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The effects of lactic acid bacteria-fermented lemon juice on blood pressure regulation and allergic responses in rodents

Cheng-Chih Tsai*, **Li-Yun Lin, Lan-Chun Chou**

Department of Food Science and Technology, HungKuang University, Taichung City 43302 Taiwan

*Corresponding author, e-mail: tsaicc@sunrise.hk.edu.tw

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ABSTRACT: Lemon juice contains numerous biologically active substances that have potential health benefits. In this study, we evaluated the biotransformation characteristics and functional properties of lactic acid bacteria-fermented lemon juices and examined their effects on regulating blood pressure. Fermentation of Pingtung Eureka lemon juice with a mixture of five selected strains of lactic acid bacteria was found to be the most effective in enhancing juice γ-aminobutyric acid (GABA) levels, with an increases in yield of up to 7.37 ± 0.39 g/l. Among the different lemon varieties fermented with *Lactobacillus fermentum* LF33, Taichung Eureka and Nantou Eureka gave the highest fermentation yields of GABA at 4.19 ± 0.17 and 5.35 ± 0.36 g/l, respectively. In spontaneously hypertensive rats that were administered fermented lemon juice to assess to the effects on blood pressure, we observed that diastolic, systolic, and mean blood pressures, as well as heart rate, were significantly lower than those in control rats ($p < 0.1$). Fermented lemon juice was also found to contribute to a significant increase in serum IgG concentrations ($p < 0.05$). Finally, we evaluated the efficacy of Taichung Limon and Pingtung Limon juice fermentation broths on the allergic effect of ovalbumin in sensitized BALB/c mice, and found that these fermented juices significantly reduced ovalbumin-induced IgE antibody levels ($p < 0.05$). In conclusion, we speculate that juice-containing substances, such as GABA, have a calming effect on ganglia, which could contribute to stabilizing heart rate, and that consumption of fermented lemon juice may have the effect of reducing levels of IgE antibodies.

KEYWORDS: fermented lemon juice, γ-aminobutyric acid, blood pressure regulation, OVA-sensitized BALB/c mice

INTRODUCTION

Lemon is a widely cultivated plant in the genus Citrus, the fruits of which are mainly used for juicing. The lemonade produced from lemon juice contains 5%–6% citric acid, which imparts a sour taste and is widely used in food processing [1]. Lemonade also contains a number of valuable nutrients, including alkaloids, flavonoids, tannins, polyphenols, and saponins [1]. Along with other nutrient constituents (vitamins, minerals, dietary fiber, essential oils, and carotenoids), polyphenols have been shown to be beneficial to human health with regards to lowering blood lipids and blood glucose and exerting anti-cancer and anti-oxidant effects [2]. In this context, biochemical conversion involves the use of bacteria or other microorganisms to degrade biomass via anaerobic digestion, fermentation, or composting [3], and previous studies have shown that the lactic acid bacterial fermentation of food substrates can improve the conversion of natural plant products and effectively enhance their functionality, including anti-oxidant, anti-cancer, anti-inflammatory, and anti-allergenic properties [4, 5].

Certain constituents of lemon have been reported to be effective in regulating blood pressure [6], among which is *S*-limonene, a component of lemon essential oil. The literature indicates that oral administration of high concentrations of *S*-limonene can contribute to reducing physical and physiological stress. Furthermore, lemonade and lemon flavonoids have been shown to have inhibitory effects on spontaneous hypertension in rats [7]. A further study has reported that sucrose is rapidly degraded during the latter stages of citrus fruit maturation, and a recent study has shown that the γ-aminobutyric acid (GABA) content of lemon juice is effective in regulating blood pressure [8]. Limón et al [9] investigated the effects of solid-state *Bacillus subtilis* fermentation, or liquid *Lactobacillus plantarum* fermentation for 48 to 96 h on aqueous extracts from *Lablab purpureus* and *Phaseolus vulgaris*, respectively, and found that solid-state fermentation extracts have higher levels of water-soluble phenolic compounds and anti-oxidant activity, whereas liquid fermentation extracts shows potential anti-hypertensive activity attributable to GABA and angiotensin-converting enzyme inhibitor activities (>90%). Consequently, with respect to value-added functional food production, fermentation is a process that warrants further research focusing on the yields of potential hypertension-preventing bioactive components.

