

Full Length Research Paper

Effects of anti-hypertension and intestinal microflora of spontaneously hypertensive rats fed gamma-aminobutyric acid-enriched Chingshey purple sweet potato fermented milk by lactic acid bacteria

Cheng-Chih Tsai^{1*}, Tsai-Hsin Chiu², Cheng-Ying Ho¹, Pei-Pei Lin¹ and Tsung-Yen Wu³

¹Department of Food Science and Technology, HungKuang University, Shalu District, Taichung City, Taiwan, Republic of China.

²Department of Food Science, National Penghu University, Makung City, Penghu Hsien, Taiwan, Republic of China.

³Agricultural Research Institute, Council of Agriculture, Taichung City, Taiwan, Republic of China.

Accepted 8 March, 2013

This study investigated the anti-hypertensive effect of gamma-aminobutyric acid (GABA)-rich Chingshey purple sweet potato (CPSP) fermented-milk with three different lactic acid bacteria (LAB) strains including *Lactobacillus acidophilus* BCRC 14065, *Lactobacillus delbrueckii* ssp. *lactis* BCRC 12256, and *Lactobacillus gasseri* BCRC 14619. In a chronic administration study, the results showed that both systolic blood pressure (SBP) and diastolic blood pressure (DBP) were significantly decreased ($P < 0.05$) in spontaneously hypertensive rats (SHR) after receiving CPSP fermented-milk for 8 weeks. Blood pressure-lowering effects were typically measured after 5 weeks of treatment. Maximum blood pressure reductions were approximately 37 (SBP) and 22 mmHg (DBP) when compared with the control group. There was no significant difference in SBP and DBP before and after the 8 weeks of feeding CPSP fermented-milk to the normotensive Wistar Kyoto rats. In a single administration study, treatment with 100% CPSP fermented-milk elicited a significant decrease ($P < 0.05$) in SBP as compared to the control group. In SHR feces, consumption of CPSP fermented-milk significantly increased the fecal populations of *Bifidobacterium* spp. numbers (1 to 2 log CFU/g feces) ($P < 0.05$) and *Lactobacillus* spp. numbers (~0.5 log CFU/g feces) ($P < 0.05$) as compared to the control group, and decreased *Clostridium perfringens* numbers (1 to 1.5 log CFU/g feces) ($P < 0.05$). These findings demonstrated that oral administration of GABA-rich CPSP fermented-milk had antihypertension and improved the intestinal microflora balance.

Key words: Gamma-aminobutyric acid, Chingshey purple sweet potato, *Lactobacillus*, spontaneously hypertensive rats, anti-hypertension.

INTRODUCTION

Hypertension has been considered a significant risk factor for cardiovascular disease (CVD) that accounted for a major cause of death in industrialized countries (Novo et al., 2009). Clinically, essential (primary)

hypertension is the most prevalent type, affecting 90 to 95% of total patients and its contributing factors include a sedentary lifestyle, stress, visceral obesity, potassium deficiency, obesity, salt (sodium) sensitivity, alcohol

intake and vitamin D deficiency (Kyrou et al., 2006). Patients with CVD rely on chronic medication to control their blood pressure within normal range (Kyrou et al., 2006). Antihypertensive drugs are the most common choice for hypertension treatment, but chemical medication may cause side effects such as dry coughs, dizziness and fatigue (Alderman, 1996).

Gamma-aminobutyric acid (GABA) is an ubiquitous amino acid involved in cardiovascular function regulation such as lowering blood pressure and heart rate (Inoue et al., 2003). GABA can be generated from catalyzation of the decarboxylation of L-glutamate by glutamate decarboxylase (GAD). Anaerobic fermented GABA tea (Tsushida and Murai, 1987) and germinated brown rice (Saikusa et al., 1994) are rich in GABA and may be produced from bacteria metabolism (Smith et al., 1992), fungi (Kono and Himeno, 2000) and saccharomycetes (Hao and Schmit, 1993). Many relevant literatures validated GABA production by lactobacilli including investigations on detaching *Lactobacillus brevis* from pickles (Ueno et al., 1997), the distillation residues of alcohol (Yokoyama et al., 2002), separating *Lactobacillus lactis* from cheese (Nomura et al., 1998), and severing *Lactobacillus paracasei* from traditional Japanese fermented foods (Komatsuzaki et al., 2005).

Most probiotic lactic acid bacteria (LAB) are isolated from the intestinal microflora of healthy human subjects. Owing to the usage of LAB in milk fermentation, the microbes and metabolic products generated from this process may improve human health by increasing nutritional value such as releasing free amino acids and synthesizing vitamins (Parvez et al., 2006). Some studies reported the production of Angiotensin I converting enzyme (ACE) inhibitory or anti-hypertensive peptides, immuno-modulatory anti-oxidative and antimicrobial peptides by LAB fermentation (Korhonen and Pihlanto, 2006). Probiotic may prevent cardiovascular diseases such as hypertension through the production of a bioactive peptide that may have ACE inhibitory activity (Turpin et al., 2010). In recent years, many studies have therefore focused on GABA production by using LAB as bacterial cell factories due to the potential bioactive component of GABA in foods and pharmaceuticals (Cho et al., 2007; Kim et al., 2009; Komatsuzaki et al., 2005; Yokoyama et al., 2002). The consumption of GABA-enriched foods has been reported to depress elevation of systolic blood pressure in spontaneously hypertensive rats (SHR) and mildly hypertensive humans (Hayakawa et al., 2004; Inoue et al., 2003).

