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Lactic fermentation and antioxidant activity of Zingiberaceae plants in Taiwan

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Abstract
The present study evaluated functional properties of lactic-fermented ginger products. Three Zingiberaceae species were used as the substrate for fermentation using three lactic acid bacteria. The fermentation process ended 35–40 h after inoculation and reached a pH value of 3.5–4.0. Total antioxidant performances were 68–75%, and were best observed using *Bifidobacterium longum* as the starter in three ginger samples. DPPH scavenging was on average 70%, with free radical anion scavenging and peroxide removal effects of 30.6% and 43.7%, respectively. The product acceptance survey showed that the 100% fermented juice without a mixture with non-fermented ginger juice obtained the highest score in overall performance. The lactic-fermented *Vanoverberghia* and *Hedychium* ginger species retained an antioxidant activity and DPPH scavenging activity of on average 70%. This study may suggest a new way of ginger food processing with high functionality. Also, it may help to popularize the growing and processing of endemic ginger plants in Taiwan.

Keywords: *Antioxidant, lactic fermentation, reducing power, Zingiberaceae*

Introduction

The Zingiberaceae plants, commonly known as ginger, have 50 genera and over 1,300 species worldwide (Williams et al. 2004). Most of them have strong aromatic and medicinal properties. The rhizome of the Zingiberaceae plant in powder form, turmeric, a source from *Curcuma* sp., is widely applied as a food additive in many Asian countries. Medicinal functions for treatment of diseases are also widely recorded in many traditional remedies (Ammon 1991; Charles and Charles 1992; Miquel et al. 2002).

Over the past few decades, there are increasing cases of isolation of novel lactic acid bacteria strains that exert a beneficial health effect to humans. Such strains are termed probiotic. Probiotics are major lactic acid bacteria; after ingestion in certain numbers, they help to promote health benefits beyond inherent basic nutrition in a certain number (Parker 1974). Lactic-fermented food has also been identified in previous studies, with evidence demonstrating its functionality in promoting gastrointestinal health (Parker 1974; Sarrela et al. 2002).

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Zingiberaceae plants are rich in functional chemicals, including terpenes, alcohols, ketones, flavonoids, carotenoids and phytoestrogens (Habsah et al. 2000; Skrzypezac-Jankun et al. 2000). These components have been reported for many antifungal, antioxidant, insecticidal and anti-inflammatory activities (Sirat 1994; Sirat and Liamen 1995; Sirat et al. 1996; Mau et al. 2003; Suhaj 2006). Some widely consumed ginger has a long human food history worldwide, based on previous investigation on Taiwan endemic ginger plants' properties in antioxidant and antimicrobial activities (Chen et al. 2008), and the present study evaluates functional properties of a new lactic-fermented ginger product prototype. The fermentation condition and functional characteristics were investigated. *Vanoverberghia* sp. is an endemic species found in local Lanyu island in 2000. *Hydychium* is used as a traditional remedy for flu and fever by aborigines in Taiwan since ancient times (Funakoshi and Ohashi 2000). The previous study by Chen et al. (2008) indicates their antioxidative and free radical reducing properties. *Vanoverberghia* is only found in Lanyu island and there has been a lack of incentive to use it commercially since its discovery. To promote their value and processing, lactic fermentation may be an optimum way to preserve its nutrient and functional properties.

Despite previous study indicating the antimicrobial activity of ginger extract (Habsah et al. 2000; Wilson et al. 2005; Chen et al. 2008), our preliminary test on ginger juice supports growth of lactic acid bacteria. This encouraging result may turn ordinary ginger material into a high-value added product. The initial conditions of the ginger material are presented in Table I. The viable bacteria count is 4.5×10^3 colony-forming units (CFU)/ml. Notably, the total solid soluble is 2.8°Brix; preliminary experiments gave a significant growth of lactic acid bacteria, and thus no additional carbon source was given. The present study is hoped to contribute a positive outcome in promoting the use of endemic ginger plants in Taiwan.

Materials and methods

Materials preparation

Three gingers (*Curcuma domestica* Valet, *Hedychium coronarium* Koenig and *Vanoverberghia sasakiana* H. Funak. & H. Ohashi) were obtained from a traditional market and Taitung Agricultural Improvement Station, Council of Agriculture. The selection of these species is based on their high functionality as an antioxidant indicated in our previous study (Chen et al. 2008). After arrival at the laboratory, samples were washed and peeled. Afterward, 150 ml sterile water was added to 150 of peeled gingers and then blended into juice using a commercial food processor (CookPot JF-102, RTmart, Taipei, ROC) under room temperature. The ginger juice was directly use for inoculation without any further treatment.