Previous studies have also reported that lactic acid bacteria-fermented orange juice shows anti-allergenic activities in animal experiments and clinical trials, and recent studies have found that the metabolites produced during the lactic acid bacteria fermentation of lemons promote antibacterial activity and immunity that are beneficial to humans [4]. Metabolites, such as γ-aminobutyric acid (GABA), have shown anti-hypertensive and anti-depressant effects in humans following oral administration [10]. The GABA produced by lactic acid bacteria can be used as a natural alternative for manufacturing GABA-enriched fermented foods [10]. Furthermore, components of the cell walls of *Lactobacillus*, including peptidoglycan, polysaccharides, and teichoic acid, have been established to have immunoregulatory properties [11], and it has been widely demonstrated that different strains stimulate diverse immune responses via different pathways. In mice injected with ovalbumin (OVA) to induce allergic reactions, supplementation of the diet with *Lactobacillus* has been found to enhance the production of antibodies, with the results revealing that serum IgG1 levels increased in mice fed with *Lactobacillus delbrueckii* subsp. *bulgaricus*, *L. casei*, or *L. acidophilus*, and serum IgG2a levels increased in mice fed *L. acidophilus* [12].

Studies have also found that anaerobically fermented Jialong tea [13] and sprouted rice [14] are rich in GABA, which can be produced by the metabolism of bacteria [15], fungi [16], and yeast [17]. GABA has also been shown to be produced by the lactic acid bacteria isolated from Japanese traditional fermented food; for example, *L. brevis* isolated from kimchi [18] and the residues remaining after the distillation of alcohol [19], and *L. paracasei* and *L. lactis* isolated from cheese [20]. Hayakawa et al [21] examined the contents of GABA in commercially available products (yogurt, kimchi, and vegetable juice) and accordingly found that the content in kimchi was higher than that in other products. In the food industry, it is important to identify different types of lactic acid bacteria that produce high levels of GABA, as different species and strains are typically characterized distinct fermentation characteristics, such as acid production and flavor. Lactic acid bacteria such as *Lactobacillus* and *Lactococcus* produce GABA via the activity of glutamate decarboxylase, the GAD gene and biochemical properties of which have been studied [22], and Ueno et al [18] purified GAD from *L. brevis* and determined its biochemical properties.

Among the *Lactobacillus* strains we assessed in the present study, the PM229 strain of *L. plantarum* has previously been shown to have antagonistic activity against the growth, adhesion, and invasion of *Klebsiella pneumoniae*, *Gardnerella vaginalis*, and *Staphylococcus saprophyticus* [23, 24]; *L. plantarum* LP 10069-fermented lemon juice has been demonstrated to have potential utility with respect to its anti-pathogenic bacteria and anti-allergy properties [25]; and *L. acidophilus* LAP5 and *L. fermentum* LF33 from swine and poultry have been found to have antagonistic activities against *Salmonella* infection [26]. In the present study, we evaluated the biotransformation characteristics of lactic acid bacteria-fermented lemonade, assuming that it can be bio-converted to yield GABA components, and investigated its synergistic functional effects in animal experiments with respect to blood pressure regulation and anti-allergenic effects.

MATERIALS AND METHODS

Bacterial culture

For the purposes of the present study, we used the following five strains of *Lactobacillus*, which were either isolated in our laboratory or obtained from the Bioresource Collection and Research Center (BCRC) and were selected on the basis of their suitability for fermentation [25]: *L. plantarum* PM229, *L. plantarum* LP 10069, *L. delbrueckii* subsp. *bulgaricus* BCRC 10696, *L. acidophilus* LAP5, and *L. fermentum* LF33. These strains, maintained at −80 °C, were activated twice in a 1% liquid suspension and cultured at 37 °C for 20 h in MRS broth (DIFCO, Detroit, Michigan, USA) containing 0.05% cysteine.