Currently, vegetable raw materials were utilized as the substrates in lactobacilli-fermented products such as soymilk, rice milk or vegetable fermented-milk (Manjunath and Ranganathan, 1989; Oh et al., 2002; Pham and Shah, 2008). In this study, Chingshey purple sweet potato (CPSP) (*Ipomoea batatas* [L.] Lam, Chingshey, Taichung, Taiwan) was used as the substrate for fermentation with milk and lactobacilli, to produce CPSP fermented-milk. In

our previous experiments, CPSP was high in total phenolic contents, total anthocyanin contents and antioxidant activities (Wu et al., 2009). We have also examined the GABA content, organic acids and cytotoxicity of fermented CPSP milk to assess its antioxidant activity and functional properties (Wu et al., 2012). In this study, we compared the effects of single-dose or chronic oral administration of GABA-rich CPSP fermented-milk on the blood pressure and improvement in the intestinal microflora of SHR.

MATERIALS AND METHODS

Bacteria strains and their growth conditions

LAB strains were purchased from the Bioresources Collection and Research Center (BCRC), Hsin-Chu, Taiwan. Stock cultures of the LAB strains were maintained at -80°C in 20% glycerol. Before experimental usage, bacteria were propagated twice in Lactobacilli deMan Rogosa Sharpe (MRS) broth (Difco, Maryland, USA) containing 0.05% L-cysteine each overnight at 37°C.

Preparation of CPSP fermented milk

The CPSP were acquired from the Taiwan Agricultural Research Institute and cleaned with tap water and stored at 4°C prior to experimentation. The CPSP was peeled, cut into 1 cm slices before steaming at 100°C for 20 min. The cooked spuds were then homogenized to produce CPSP, and mixed with 10% skimmed milk powder, 0.05% protease and 3% whey protein for pasteurization (121°C, 15 min). Three different LAB strains, that is, *Lactobacillus acidophilus* BCRC 14065 (LA), *Lactobacillus delbrueckii* ssp. *lactis* BCRC 12256 (LDL), *Lactobacillus gasseri* BCRC 14619 (LGA) or multi-species combination (LA + LDL + LGA), were added to the CPSP mixture and incubated at 37°C for 24 h. The final fermented CPSP milk product was stored at 4°C in the refrigerator for the following experimental procedure.

Titration of acidity

Determination of acidity is based on the Liu and Chang (2004). Nine grams of sample were mixed with equal amount of distilled water. Then, 0.5 ml of 1% phenolphthalein indicator was added and mixed with the sample mixture. 0.1 N of NaOH solution was added until the mixed solution turned into a light red color that persisted for at least 30 s, signifying the end of titration. We recorded the amount of NaOH solution in mL and calculated the titration of acidity (as % lactic acid per gram sample) of the CPSP fermented-milk sample using the following equation:

$$\text{Lactic acid (\%)} = (N \times F \times V \times 0.09 / \text{sample weight}) \times 100$$

*N = 0.1 (the normality of NaOH solution); **F is the titer of NaOH solution as adjusted by the first standard acid (potassium hydrogen phthalate); ***V is the amount of NaOH solution used for titration (ml).

Determination of GABA

Determination of GABA content in CPSP fermented-milk was done using the revised method by Horie and Kohata (2002). One milliliter of CPSP fermented-milk was de-proteined via 10% 5-sulfosalicylic

acid (SSA), centrifuged, and given a supernatant comprised of o-phthalate / 2-mercaptoethanol (OPA/ MCE) derivative. Five microliters of the above solution was directly analyzed by HPLC (HITACHI, Pump L-2130); using Phenomenex C-18 column and a fluorescence detector (HITACHI, FL Detector L-2485), wavelengths $\lambda_{ex} = 375 \text{ nm}$ and $\lambda_{em} = 460 \text{ nm}$ were measured. Elution buffer A contained 80% (v/v) 0.1 M acetate buffer (pH 7.2), 19% methanol and 1% tetrahydrofuran. Elution buffer B contained 90% methanol and 10% sodium acetate buffer. GABA was eluted by a linear gradient from 85 to 0% elution buffer A over 35 min at a flow rate of 1 ml/min; the column temperature was 37°C.

Rats and feeding

This study used specific pathogen-free rats. The animal research procedure was approved by The Institutional Animal Care and Use Committee of Hungkuang University, Taichung County, Taiwan (approval No. 96027). Inbred rat strains of 6-week-old SHR and WKY (Wistar Kyoto) rats were purchased from the National Laboratory for Animal Breeding and Research Center, Taipei, Taiwan. These rats were raised at $20 \pm 2^\circ\text{C}$ with the relative humidity of $55 \pm 5\%$ under 12-h light cycle. The rats were fed a Laboratory rodent diet 5001 *ad libitum* (Manufactured by PMI Nutrition International Inc., Richmond, Indiana, USA) for 2 weeks prior to the study.

At day zero, the rats were randomly allocated into eight groups (four groups were SHR and four groups were WKY). The rats were fed by oral administration of a single 2.5-ml dose of CPSP fermented-milk or sterilized water daily for 8 weeks. Each experimental group consisted of 6 rats. The control group was fed sterilized water. The positive control group was fed anti-hypertensive medicine such as Captopril (15.6 mg/kg body weight/day). The low GABA concentration group was fed 10% CPSP fermented-milk (60 μg GABA/ mL); the normal GABA concentration group was fed 100% pure CPSP fermented-milk (600 μg GABA/ mL). During the experiment, the rat weight and blood pressure were measured biweekly.

Blood pressure and heart rate measurements

Using the tail-cuff method (Hussein et al., 2005), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured biweekly from the 8th to 15th week of age. The rats were lightly supported in a mesh holder made of cloth and maintained at $37 \pm 1^\circ\text{C}$ (Model THC-1 Digital Thermo, Softron, Tokyo, Japan). Blood pressure was measured indirectly from the tail artery with a tail cuff apparatus (BP-98A, Softron, Tokyo). To avoid circadian cycle influence, arterial blood pressure measurements were performed at the same time of the day (between 09:00 a.m. and 13:00 p.m.). The values presented were the average of three separate measurements.