Table I. Initial conditions of *Curcuma* ginger juice material ($n = 3$).

Measurement	Result
Viable bacteria count	$4.5 \pm 0.8 \times 10^3$ CFU/ml
Titratable acid (citric acid)	$0.26 \pm 0.04\%$
pH value	6.4
Total solid soluble	2.8 ± 0.3°Brix

Strains *Lactobacillus acidophilus* BCRC 14079, *Lactobacillus casei* subsp. *casei* BCRC 12248 and *Bifidobacterium longum* BCRC 14602 were purchased from Bioresources Collection and Research Center, Hsinchu, ROC. The strains were subcultured twice on a MRS (de Man, Rogosa and Sharpe) plate (BD Difco, NJ, USA) according to the user manual.

Total soluble solids, titratable acid and pH determination

Fermented ginger juice was filtered with an 11-cm diameter Whatman #1 filter (Post Apple Scientific, Inc., North East, PA, USA). The total soluble solids were determined using a Hand Refractometer (ATAGO, Tokyo, Japan). The recorded data were presented as °Brix. Titratable acid (citrus acid) was determined using the method described by Policegoudra and Aradhya (2007), as it is the major titratable component of ginger juice. Fermented juice was filtered with a Whatman #1 filter, and 20 g filtered juice was titrated using 0.1 M NaOH at room temperature to a pH value of 8.1. Fermented juice was filtered with a Whatman #1 filter and measured by a pH Vision 6071 microcomputer (Jenco Electronics Ltd., Taiwan, ROC).

Fermentation of ginger juice

Strains *L. acidophilus* BCRC 14079, *L. casei* subsp. *casei* BCRC 12248 and *B. longum* BCRC 14602 were used as fermentation starters based on the previous study of Chen et al. (2008) and Tien et al. (2005). The subcultured starters were cultured in MRS (de Man, Rogosa and Sharpe plate) broth for 24 h at 37°C and reached an OD_{600} of 1.0, which is equivalent to 10^6 CFU/ml. Five milliliters of starter (5×10^6 CFU/ml) was inoculated into 150 ml ginger juice. The fermentation process was carried at 37°C for 60 h. To standardize the growing conditions of fermentation and to avoid growth of other pathogens, fermented ginger juice with a viable bacterial count greater than 10^8 CFU/ml was heated at 90°C for 1 min on heating plate according to Chen (2002). The viable bacteria count was carried out by the serial dilution plate count method. The fermented samples were dried with a freeze-dryer (Christ Freeze dryer Alpha 1-2/LD-2, Vacuum pump RZ-5; Kuhner, Basel, Switzerland) for 48 h. Dried samples were subsequently milled using a commercial hand-carry milling machine (TSK-U928S EUPA, Hypermart, Taipei, Taiwan). The preparation of methanolic extract was described by Chen et al. (2008). The samples were ready for further testing.

Total antioxidant activity

The antioxidant capacity of samples was measured using the method as described by Miller and Rice-Evans (1997) and Arnao et al. (2001) with little modification. Peroxidase (4.4 units/ml; Sigma-Aldrich, St Louis, MO, USA), H_2O_2 (50 μM; Merck, Darmstadt, Germany), 2,2-azino-bis (3-ethylbenz-thiazoline-6-sulphonic acid) (100 μM; Sigma-Aldrich) and distilled water (1 ml) were mixed and kept in the dark for 1 h for reaction. One milliliter of plant extract was subsequently added and determined for absorbance at 734 nm. The antioxidant capacity was calculated by the following formula:

$$\text{Total antioxidant activity (\%)} = [1 - (A_{734\ \text{nm}}\ \text{sample}/A_{734\ \text{nm}}\ \text{control})] \times 100\%$$

Reducing power

The reducing power was measured according to the method described by Duh and Yen (1997). One milliliter of plant extract, phosphate buffer (0.2 M, pH 6.6, 0.5 ml; Merck), and potassium hexacyanoferrate solution (1% v/w, 2.5 ml; Merck) were placed in a test tube and heated at 50°C for 20 min. The tube was cooled on ice and 0.5 ml 10% trichloroacetic acid (Merck) was added. After centrifugation at 3,000 $\times g$ for 10 min, an 1 ml aliquot of supernatant was mixed with 1 ml distilled water and 0.1 ml ferric chloride (0.1%; Merck), and then the reaction maintained for 10 min. Finally, the absorbance at 700 nm was measured. Increased absorbance of the reaction mixture indicates higher reducing power.