Preparation of lemonade fermentation broth

The Eureka lemon and Limon varieties used in this study were purchased from suppliers in Taichung, Nantou, and Pingtung. As described by Islam et al [27], fresh lemons were washed, rinsed with distilled water, and dried prior to juicing. The lemon juice thus obtained was passed through a 100-mesh filter and centrifuged at $6080 \times g$ for 10 min. The pH of the resulting supernatant was adjusted to 3.5 with sodium bicarbonate [27], and inulin fiber or glucose was then added to adjust the sugar content to 15 °Brix. Thereafter, the juice was heat treated at 90 °C for 5 min and then cooled. Lactobacillus strains were activated, centrifuged at 8000 rpm for 10 min, and resuspended in sterile water. Each of the five selected Lactobacillus strains (2%, 8 log CFU) was added to 500 ml of lemon juice for fermentation at 30 °C for 72 h [28]. The fermented lemon juice thus obtained was stored at −80 °C.

Measurement of GABA content

The GABA content was analyzed using a modified version of the method described by Horie and Kobata [29]. Lemon fermentation broth (1 ml) was treated with 10% 5-sulfosalicylic acid for deproteinization, followed by centrifugation. The supernatant was collected and mixed with an *o*-phthalate/2-mercaptoethanol derivatization solution, 5-µl aliquots of which were analyzed by high-performance liquid chromatography (PumpL-2130; Hitachi) using a Phenomenex C-18 column (Gemini 5u C 18 110A, 250×4.6 mm). The excitation and emission wavelength settings of the fluorescence detector (FL Detector L-2485; Hitachi) were set to 375 nm and 460 nm, respectively. Elution solution A contained 80% (v/v) of 0.1 M sodium acetate buffer (pH 7.2), 19% methanol, and 1% tetrahydrofuran. Elution solution B contained 90% methanol and 10% sodium acetate buffer. The time taken for elution solution A to decrease from 85% to 0% was 35 min. The flow rate was 1 ml/min, and the column temperature was 37 °C. To determine GABA concentrations, we used an analytical standard marker of γ-aminobutyric acid (molecular weight: 103.12) (Merck KGaA, Darmstadt, Germany) and the equation $y = 6E + 08x - 718199$, ($R^2 = 0.9999$).

Animal experiments: Blood pressure regulation

The protocol used in this experiment was approved by HungKuang University, Taichung, Taiwan (approval number: HK-10801). Experimental animals were purchased from BioLASCO Taiwan Co., Ltd. and reared in an animal room at a temperature of 23 ± 1 °C and relative humidity of 60% ± 3%, under a 12-h light:12-h dark photoperiod. During the study period, the animals were given ad libitum access to food and water. To determine the effects of fermented lemon juice on systolic and diastolic blood pressure in a hypertensive animal model, fermented lemon juice was administered to spontaneously hypertensive rats (SHRs) [30], thereby enabling us to evaluate blood pressure regulation effects. Using the aforementioned GABA measurement protocol, we screened for fermented lemonade with high GABA levels. The rats were divided into three experimental groups, each containing eight animals: a control group (fed only with a standard feed), a lactic acid bacteria-fermented lemonade group, and a positive control group (treated with a commonly used hypertension medication). Blood pressure was measured using the tail-pulse method. The experiment included observations of the general condition, weight, and growth status. Blood pressure was measured 1 week prior to the experiment when the animals were awake and resting at a room temperature of 25 °C. Indirect blood pressure measurement by tail pulse necessitates the insulation of rats, and the temperature of the thermostatic box and insulation duration were recorded. During the experimental period, blood pressure was measured once a week and included the recording of heart rate and systolic, diastolic, and mean blood pressures, the values of which are presented as the means of three to five measurements.