Bifidobacterium, *Lactobacillus* and *Clostridium perfringens* numbers determination in fecal bacteria microflora

The microflora improvement in the intestine was conducted in accordance with the guidelines for health food improvement and gastrointestinal function evaluation from the Department of Health, Executive Yuan, Taiwan, R.O.C. On days 0, 14, 28, 42 and 56, feces samples were collected from each rat to determine fecal bacterial flora. Probiotic microorganisms were isolated from feces in which the media and methods used were that of Liu et al. (2006) and the probiotic micro-organism count was determined biweekly. Approximately 0.5 g (wet weight) of feces was suspended in 4.5 mL anaerobic solution and serially diluted in an anaerobic workstation

to obtain different concentrations. After serial dilutions, 1 mL aliquots were spread onto *Bifidobacterium* iodoacetate medium-25 (BIM-25) agar for *Bifidobacterium* spp., MRS agar with bromocresol green for *Lactobacillus* spp. and Tryptose-sulfite-D-cycloserine (TSC) agar for *C. perfringens*. The plates were incubated under anaerobic conditions at 37°C for 48 h. The results were expressed as log₁₀ CFU per gram of feces (wet weight).

Statistical analysis

Data were presented as means \pm standard error (SEM) of the three replicates. SPSS V.13 was used for analysis (SPSS Inc., Chicago, USA). One-way analysis of variance (ANOVA) and Deng-style new multiple range test (Duncan's New Multiple Range Test) were applied to determine the differences among the means. P values <0.05 were regarded as significant.

RESULTS

GABA content measurement

In the previous study, a mixture of 10% skimmed milk powder, 0.05% protease and 3% whey protein were the best fermentation condition for the CPSP fermented-milk (Wu et al., 2012). GABA contents were measured in the stored CPSP fermented-milk. The produced CPSP fermented-milk remained stable when placed below 4°C for 0, 7, 14, 21 and 28 days. GABA content within a month, show that GABA content was stable in the environment (Figure 1).

pH value and titratable acidity measurement in CPSP fermented-milk

The three lactobacilli, *L. acidophilus* BCRC 14065, *L. delbrueckii* ssp. lactis BCRC 12256, and *L. gasseri* BCRC 14619, were cultured and then mixed with each other into CPSP milk before sampling. The findings showed that pH values declined to pH 4 and titratable acidity increased up to about 0.3% during fermentation period (Figure 2).

Changes in feed, water intake and body weight

Throughout the experimental period, the daily feed and water intake of the SHR groups increased normally (data not shown). The SHR group body weights were not significantly different during the experiment (Figure 3).

Chronic anti-hypertensive effect of CPSP fermented-milk on DBP and SBP in the SHR and WKY rat

We investigated the anti-hypertensive effect of GABA-rich CPSP fermented-milk upon chronic administration. The change in diastolic pressure was observed in the SHR

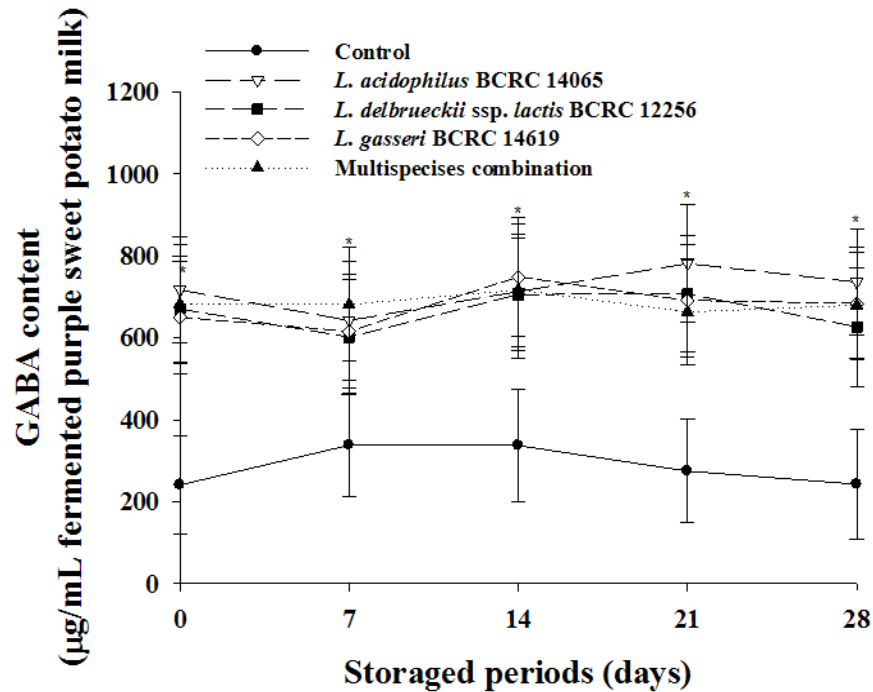


Figure 1. GABA content in CPSP fermented-milk inoculated with different lactic acid bacterium during a 28-day storage period at 4°C. Each value is the mean \pm standard deviation ($n = 3$). *Comparison of different lactic acid bacterium (single or multiple strains) with control showing significant differences in the same day by Duncan's multiple range test ($P < 0.05$).

