents had chromatographic grade.

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

The raw tubers were skinned, cut to 0.5-cm thick slices, lyophilized, milled, and then passed through a 100-mesh sieve. Then, twice the volume of distilled water was added into the lyophilized yam sample and heated to 95℃ 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.

3. Preparation of lactic acid bacteria fermented *Dioscorea opposita* Thunb. solution

The fermented *Dioscorea opposita* Thunb. was prepared as follows: The mixture of *L. bulgaricus* and *S. thermophilus* (LAB culture) in the ratio of 1:1 was incubated in MRS broth at 37℃ for 24 h and centrifuged. The precipitant was washed with distilled water 3 times and used as fermenting microorganism. The raw and extracted powders were sterilized at 60℃ for 30 min, and three different concentrations of powder (2, 4, and 6 %) were made with distilled water and inoculated by LAB culture for lactic acid bacteria fermented *Dioscorea opposita* Thunb. (LFY) for 0, 8, 16, 24 and 32 h with 200 rpm shaking.

4. Titratable acidity (TA)

The TA values of each LFY sample were determined after mixing the sample with 9 mL of hot distilled water (90℃) and titrating with 0.1 N NaOH containing 0.5% phenolphthalein as an indicator to an end point of faint pink color. All samples were measured in triplicate.

5. Microbial analysis

MRS plate count agar (Difco Laboratories, Detroit, MI, USA) was used for lactic acid bacteria counting. One milliliter of LFY samples was diluted with 9 mL of sterile peptone and water diluents. Subsequent dilutions of each sample were plated in triplicate and incubated at 37℃ for 48 h.

6. Viscosity

The viscosity of LFY samples (100 mL) was measured after mixing the LFY sample for 5 min at room temperature using a Brookfield viscometer (Model LVDV I+, Version 3.0, Stongton, MA, USA) with a spindle no 2 at 60 rpm. All samples were measured in triplicate.

7. Measurement of allantoin and diosgenin

To ten milliliters of LFY sample, 10 to 15 times of 95% ethanol (v/v) was added, and the mixture was ultrasonicated using sonicator (Branson 3210, Branson Ultrasonics Corporation, Danbury, CT, USA) for 10 min at 4℃ to be stored for over-night. The supernatant evaporated on a temperature-controlled water bath (Koma, Prime Tech, Seoul, Korea) at 40℃ for 5 min. Four mL of distilled water was added and ultrasonicated for 30 min at 4℃. One mL of sample was taken and centrifuged at 7,700 × g for 15 min. The supernatant was then filtered through a 0.45 μm nylon syringe filter (Whatman International Ltd., Maidstone, England) and then injected for analysis.

Allantoin and diosgenin were analyzed by Agilent 1,200 Series HPLC system with Agilent 1,200 series diode-array detector (Agilent technology, Santa Clara, CA, USA) and Waters SunFire^{TM} C18 5 μm (4.6 mm×250 mm) column (Milford, MA, USA), with isocratic elution buffer. The conditions were as follow: For allantoin, Mobile phase: $CH_3CH_2OH/CHCl_3/H_2O$ (0.5/0.012/100); Flow rate: 0.2 mL/min for 1 to 5 min, increasing to 0.5 mL/min after 5 to 15 min and to 0.2 mL/min after 15 to 20 min; UV wavelength: 215 nm; Injection volume: 20 μL. For diosgenin, Mobile phase: $CH_3CHOHCH_3/CH_3CN/H_2O$ (60/30/10); Flow rate: 0.7 mL/min; UV wavelength: 213 nm; injection volume: 20 μL. The allantoin and diosgenin were quantified using the respective external standards. A standard graph for each was prepared by plotting concentration versus area. Quantification was carried out from integrated peak areas of the sample and corresponding standard graph.

8. Statistical analysis

All statistical analyses were performed using SAS version 9.0 (SAS Institute Inc., 2002). An ANOVA was performed using the general linear models procedure to determine significant differences among the samples. Means were compared by using Duncan's multiple range test (p<0.05).

Results and Discussion

1. TA

The changes in TA values of LFY samples fermented at 37℃ for 32 h are shown in Fig. 1. The values of TA for all samples studied were greatly increased at 8 h. It could be suggested that the *L. bulgaricus* initiate the fermentation by hydrolyzing polymeric carbohydrates and generated fermentable sugars which are then used by the *S. thermophilus* and this results in a faster increase in TA within 8 h of fermentation (Achi and Akubor, 2000). When higher concentration of yam (6%) was added, the TA values were significantly higher within both samples ($P<0.05$). These results may be attributed to the higher content of the carbohydrate and subsequent conversion of the sugars to acid by the fermenting microbes (Daeschel *et al*., 1987; Park *et al*., 2010). The extracted LFY sample at all levels (2, 4, and 6%) showed higher TA values which were coincide with lower pH values (Data not shown). Simple sugars produced during extraction process are the primary substrates fermented by lactic acid bacteria and a higher concentration of these substrates will result in a more rapid fermentation process (Andersson *et al*., 1990). Some researchers also observed that the fermentation of *Dioscorea batatas* Decne. in solution 5 and 10% by the combination culture of *L. acidophilus*, *S. thermophilus* and *B. bifidus* at 37℃ for 20 h showed 0.24 TA. Thus it confirmed that raw and extracted yam powder can aid in the symbiotic growth of LAB cultures.