Anti-allergenic effects of fermented lemonade

The protocol for this experiment was approved by HungKuang University, Taichung, Taiwan (approval number: HK-10710). Seven-week-old BALB/c mice were divided into the following groups, each containing seven animals: a control group (fed only with a standard feed), a sensitized group (fed with the standard feed but sensitized with OVA), and a lactic acid bacteria-fermented lemonade group. OVA (0.5 mg) in phosphate-buffered saline (containing 2 mg aluminum hydroxide adjuvant) was administered to mice via intraperitoneal injection, with each mouse being sensitized twice at the ages of 10 and 12 weeks using a dose of 0.1 ml/10 g body weight. Blood samples were collected at weeks 7, 10, 12, and 13 (at euthanasia). The mice were euthanized on day 49 after feeding with lactic acid bacteria fermentation products, and blood samples were collected. Retro-orbital blood was collected and allowed to stand for 1 h to separate the serum, which was then centrifuged at $3000 \times g$ for 20 min at 4 °C. The supernatant was collected and stored at −20 °C for further analysis of specific antibodies. The BD OptEIA™ Sets (BD Biosciences, CA, USA) for OVA-specific IgE, mouse IgG1, and mouse IgG2a were used for measurement [31].

Statistical analysis

For the purposes of statistical analyses, we used the Statistical Package for the Social Sciences, version 12.0. Experiments were conducted at least three times, and all data are presented as the means ± standard deviation (SD). One-way analysis of variance (ANOVA) was used for each experimental group, and Duncan's new multiple-range test was used to analyze differences in mean values among the experimental groups. p-values < 0.05 or < 0.1 were considered statistically significant.

RESULTS AND DISCUSSION

Measurement of GABA content

As shown in Table 1, the limes and lemons from different regions used for fermentation studies are listed in Table 1. Prior to fermentation, GABA levels in lime juice were between 2.35 and 3.20 g/l, which were significantly higher than that in the Eureka varieties of lemon from different regions (0.85–1.94 g/l) ($p <$ 0.05). However, after probiotic fermentation, the fruit juice obtained from the Eureka varieties showed a broader variation in GABA content. Pre-fermentation, the GABA content in Pingtung Eureka lemons was 0.85 ± 0.23 g/l, which was raised to 7.37 ± 0.39 g/l and 6.16 ± 0.93 g/l after fermentation using a mixture of the five assessed probiotic strains (LAP5 + LF33 + LP10069 + BCRC10696 + PM229) and LP 10069 alone, respectively. The LF33 strain was found to be the most effective with respect to increasing the GABA content in Taichung Eureka lemons, from 1.94 ± 0.04 to 4.19 ± 0.17 g/l, whereas a mixture of the strains increased levels to 3.43 ± 0.08 g/l. LF33 also promoted the highest increase in GABA content in Nantou Eureka lemons (from 1.02 ± 0.23 to 5.35 ± 0.36 g/l), comparing with the 2.99 ± 0.76 and 1.79 ± 0.21 g/l obtained with the mixed strains and LP 10069, respectively. Among the varieties of limes assessed, probiotic fermentation increased the GABA content in Nantou limes (from 2.35 ± 0.61 to 3.68 ± 0.42 g/l) but had no appreciable effect on GABA concentration in Pingtung and Taichung limes. The highest GABA concentrations were obtained when using the mixed strains to ferment Pingtung Eureka lemonade, followed by the fermentation of Taichung Eureka lemonade. To increase component diversity in the fermentation broth for subsequent animal experiments used to evaluate blood pressure regulation effects, we prepared a 1:1 mixture of Ping-

Table 1 Determination of γ-aminobutyric acid content in Limon and lemon juice from different origins after fermentation by lactic acid bacteria.