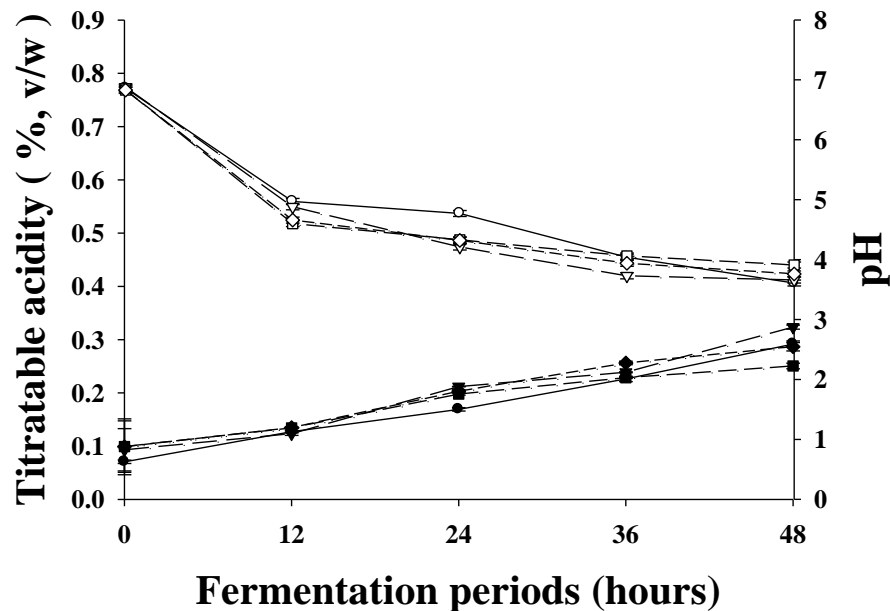


Figure 2. pH and titratable acidity effect in CPSP fermented-milk inoculated with different lactic acid bacterium during a 48-h fermentation period at 37°C. *L. acidophilus* BCRC 14065 (●): pH value and (○): titratable acidity; *L. delbrueckii* ssp. *lactis* BCRC 12256 (▼): pH value and (▽): titratable acidity; *L. gasseri* BCRC 14619 (■): pH value and (□): titratable acidity; multispecies (◆): pH value and (◇): titratable acidity. Each value is mean \pm standard deviation ($n = 3$).

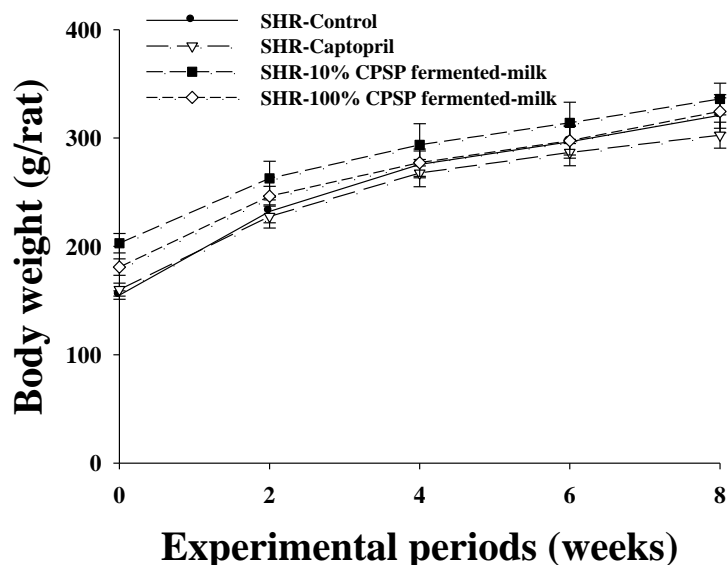


Figure 3. Body weights of SHR rats were fed sterilized water, Captopril, 10 or 100% CPSP fermented-milk during the 8 weeks treatment periods. Each value is mean \pm standard deviation (n = 6).

Table 1. Diastolic blood pressure of WKY and SHR fed different doses of CPSP fermented-milk for 8 weeks.

Groups	DBP (mm Hg) ¹				
	1 week	3 weeks	5 weeks	7 weeks	8 weeks
SHR-Control	95.50 \pm 16.92 ^{ab}	118.83 \pm 15.41 ^{ab}	135.00 \pm 16.77 ^a	136.50 \pm 6.28 ^a	139.83 \pm 2.48 ^a
SHR-Captopril	82.71 \pm 15.00 ^b	110.00 \pm 14.64 ^b	116.50 \pm 14.05 ^a	97.33 \pm 18.95 ^b	95.67 \pm 10.67 ^b
SHR-10% PSP yogurt	103.50 \pm 16.27 ^a	126.57 \pm 8.44 ^a	130.25 \pm 25.72 ^a	114.67 \pm 30.03 ^{ab}	101.50 \pm 33.92 ^b
SHR-100% PSP yogurt	112.38 \pm 12.23 ^a	120.75 \pm 10.78 ^{ab}	114.88 \pm 17.87 ^a	91.63 \pm 14.31 ^b	88.88 \pm 14.02 ^b
WKY-Control	65.67 \pm 6.11 ^c	78.67 \pm 11.06 ^c	77.33 \pm 6.11 ^b	84.67 \pm 3.06 ^c	77.33 \pm 5.13 ^c
WKY-Captopril	62.67 \pm 9.45 ^c	79.00 \pm 10.54 ^c	87.00 \pm 4.58 ^b	78.67 \pm 4.93 ^c	77.00 \pm 4.00 ^c
WKY-10% PSP yogurt	90.67 \pm 2.52 ^c	92.67 \pm 14.01 ^c	84.33 \pm 3.06 ^b	78.00 \pm 2.65 ^c	80.00 \pm 2.65 ^c
WKY-100% PSP yogurt	79.00 \pm 5.29 ^c	87.33 \pm 10.79 ^c	76.00 \pm 10.54 ^b	84.33 \pm 5.51 ^c	79.33 \pm 5.69 ^c

¹Data are expressed as means \pm S.E.M; (n = 6). ^{a,b,c,d}Values in the same column with different letters indicate significant difference ($P < 0.05$) by one-way ANOVA.

and WKY feeding period (Table 1). A significant decrease in DBP was observed from 7 to 8 weeks after the start of chronic feeding with 10 and 100% GABA-rich CPSP fermented-milk, respectively, than with the control. Nonetheless, there was no significant difference in the DBP before and after the 8 weeks of feeding in the WKY normal rats fed with de-ionized water, Captopril, 10 and 100% CPSP fermented-milk.