DPPH free radical scavenging activity

The DPPH (Sigma-Aldrich) removal method was as referred by Shimada et al. (1992). Briefly, 1 ml methanolic extract and 5 ml freshly prepared 0.1 mM DPPH methanolic solution were thoroughly mixed and kept in the dark for 60 min. The absorbance of the reaction mixture at 517 nm was measured with a spectrophotometer. The blank was prepared by replacing the ginger extract with methanol (1 ml). The percentage of free radical scavenging activity was calculated as follows:

$$\text{Scavenging effect (\%)} = [1 - (A_{517\ \text{nm}}\ \text{sample}/A_{517\ \text{nm}}\ \text{blank})] \times 100\%$$

Scavenging effect against superoxide anion radicals

Using Robak and Gryglewski's (1988) method, 120 μM phenazine methosulfate (Sigma-Aldrich), 936 μM α-nicotinamide-adenine-dinucleotide (Sigma-Aldrich) and 300 μM nitro-blue tetrazolium (Sigma-Aldrich) solution were mixed with 50 μl fermented ginger juice. After 5 min reaction, the absorbance at 560 nm was measured, the percentage of free radical scavenging activity was calculated as follows:

$$\text{Scavenging effect (\%)} = [1 - A_{560\ \text{nm}}\ \text{sample}/A_{560\ \text{nm}}\ \text{blank})] \times 100\%$$

Scavenging effect against hydrogen peroxide

The method was that referred to Pick and Mizel (1981). Fifty microliters of fermented ginger juice was added with 50 μl of 5 mM H_2O_2 and mixed thoroughly. After 20 min reaction under room temperature, 100 μl horseradish peroxidase-phenol red (HRPase, 300 μg/ml; phenol red, 4.5 mM; Sigma-Aldrich) was added and reacted for 10 min. Absorption at 610 nm was recorded and calculated as follows:

$$\text{Scavenging effect (\%)} = [1 - (A_{610\ \text{nm}}\ \text{sample}/A_{610\ \text{nm}}\ \text{blank})] \times 100\%$$

Sensory testing

Sensory testing of the product was evaluated by 25 students with a food processing background from Department of Horticulture, National Taiwan University. Fermented ginger was mixed with fresh ginger juice in 0:1, 1:1 and 1:0 ratios. The testers were