LAB Strain	γ-aminobutyric acid (g/l)					
	PE	TE	NE	PL	TL	NL
Control	0.85 ± 0.23^{eF}	1.94 ± 0.04^{dD}	1.02 ± 0.23^{fE}	3.20 ± 0.12^{aA}	2.87 ± 0.31^{aB}	2.35 ± 0.61^{eC}
Co-culture	7.37 ± 0.39^{aA}	3.43 ± 0.08^{bB}	2.99 ± 0.76^{bD}	3.05 ± 0.19^{bC}	2.16 ± 0.72^{cE}	2.17 ± 0.38^{fE}
LAP5	1.33 ± 0.09^{dD}	0.38 ± 0.43^{gF}	1.03 ± 0.53^{dE}	2.91 ± 0.04^{cB}	1.97 ± 0.25^{dC}	3.68 ± 0.42^{aA}
LF33	4.97 ± 0.02^{cB}	4.19 ± 0.17^{aC}	5.35 ± 0.36^{aA}	2.35 ± 0.09^{fD}	1.88 ± 0.30^{fE}	2.36 ± 0.13^{eD}
LP10069	6.16 ± 0.93^{bA}	2.34 ± 0.36^{cC}	1.79 ± 0.21^{cE}	2.35 ± 0.64^{fC}	2.15 ± 0.15^{cD}	2.72 ± 0.17^{cB}
BCRC10696	0.74 ± 0.86^{fF}	1.57 ± 0.28^{eC}	0.92 ± 0.32^{eE}	2.60 ± 0.32^{eA}	1.21 ± 0.41^{eD}	2.54 ± 0.24^{dB}
PM229	0.68 ± 0.65^{gF}	0.96 ± 0.49^{fD}	0.93 ± 0.46^{eE}	2.77 ± 0.27^{dB}	2.35 ± 0.09^{bC}	3.27 ± 0.82^{bA}

PE: Pingtung Eureka; PL: Pingtung Limon; TE: Taichung Eureka; TL: Taichung Limon; NE: Nantou Eureka; NL: Nantou Limon.
^{a,b,c,d,e,f} values in the same column with different superscripts mean significant difference ($p < 0.05$).
^{A,B,C,D,E,F} values in the same row with different superscripts mean significant difference ($p < 0.05$).

Table 2 Diastolic blood pressure of SHR rats fed with different test substances for eight consecutive weeks.

	DBP (mm Hg)		
	Control	Captopril	FTPE
0 week	114.00 ± 8.26^{a}	123.13 ± 23.15^{a}	116.00 ± 7.41^{a}
1 week	125.75 ± 11.46^{a}	139.50 ± 22.35^{a}	118.75 ± 16.44^{a}
2 week	117.25 ± 10.35^{a}	120.75 ± 12.79^{a}	129.63 ± 18.86^{a}
3 week	111.75 ± 32.39^{a}	108.13 ± 15.16^{a}	112.86 ± 9.49^{a}
4 week	125.63 ± 11.86^{a}	120.13 ± 13.57^{a}	128.71 ± 19.15^{a}
5 week	135.29 ± 23.55^{a}	112.00 ± 9.76^{b}	125.83 ± 12.45^{ab}
6 week	139.50 ± 17.08^{a}	111.75 ± 14.40^{b}	115.71 ± 18.30^{b}
7 week	136.00 ± 8.94^{a}	113.00 ± 16.97^{b}	128.00 ± 11.42^{ab}
8 week	148.17 ± 14.52^{a}	129.25 ± 10.95^{b}	127.20 ± 18.05^{b}

Captopril: antihypertensive drugs; FTPE: fermented Taichung Eureka juice + fermented Pingtung Eureka juice.
^{a,b} values in the same column with different superscripts mean significant difference ($p < 0.05$).

tung Eureka and Taichung Eureka broths fermented using the mixed lactic acid bacterial strains.

It has previously been established that the PM229 and LP10069 strains are the most suitable for fermentation, with bacterial counts being maintained at 7–8 log CFU/ml after fermentation for probiotic activity [25]. Given the minimal differences in *Lactobacillus* counts among lemon juices supplemented with glucose or inulin, we selected glucose as the carbon source for fermentation, based on cost considerations. Following fermentation with different strains, the *Lactobacillus* counts of Taichung and Nantou Limon lemon fermentation broths were 7.04–8.02, and 5.80–7.10 log CFU/ml, respectively, whereas those for Taichung Eureka and Nantou Eureka fermented lemon juices were 8.08–8.18 and 7.99–8.79 log CFU/ml, respectively [25].