The time course of the changes in SBP during the SHR and WKY feeding period is shown in Table 2. In the SHR control group, the SBP was 161 \pm 10.68 mmHg at one week, and it increased gradually with age and reached 198.2 \pm 17.67 after 8 weeks of experimental periods ($P < 0.05$). Both the 10 and 100% GABA-rich CPSP fermented-milk groups showed significant difference in

SBP after 5 weeks of feeding ($P < 0.05$). The CPSP fermented-milk had dose-dependent anti-hypertensive effects on SBP. At the end of the test period, the SBP values of the 10 or 100% GABA-rich CPSP fermented-milk were significantly lower than that for the SHR control group. The SBP of normal WKY fed with de-ionized water, Captopril or CPSP fermented-milk did not show a significant difference.

Effect of a single oral administration of 100% CPSP fermented-milk on SBP in SHR

Figure 4 shows changes in average resting SBP for SHR upon single administration of 100% CPSP fermented-

Table 2. Systolic blood pressure of WKY and SHR fed different doses of CPSP fermented-milk for 8 weeks.

Groups	SBP (mm Hg) ¹				
	1 week	3 weeks	5 weeks	7 weeks	8 weeks
SHR-Control	161.00 ± 10.68 ^a	174.83 ± 8.30 ^a	202.00 ± 14.09 ^a	197.17 ± 23.75 ^a	198.17 ± 17.67 ^a
SHR-Captopril	138.14 ± 8.69 ^b	156.83 ± 12.27 ^b	155.00 ± 12.23 ^b	147.33 ± 10.52 ^c	145.17 ± 6.05 ^c
SHR-10% PSP yogurt	165.63 ± 11.86 ^a	180.14 ± 12.89 ^a	169.50 ± 9.70 ^b	165.50 ± 4.81 ^b	164.33 ± 3.78 ^b
SHR-100% PSP yogurt	167.50 ± 10.68 ^a	186.00 ± 5.95 ^a	165.75 ± 13.55 ^b	152.25 ± 9.29 ^{bc}	148.50 ± 5.76 ^c
WKY-Control	115.67 ± 2.52 ^c	138.00 ± 5.57 ^c	119.33 ± 1.15 ^c	121.33 ± 2.08 ^d	121.33 ± 2.52 ^d
WKY-Captopril	112.67 ± 13.32 ^c	134.67 ± 5.03 ^c	132.67 ± 2.52 ^c	127.33 ± 3.21 ^d	125.00 ± 2.65 ^d
WKY-10% PSP yogurt	141.67 ± 4.93 ^c	143.67 ± 7.58 ^c	131.00 ± 2.65 ^c	128.67 ± 2.08 ^d	128.00 ± 2.65 ^d
WKY-100% PSP yogurt	152.00 ± 4.36 ^c	142.00 ± 5.57 ^c	135.67 ± 5.51 ^c	132.33 ± 2.08 ^d	131.33 ± 3.51 ^d

¹Data are expressed as means ± S.E.M; (n = 6). ^{a,b,c,d}Values in the same column with different letters indicate significant difference ($P < 0.05$) by one-way ANOVA.

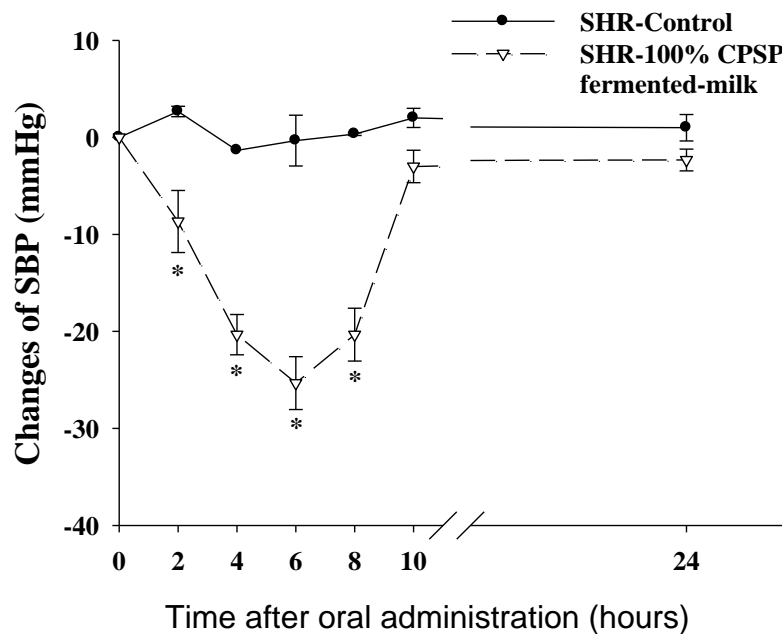


Figure 4. Systolic blood pressure of SHR fed 100% CPSP of purple sweet potato fermented-milk for 0 to 24 h at 7th week. Each value is mean ± standard derivation (n = 3). * Duncan's multiple range test determined the means significant differences from control ($P < 0.05$).

milk. In the SHR, the result showed independent time effects by the 100% CPSP fermented milk group. Two hours after administration, 100% CPSP fermented-milk group showed decreased SBP by 8.67 ± 3.2 mmHg when compared with the control group. Six hours after administration, SBP was decreased, 25.33 ± 2.73 mmHg. Ten hours after administration, SBP returned to the baseline value.