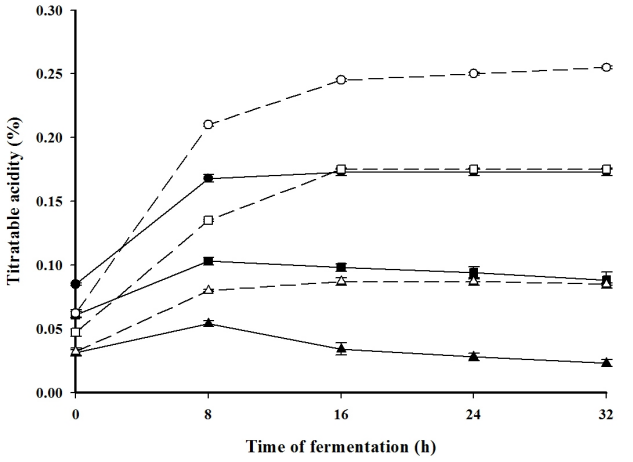

Fig. 1. Changes in titratable acidity of powdered *Dioscorea opposita* Thunb. fermented with *Lactobacillus bulgaricus* and *Sptreptococcus thermophilus* at 37℃ for 32 h.
—▲—, 2% raw LFY; —■—, 4% raw LFY; —●—, 6% raw LFY; —△—, 2% extracted LFY; —□—, 4% extracted LFY; —○—, 6% extracted LFY.

2. Microbial counts

The changes in LAB counts of LFY samples fermented at 37℃ for 32 h are shown in Table 1. At 0 h of fermentation, the mean microbial counts of the raw LFY samples were greater than those of extracted LFY samples at all concentrations (2, 4, and 6%). The dramatic increase in microbial cell count was found in 6% raw LFY sample at 8 h of fermentation. Moreover, increasing the concentration of raw powder from 2 to 6 % at 32 h, fermentation resulted in an increase of the mean microbial count from 2.25 to 4.65×10^8 CFU/mL. These findings can be explained by the fact that higher viscous mucilage and starch in raw yam powder enhance the growth of LAB during 32 h of fermentation. In our preliminary study, we also found that the raw yam fermented by *L. bulgaricus* at 37℃ for 32 h was counted in 3.35×10^8 CFU/mL which was lower than in our present study. Thus, it proves that symbiotic growth of LAB species enhances the microbial count in raw fermented yam. However, the extracted LFY samples doesn't follow the increasing trend at various levels (2, 4, and 6%) during fermentation for

Table 1. Changes in microbial count of powdered *Dioscorea opposita* Thunb. fermented by *Lactobacillus bulgaricus* and *Sptreptococcus thermophilus* at 37℃ for 32 h (CFU/mL)

Dioscorea opposita Thunb.	Concentration (%)	Fermentation time (h)				
		0	8	16	24	32
Raw LFY	2	1.33×10^8	1.75×10^8	2.00×10^8	2.35×10^8	2.25×10^8
	4	1.35×10^8	2.02×10^8	2.85×10^8	2.83×10^8	2.81×10^8
	6	1.41×10^8	3.15×10^8	5.02×10^8	5.05×10^8	4.65×10^8
Extracted LFY	2	1.11×10^8	1.36×10^8	2.30×10^7	2.14×10^6	4.38×10^5
	4	1.12×10^8	1.34×10^8	1.08×10^8	8.56×10^6	2.50×10^6
	6	1.11×10^8	1.61×10^8	2.37×10^8	1.35×10^8	9.50×10^7

LFY: Lactic acid bacteria fermented yam.

32 h. Based on these data, raw yam powder was little more effective for fermentation by LAB cultures than the extracted powder.

3. Viscosity

The change in viscosity of LFY samples is presented in Fig. 2. Dramatic decrease was found in the raw LFY sample regardless of concentration and no difference was found at the end of fermentation (p<0.05). Especially, the viscosity of 6% raw LFY sample was the highest value among samples, which then decreased after 8 hr of fermentation and continuously decreased thereafter. This was explained by fact that raw yam starch and other viscous substance undergo acid hydrolysis during fermentation result in rapid decrease in viscosity. 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. Mali *et al*. (2003) also observed that viscosity of yam decreased drastically as a consequence of starch hydrolysis promoted by acidic heat treatment. The present study indicated that if there were any viscous materials in the yam samples, fermentation by LAB may decrease the viscosity.