Changes in blood pressure

As shown in Tables 2 and 3, there were no significant differences in diastolic, systolic, or mean blood pressures or heart rate in pre-treatment SHRs among all groups ($p > 0.05$). However, at week 8 of treatment, there were significant reductions in diastolic, systolic, and mean blood pressures between the two treatment groups and the control group ($p < 0.05$), thereby indicating that fermented lemonade has the potential to bring about long-term blood pressure reductions in rats. In addition, when measured at week 7, the heart rate of rats fed with fermented lemonade (346 ± 36.06 bpm) was lower than that of rats in the control group (387.83 ± 30.75 bpm) and drug-treatment group (370.13 ± 55.23 bpm). At week 8, the difference between the treatment groups and the control group was between 30 and 61 bpm. On the basis of these findings, we speculate that there are substances in lemonade, such as GABA, that have a calming effect on neurons, thereby stabilizing heart rate [8].

Several antihypertensive drugs are known to have considerable side effects. The captopril used in the present study cannot penetrate the blood-brain barrier or inhibit the synthesis of angiotensin, alter the production of prostaglandin, or regulate the activity of the adrenergic nervous system. Drug doses are based on recommendations of the Drug Education Resources Centre. The human dose for captopril covers four levels, namely, low, medium, medium-high, and high, corresponding to 37.5, 75, 150, and 450 mg/day in adults, which are converted the equivalent doses for animals. In this regard, Qian et al [32] have suggested that captopril doses of between 10 and 50 mg/kg BW/-day are suitable for animals, which are equivalent to human doses of 96 and 480 mg/kg, corresponding to medium and high captopril doses, respectively. Consequently, in the present study, we used a captopril dose 15.6 mg/kg BW/day (equivalent to the medium-high dose in humans) for treating animals in the positive control group. The amount of lemonade administered to rats, was calculated from the recommended dose for human adults (50 ml/60 kg) to obtain a daily dose of 5.2 ml/kg for rats, as described in Chen et al [33].

Hata et al [34] studied the effects of calpis, a

Table 3 Systolic blood pressure, average blood pressure and heart rate of SHR rats fed with different test substances for eight consecutive weeks.

Week	SBP (mm Hg)			MBP (mm Hg)			HR (beat/min)		
	Control	Captopril	FTPE	Control	Captopril	FTPE	Control	Captopril	FTPE
0	157.88 ± 11.47^a	156.75 ± 17.47^a	159.38 ± 15.06^a	128.63 ± 7.15^a	134.38 ± 20.22^a	130.50 ± 9.68^a	394.63 ± 46.93^a	380.88 ± 98.34^a	433.63 ± 51.21^a
1	168.75 ± 21.97^a	177.25 ± 41.04^a	171.88 ± 28.05^a	140.00 ± 11.98^a	152.00 ± 27.58^a	136.25 ± 19.14^a	410.25 ± 76.12^a	460.88 ± 89.46^a	406.75 ± 86.70^a
2	176.50 ± 22.30^a	172.50 ± 9.84^a	180.00 ± 19.83^a	136.88 ± 13.67^a	138.13 ± 9.06^a	146.00 ± 17.17^a	386.50 ± 50.39^a	423.63 ± 70.38^a	377.25 ± 42.30^a
3	168.25 ± 18.42^a	164.75 ± 5.82^a	166.29 ± 30.30^a	130.50 ± 25.56^a	124.38 ± 3.96^a	130.57 ± 12.74^a	355.00 ± 66.86^a	371.25 ± 44.02^a	347.29 ± 50.84^a
4	184.63 ± 8.53^a	158.75 ± 18.54^b	183.57 ± 17.52^a	145.13 ± 7.74^{ab}	132.75 ± 12.75^a	146.86 ± 16.12^b	394.13 ± 32.62^a	376.75 ± 40.54^a	407.43 ± 43.74^a
5	187.00 ± 14.27^a	154.50 ± 22.83^b	168.71 ± 17.63^b	150.75 ± 18.09^a	128.75 ± 13.18^b	141.57 ± 12.63^{ab}	376.25 ± 34.28^{ab}	326.50 ± 68.12^b	348.71 ± 85.98^{ab}
6	194.29 ± 10.78^a	159.88 ± 24.69^c	174.43 ± 11.24^{bc}	156.75 ± 11.50^a	127.63 ± 16.10^b	135.00 ± 12.18^b	360.38 ± 44.91^a	352.38 ± 60.77^a	353.43 ± 23.73^a
7	197.00 ± 14.87^a	170.75 ± 20.69^c	174.00 ± 11.93^{bc}	156.33 ± 10.27^a	134.00 ± 16.21^b	144.83 ± 10.28^{ab}	387.83 ± 30.75^a	370.13 ± 55.23^{ab}	346.00 ± 36.06^b
8	199.33 ± 8.12^a	170.88 ± 24.02^b	189.40 ± 20.77^{ab}	165.33 ± 10.39^a	143.00 ± 13.54^b	148.00 ± 16.37^b	392.17 ± 34.07^a	389.00 ± 50.45^a	362.00 ± 30.73^{ab}