Effect on microfecal *Bifidobacterium* spp., *Lactobacillus* spp. and *C. perfringens* numbers

The findings showed that the viable count of *Bifidobacterium*

in excrements of SHR was 10^7 CFU/g feces in the beginning of the experiment. The viable count of *Bifidobacterium* in the groups fed with 10 and 100% CPSP fermented-milk was above 10^8 CFU/g feces in the eighth week of experiment. It significantly increased ten times more than the control group and the Captopril group (Figure 5A).

The *Lactobacillus* viable count in the SHR was 10^7 CFU/g feces at week zero. After eight weeks of experimental period, the viable *Lactobacillus* count in the groups fed with 10 and 100% CPSP fermented-milk were remarkably increased as compared to the control group and the Captopril group (Figure 5B).

C. perfringens number in the SHR was about 10^5 CFU/g

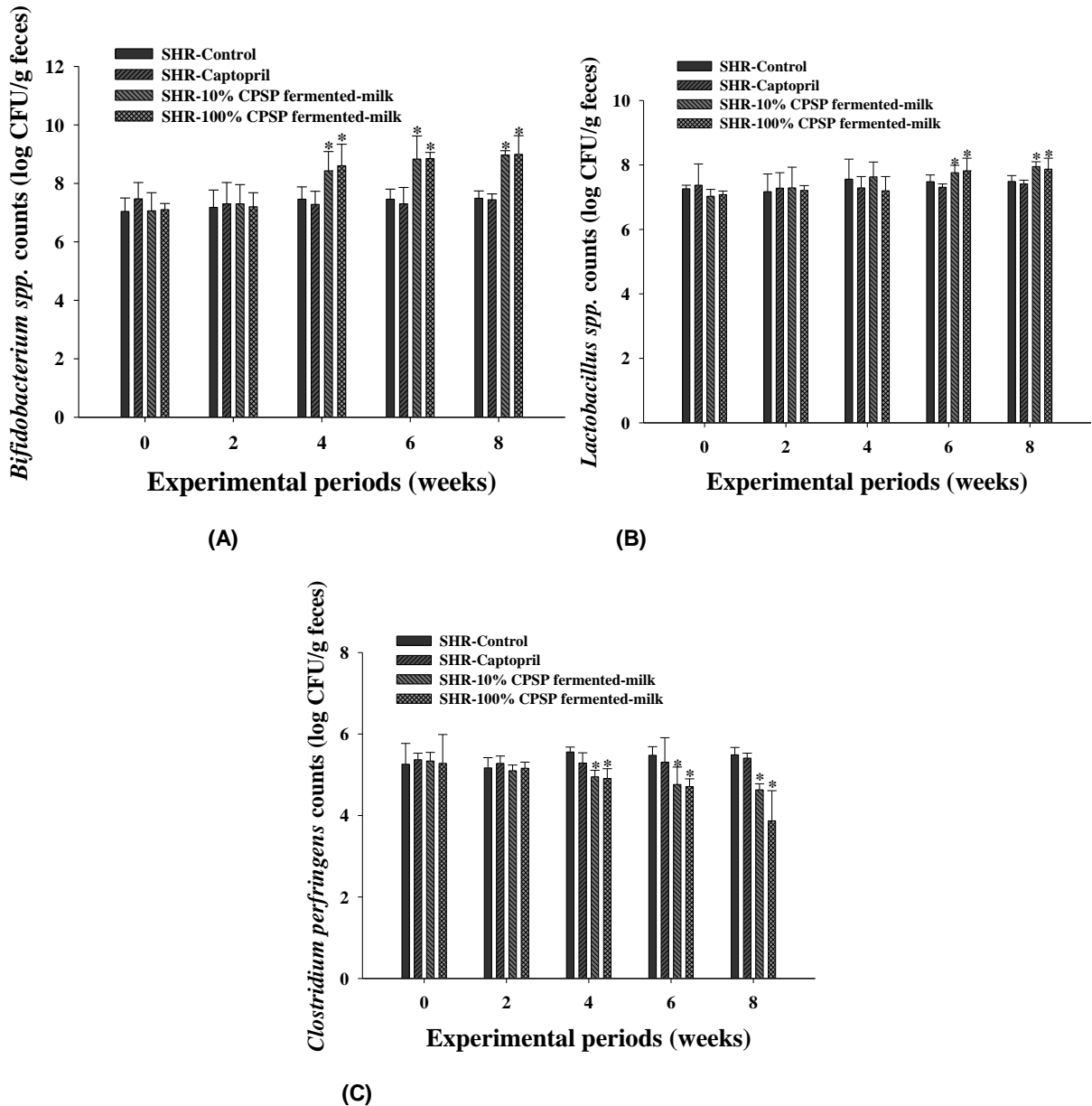


Figure 5. Total counts of (A) *Bifidobacterium*, (B) *Lactobacillus* and (C) *C. perfringens* in feces of SHR after oral administration of CPSP fermented-milk. Each value is mean \pm standard derivation (n = 6). * Duncan's multiple range test determined the means significant differences from control (P < 0.05).

feces at week zero. The viable *C. perfringens* count in the groups fed with 10 and 100% CPSP fermented-milk were remarkably decreased, 1-1.5 log CFU/ g feces (P < 0.05) as compared to the control and Captopril group after six weeks (Figure 5C).