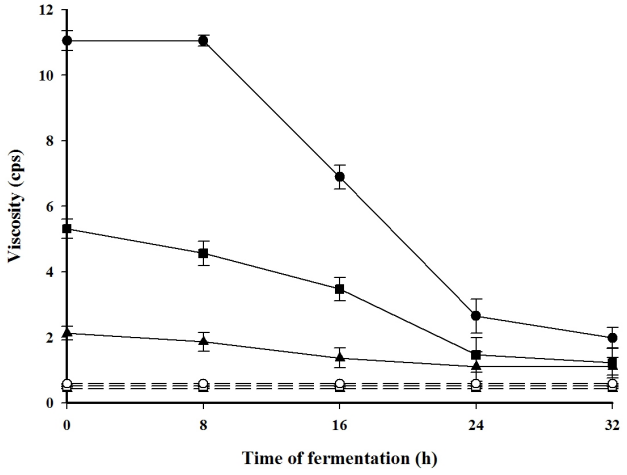

Fig. 2. Changes in viscosity of powdered *Dioscorea opposita* Thunb. fermented with *Lactobacillus bulgaricus* and *Sptreptococcus thermophilus* at 37℃ for 32 h.
—▲—, 2% raw LFY; —■—, 4% raw LFY; —●—, 6% raw LFY; —△—, 2% extracted LFY; —□—, 4% extracted LFY; —○—, 6% extracted LFY.

4. Allantoin and diosgenin

The changes in allantoin and diosgenin contents of LFY samples are presented in Tables 2 and 3. There was a significant difference in allantoin content between raw and extracted LFY samples (p<0.05). This was due to the higher concentrations of allantoin found in raw yam (Yoon *et al*., 2008) than extracted yam powder. However, extraction process does not affect the content of diosgenin (Data not shown). Further the fermentation process itself does not affect allantoin and diosgenin of both raw and extracted LFY sample through out fermentation period of 32 h (Tables 2 and 3). In our preliminary study, we also observed that allantoin content of both raw and extracted yam samples fermented by *L. bulgaricus* does not change during fermentation. Based on these results, it confirmed that the allantoin and diosgenin content does not effect during fermentation by LAB cultures and it can be used as a potential nutraceutical ingredient in various LAB fermented products for its health beneficial role.

Table 2. Changes in allantoin content of powdered *Dioscorea opposita* Thunb. fermented by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* at 37℃ for 32 h[1)] (mg/mL)

Dioscorea opposita Thunb.	Concentration (%)	Fermentation time (h)				
		0	8	16	24	32
Raw LFY	2	0.14±0.01[a]	0.13±0.02[a]	0.14±0.02[a]	0.14±0.01[a]	0.13±0.02[a]
	4	0.25±0.01[a]	0.28±0.01[a]	0.26±0.01[a]	0.26±0.01[a]	0.27±0.01[a]
	6	0.38±0.01[a]	0.37±0.01[a]	0.39±0.01[a]	0.39±0.01[a]	0.40±0.01[a]
Extracted LFY	2	0.08±0.01[a]	0.08±0.01[a]	0.08±0.01[a]	0.08±0.01[a]	0.08±0.01[a]
	4	0.18±0.01[a]	0.19±0.01[a]	0.17±0.01[a]	0.19±0.01[a]	0.18±0.01[a]
	6	0.26±0.01[a]	0.25±0.01[a]	0.27±0.02[a]	0.28±0.06[a]	0.27±0.03[a]

[1)] Values within the same row with different superscripts are significantly different at p<0.05.
LFY: Lactic acid bacteria fermented yam.

Table 3. Changes in diosgenin content of powdered *Dioscorea opposita* Thunb. fermented by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* at 37℃ for 32 h[1)] (mg/mL)

Dioscorea opposita Thunb.	Concentration (%)	Fermentation time (h)				
		0	8	16	24	32
Raw LFY	2	0.60±0.05[a]	0.65±0.03[a]	0.62±0.06[a]	0.62±0.04[a]	0.60±0.06[a]
	4	1.32±0.03[a]	1.26±0.13[a]	1.27±0.07[a]	1.31±0.04[a]	1.32±0.04[a]
	6	1.91±0.02[a]	1.96±0.07[a]	1.94±0.09[a]	1.90±0.05[a]	1.87±0.20[a]
Extracted LFY	2	0.62±0.03[a]	0.64±0.01[a]	0.64±0.05[a]	0.60±0.07[a]	0.60±0.01[a]
	4	1.29±0.08[a]	1.29±0.06[a]	1.34±0.10[a]	1.31±0.05[a]	1.26±0.12[a]
	6	1.90±0.07[a]	1.92±0.14[a]	1.93±0.06[a]	1.93±0.07[a]	1.84±0.04[a]

[1)] Values within the same row with different superscripts are significantly different at $p<0.05$.
LFY: Lactic acid bacteria fermented yam.

In conclusion, raw yam powder fermented by combination culture of LAB can enhance the symbiotic growth of both *L. bulgaricus* and *S. thermophilus*. Further fermentation of the yam with LAB doesn't affect the phytochemical content, such as allantoin and diosgenin of fermented yam powder. Thus raw yam powder fermented by the symbiotic culture of LAB may serve as an innovative nutraceutical food product with the supplement of allantoin and diosgenin.

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