Captopril: antihypertensive drugs; FTPE: fermented Taichung Eureka juice + fermented Pingtung Eureka juice.
^{a,b} values in the same column with different superscripts mean significant difference ($p < 0.1$).

Table 4 The effect of lemon juice fermented by probiotics on immune- globulin in the serum of OVA-sensitized mice.

	Control	FTPL	OVA
IgE (pg/ml)	0 ± 1.25^a	13.52 ± 10.95^b	32.39 ± 7.82^c
IgG1 (µg/ml)	12.41 ± 1.71^a	20.37 ± 18.17^{ab}	44.35 ± 17.10^b
IgG2a (ng/ml)	65.22 ± 11.20^a	85.74 ± 8.51^{ab}	72.73 ± 23.97^a

FTPL: fermented Taichung lemon + fermented Pingtung lemon juices. ^{a,b} values in the same column with different superscripts mean significant difference ($p < 0.05$).

product of milk fermented by *Lactobacillus helveticus* and *Saccharomyces cerevisiae*, in 30 elderly patients with hypertension, many of whom had been treated with anti-hypertensive drugs. The patients were divided into two groups, those in one of which, were requested to drink 95 ml of calpis daily for 8 weeks, whereas patients in the other (placebo) group drank the same amount of artificial yogurt. After drinking calpis, systolic blood pressure was found to be significantly reduced in the fourth (-9.4 ± 3.6 mm Hg) and eighth (-14.1 ± 3.1 mm Hg) weeks, and the diastolic blood pressure had decreased significantly (-6.9 ± 2.2 mm Hg) by week 8. In contrast, no significant reductions in blood pressure were detected among the placebo group patients. Otherwise, the two groups showed no significant changes with respect other indicators, such as pulse rate, body weight, and serum biochemical values. Similarly, Fuglsang et al [35] found that milk fermented with two strains of *L. helveticus* (CHCC637 and CHCC641) contained numerous types of angiotensin-converting enzyme inhibitors and had a significant effect on lowering blood pressure in experimental animals.

Anti-allergenic effects in OVA-sensitized BALB/c mice

Having been fed a diet containing a 1:1 mixture of fermented Taichung lime and Pingtung lime juices for 3 weeks, BALB/c mice were sensitized twice with ovalbumin, and were subsequently evaluated for the production of IgE, the results of which are presented in Table 4. We accordingly found that administration of fermented lemonade and commercially available products significantly reduced OVA-induced IgE antibody levels by 58% and 68%, respectively ($p < 0.05$). Compared with the OVA-induced group, the levels of IgG1 and IgG2a in mice administered the commercially available products were significantly higher than ($p <$ 0.05). Although we detected no statistically significant differences in serum IgG1 and IgG2a concentrations in the fermented lemonade and OVA-induced groups, we did, nevertheless, observe reductions and increases of 44.35 ± 17.1 to 37 ± 18.17 µg/ml and 72.73 ± 23.97 to 85.74 ± 8.51 µg/ml in the levels of IgG1 and IgG2a, respectively. In this regard, previous studies have indicated that the production of IgG2a and IgG1 antibodies is induced by Th1 and Th2 cells, respectively [31].