DISCUSSION

The putative anti-hypertensive effect of milk after fermentation by lactic bacteria has attracted attention

over the past 20 years (Usinger et al., 2009). Research on fermented milk and hypertension has mainly focused on the content of peptides with *in vitro* inhibitory effect of ACE. However, fermented milk products contain several proteins, peptides and minerals, all with possible different anti-hypertensive modes of actions (Usinger et al., 2009). Diet modifications are one way to lower blood pressure and CPSP fermented milk could be a feasible way.

A previous research showed that the oral administration of GABA in rice germ of 26.4 mg daily or fermented black raspberry juice by GABA produced bacteria were effective

in treating neurological disorders (Kim et al., 2009). Moreover, feeding rats with fermented-milk containing GABA 10-12 mg/100 mL along with lactobacilli resulted in reduced blood pressure (Inoue et al., 2003). According to our results, the amounts of GABA produced in CPSP fermented-milk by *L. acidophilus* BCRC 14065, *L. delbrueckii* ssp. *lactis* BCRC 12256, *L. gasseri* BCRC 14619 or mixture of three strains in this study were more than enough to provide these functional benefits.

Some LAB produces peptides that are released from the milk protein by proteolysis and inhibit ACE, which is instrumental in inducing hypertension. For example, Maeno et al. (1996) purified the extracellular proteinase of *Lactobacillus helveticus* CP790, separating different peptides with two-stage anti-phase high-efficiency liquid chromatography to prove that, after oral administration with 2 mg/kg body weight, the rats continuously maintained the ability to regulate blood pressure within 2 to 10 h and presented the largest blood pressure dropping value (-31.5 ± 5.6 mm Hg) at the sixth hour. In our study, 100% CPSP fermented-milk had similar results; systolic pressure still significantly dropped (-25.33 ± 2.73 mmHg) at six hours later as compared to the control group.

The CPSP is also rich in vitamins (e.g. B1, B2, C and E), minerals (e.g. calcium, magnesium, potassium and zinc), dietary fiber, amino acid and non-fibrous carbohydrates, especially anthocyanins (Suda et al., 2003). Moreover, in our previous study, it was indicated that the anthocyanins and antioxidant ability of the fermented CPSP fermented-milk through LA, LDL and LG strains was significantly elevated (Wu et al., 2009, 2012).

In research studies, a significant anti-hypertensive effect inversely associated with blood and a vegetarian diet rich in calcium, magnesium, potassium, fiber, protein, and several amino acids, such as tyrosine and phenylalanine, has been reported (Zhao et al., 2001; Yoshimura et al., 2010). Some *in vitro* studies have demonstrated the potential benefit of anthocyanins such as radical-scavenging ability, anticancer potential, ameliorative effect on liver injury and an inhibitory effect on ACE that could contribute to the anti-hypertensive effect (Suda et al., 2003; Teow et al., 2006).

In conclusion, the GABA-rich CPSP fermented milk with *L. acidophilus* BCRC 14065, *L. delbrueckii* ssp. *lactis* BCRC 12256 and *L. gasseri* BCRC 14619 showed a significant reduction in systolic pressure and diastolic pressure in SHR, but did not affect blood pressure in normal WKY. We also found that CPSP fermented milk significantly increased the viable *Bifidobacterium* spp. and *Lactobacillus* spp. counts, and decreased *C. perfringens* number in the feces. The addition of probiotics with high GABA-producing ability in CPSP fermented milk enhanced functional and nutritive values.

ACKNOWLEDGEMENT

This research was supported by National Science Council, Taiwan, R.O.C. (NSC97-2313-B-241-004-MY3).