In recent years, it has been established that the metabolites produced during lactic acid bacteria-mediated fermentation can enhance the antibacterial and immune properties of lemons, and thereby have beneficial health effects [4]. The cell wall components of lactic acid bacteria, including peptidoglycans, polysaccharides, and teichoic acid, are known to have immunomodulatory properties [36], and cell extracts have been demonstrated to induce changes in macrophage type and enhance the phagocytotic degradation of pathogenic bacteria. Lactic acid bacteria also release certain peptides during milk fermentation, and in mice fed diets containing these bacteria, increases in the number of IgA-secreting B cells have been detected, which have been found to enhance immune function [37]. The findings of numerous studies have also revealed that different probiotic strains can stimulate different immune responses via different mechanisms. For example, in studies examining the secretion of cytokines in small intestine tissues, it has been observed that among mice treated with *L. acidophilus*, *L. casei*, *L. delbrueckii* ssp. b*ulgaricus*, or *S. thermophilus*, those that had be fed *L. casei* or *L. delbrueckii* ssp. *bulgaricus* were characterized by higher levels of both IL-10 and IL-4, whereas rats fed *L. acidophilus* showed significant increases in IL-2 and IL-12. In addition, after the injection of mice with ovalbumin to induce allergies, changes in antibody production have been observed, with amounts of IgG1 in the serum of mice fed *L. delbrueckii* ssp. *bulgaricus*, *L. casei*, or *L. acidophilus* increasing, whereas those fed *L. acidophilus* also showed increases in the levels of IgG2a [12].

Although IgE, discovered by Ishizaka et al [38], occurs in serum at very low concentrations, it plays particularly important roles in the human body; for example, mediating the immediate hypersensitivity reaction following allergen stimulation, and is associated with defense against parasites [39]. Levels of IgE tend to increase in the blood of patients with atopic asthma, and in this regard, IgE has been found to have a high affinity for mast cells and basophils. After binding, IgE induces the release of multiple bioactive substances, including inflammatory mediators such as histamine, leukotriene, and kinins, which contribute to triggering a cascade of organ responses. During allergic inflammation, Th2 cells not only induce B cells to produce allergen-specific IgE but also stimulate B cells to produce IgG1 that inhibits IgG2a. Consequently, the equilibrium between Th1 and Th2 cells influences the intensity of allergic responses and regulates the infiltration of lymphocytes and secretion of cytokines during allergic responses.

Common allergies, such as asthma, atopic rhinitis, eczema, and food allergies, are generally type 1 reactions. The antigen-induced hypersensitivity responses associated with type 1 reactions tend to be mediated by type II helper T cells, resulting in a disequilibrium between Th1 and Th2 cells. Kakiyama et al [40] demonstrated that LP0132-fermented citrus juice has beneficial effects in the treatment of Japanese cedar pollinosis. Consequently, we believe that fermented Taichung and Pingtung lime juice mixtures may have potential therapeutic application in the treatment of allergy-induced immune responses; however, further studies are required to confirm the efficacy of such treatment.

CONCLUSION

Fermentation of lemon juice using lactic acid bacteria can enhance the health-related benefits of this natural product. In this study, we used these bacteria to ferment the juice of lemons of different varieties and from different regions to determine the corresponding yields of γ-aminobutyric acid. When administered to spontaneously hypertensive rats, fermented lemon juices were found to significantly lower heart rate and diastolic, systolic, and mean blood pressures, whereas in ovalbumin-sensitized mice, these juices significantly reduced induced IgE antibody levels. On the basis of our findings, we thus believe that lactic acid bacteria-fermented juice may have anti-allergenic and/or hypertension-related regulatory effects, and should accordingly be consumed daily as a dietary health product.

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