REFERENCES

- Alderman M (1996). Sodium, potassium and blood pressure. *J. Hypertens.* 14(6):737-741.
- Cho YR, Chang JY, Chang HC (2007). Production of gamma-aminobutyric acid (GABA) by *Lactobacillus buchneri* isolated from kimchi and its neuroprotective effect on neuronal cells. *J. Microbiol. Biotechnol.* 17(1):104-109.
- Hao R, Schmit JC (1993). Cloning of the gene for glutamate decarboxylase and its expression during conidiation in *Neurospora crassa*. *Biochem. J.* 293(3):735-738.
- Hayakawa K, Kimura M, Kasaha K, Matsumoto K, Sansawa H, Yamori Y (2004). Effect of a gamma-aminobutyric acid enriched dairy product on the blood pressure of spontaneously hypertensive and normotensive Wistar-Kyoto rats. *Br. J. Nutr.* 92(3):411-417.
- Horie H, Kohata K (2002). Analysis of tea components by high-performance liquid chromatography and high-performance capillary electrophoresis. *J. Chromatogr. A.* 881(1-2):425-438.
- Hussein G, Nakamura M, Zhao Q, Iguchi T, Goto H, Sankawa U, Watanabe H (2005). Antihypertensive and neuroprotective effects of astaxanthin in experimental animals. *Biol. Pharm. Bull.* 28(1):47-52.
- Inoue K, Shirai T, Ochiai H, Kasao M, Kayakawa K, Kimura M, Sansawa H (2003). Blood-pressure-lowering effect of a novel fermented milk containing gamma-aminobutyric acid (GABA) in mild hypertensives. *Eur. J. Clin. Nutr.* 57(3):490-495.
- Kim JY, Lee MY, Ji GE, Lee YS, Hwang KT (2009). Production of gamma-aminobutyric acid in black raspberry juice during fermentation by *Lactobacillus brevis* GABA100. *Int. J. Food. Microbiol.* 130(1):12-16.
- Komatsuzaki N, Shima J, Kawamoto S, Momose H, Kimura T (2005). Production of gamma-aminobutyric acid (GABA) by *Lactobacillus paracasei* isolated from traditional fermented foods. *Food Microbiol.* 22(6):497-504.
- Kono I, Himeno K (2000). Changes in gamma-aminobutyric acid content during beni-koji making. *Biosci. Biotechnol. Biochem.* 64(3): 617-619.
- Korhonen H, Pihlanto A (2006). Bioactive peptides: Production and functionality. *Int. Dairy J.* 16(9):945-960.
- Kyrou I, Chrousos GP, Tsigos C (2006). Stress, visceral obesity, and metabolic complications. *Ann. N. Y. Acad. Sci.* 1083:77-110.
- Liu JR, Wang SY, Chen MJ, Yueh PY, Lin CW (2006). The anti-allergenic properties of milk kefir and soymilk kefir and their beneficial effects on the intestinal microflora. *J. Sci. Food Agric.* 86(15):2527-2533.
- Liu ZS, Chang SK (2004). Effect of soy milk characteristics and cooking conditions on coagulant requirements for making filled tofu. *J. Agric. Food Chem.* 52(11):3405-3411.
- Maeno M, Yamamoto N, Takano T (1996). Identification of an antihypertensive peptide from casein hydrolysate produced by a proteinase from *Lactobacillus helveticus* CP790. *J. Dairy Sci.* 79(8):1316-1321.
- Manjunath N, Ranganathan B (1989). Cytotoxic activity of lactobacilli isolated from plant and dairy sources. *Indian J. Exp. Biol.* 27(1):72-75.
- Nomura M, Kimoto H, Someya Y, Furukawa S, Suzuki I (1998). Production of gamma-aminobutyric acid by cheese starters during cheese ripening. *J. Dairy Sci.* 81(6):1486-1491.
- Novo S, Lunetta M, Evola S, Novo G (2009). Role of ARBs in the blood hypertension therapy and prevention of cardiovascular events. *Curr. Drug. Targets.* 10(1):20-25.
- Oh Y, Osato MS, Han X, Bennett G, Hong WK (2002). Folk yoghurt kills *Helicobacter pylori*. *J. Appl. Microbiol.* 93(6):1083-1088.
- Parvez S, Malik KA, Kang S, Kim HY (2006). Probiotics and their fermented food products are beneficial for health. *J. Appl. Microbiol.* 100(6):171-1185.

- Pham TT, Shah NP (2008). Effect of lactulose on biotransformation of isoflavone glycosides to aglycones in soymilk by lactobacilli. *J. Food Sci.* 73(3):158-165.
- Saikusa T, Horino T, Mori Y (1994). Accumulation of γ -aminobutyric acid (GABA) in the rice germ during water soaking. *Biosci. Biotechnol. Biochem.* 58(12):2291-2292.
- Smith DK, Kassam T, Singh B, Elliott JF (1992). *Escherichia coli* has two homologous glutamate decarboxylase genes that map to distinct loci. *J. Bacteriol.* 174(18):5820-5826.
- Suda L, Oki T, Masuda M, Kobayashi M, Nishiba Y, Furuta S (2003). Physiological functionality of purple-fleshed sweet potatoes containing anthocyanins and their utilization in food. *Jpn. Agric. Res. Q.* 37(3):167-173.
- Teow CC, Truong VD, McFeeters RF, Thompson RL, Pecota KV, Yencho GC (2006). Antioxidant activities, phenolic and β -carotene contents of sweet potato genotypes with varying flesh colours. *Food Chem.* 103(3):829-838.
- Tsushida T, Murai T (1987). Conversion of glutamic acid to gaminobutyric acid in tea leaves under anaerobic conditions. *Agric. Biol. Chem.* 51:2865-2871.
- Turpin W, Humblot C, Thomas M, Guyot JP (2010). Lactobacilli as multifaceted probiotics with poorly disclosed molecular mechanisms. *Int. J. Food Microbiol.* 143(3):87-102.
- Ueno Y, Hayakawa K, Takahashi S, Oda K (1997). Purification and characterization of glutamate decarboxylase from *Lactobacillus brevis* IFO 12005. *Biosci. Biotechnol. Biochem.* 61(7):1168-1171.
- Usinger L, Ibsen H, Jensen LT (2009). Does fermented milk possess antihypertensive effect in humans? *J. Hypertens.* 27(6):1115-1120.
- Wu TY, Lee CH, Chiu TH, Tsai SJ (2009). Studies on antioxidant capacity of hot water extracted from different sweet potato varieties. *J. Taiwan Agric. Res.* 58(1):7-16.
- Wu TY, Tsai CC, Hwang YT, Chiu TH (2012). Effect of antioxidant activity and functional properties of Chingshey purple sweet potato fermented-milk by *Lactobacillus acidophilus*, *L. delbrueckii* subsp. *lactis*, and *L. gasseri* strains. *J. Food Sci.* 77(1):2-8.
- Yokoyama S, Hiramatsu J, Hayakawa K (2002). Production of gamma-aminobutyric acid from alcohol distillery lees by *Lactobacillus brevis* IFO-12005. *J. Biosci. Bioeng.* 93(1):95-97.
- Yoshimura M, Toyoshi T, Sano A, Izumi T, Fujii T, Konishi C, Inai S, Matsukura C, Fukuda N, Ezura H, Obata A (2010). Antihypertensive effect of a gamma-aminobutyric acid rich tomato cultivar 'DG03-9' in spontaneously hypertensive rats. *J. Agric. Food Chem.* 58(1):615-619.
- Zhao G, Li Z, Gu T (2001). Antihypertension and anti-cardiovascular remodeling by phenylalanine in spontaneously hypertensive rats: effectiveness and mechanisms. *Chin. Med. J.* 114(3):270-274.