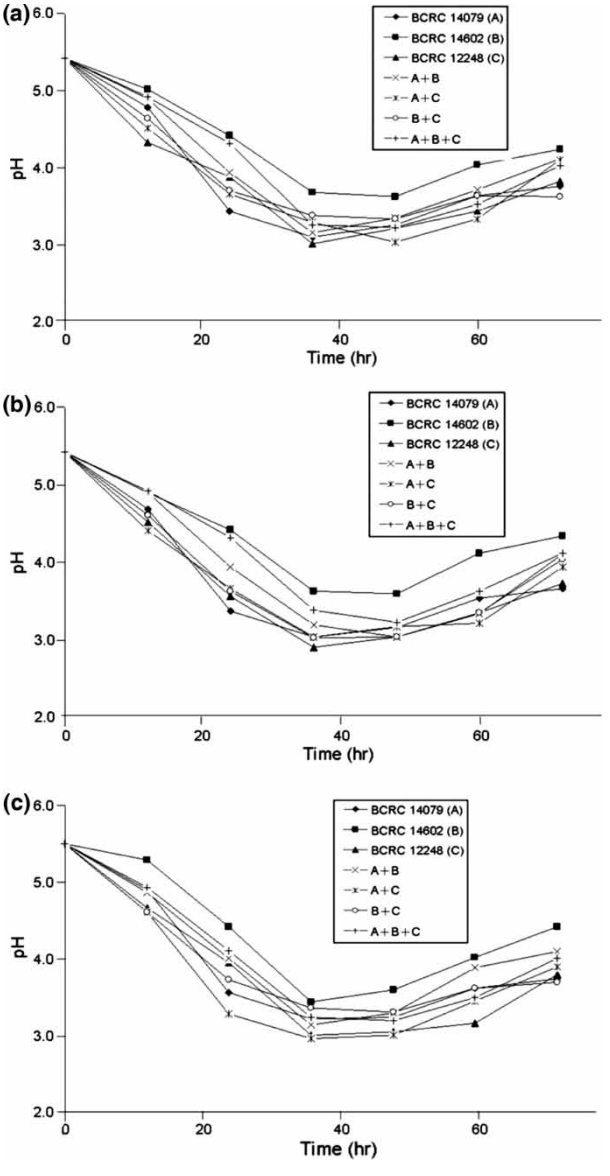

Figure 1. Changes in pH value during fermentation by different starters. (a) *Curcuma domestica* Valet. (b) *Hedychium coronarium* Koenig. (c) *Vanoverberghia sasakiana* H. Funak. & H. Ohashi. Reported values are the mean ± standard deviation ($n = 3$).

requested to evaluate the products in terms of color, aroma, sweetness, tartness and overall acceptability along a scale of zero to seven, with zero indicating the lowest and seven the highest levels of acceptance.

Statistical analysis

Experimental results were averages of triplicate analysis. The data were recorded as the mean ± standard deviation and analysis was by the statistical analysis system

Table II. Total antioxidant activity of lactic-fermentation of gingers.

	Total antioxidant capacity (%)		
	C. domestica Valet	*H. coronarium* Koenig	*V. sasakiana* H. Funak. & H. Ohashi
L. acidophilus (A)	$65.7 \pm 3.9^{B,b}$	$70.4 \pm 2.8^{AB,a}$	$66.7 \pm 4.5^{A,ab}$
B. longum (B)	$71.6 \pm 5.2^{A,a}$	$75.2 \pm 4.8^{A,a}$	$68.2 \pm 2.8^{A,a}$
L. casei (C)	$66.9 \pm 3.82^{B,a}$	$67.5 \pm 5.2^{BC,a}$	$65.3 \pm 3.7^{AB,a}$
A+B	$63.8 \pm 4.3^{B,a}$	$66.9 \pm 5.8^{BC,a}$	$61.2 \pm 3.9^{ABC,a}$
A+C	$62.5 \pm 3.8^{BC,a}$	$62.1 \pm 3.2^{C,a}$	$55.5 \pm 5.1^{C,b}$
B+C	$64.9 \pm 5.8^{B,a}$	$60.7 \pm 2.8^{B,a}$	$58.8 \pm 4.9^{BC,a}$
A+B+C	$57.4 \pm 4.8^{C,a}$	$63.5 \pm 5.3^{BC,a}$	$55.6 \pm 4.2^{C,b}$
BHA (butylated hydroxyanisole)	94.7 ± 4.7		
α-Tocopherol	95.3 ± 5.5		

Contents of BHA and α-tocopherol are 40 mg/l. Reported values are the mean ± standard deviation ($n = 3$). Data bearing different superscript letters in the same column (uppercase) and in the same row (lowercase) are significantly different ($P < 0.05$).

(SAS Inc., Cary, NC, USA). One-way analysis of variance was performed by analysis of variance procedures. Significant differences between means were determined by Duncan's multiple-range tests. Results were considered statistically significant at $P < 0.05$.

Results and discussion

Changes in pH in a 60-h fermentation process were observed as shown in Figure 1. The pH value of majority of inoculated substrates decreased from pH 5.5 to 3.0, especially BCRC 12248. The lowest pH level of the fermentation process occurred 38 h after inoculation. Notably, BCRC14602-fermented substrates retained the highest pH value among samples, with the lowest pH of 4.0 observed in *Vanoverberghia* samples (Figure 1c).

Table III. Reducing power of lactic-fermentation of gingers.

	OD_{700}		
	C. domestica Valet	*H. coronarium* Koenig	*V. sasakiana* H. Funak. & H. Ohashi
L. acidophilus (A)	$0.18 \pm 0.03^{F,a}$	$0.20 \pm 0.02^{C,a}$	$0.19 \pm 0.01^{C,a}$
B. longum (B)	$0.30 \pm 0.01^{A,a}$	$0.31 \pm 0.01^{A,a}$	$0.28 \pm 0.02^{A,a}$
L. casei (C)	$0.26 \pm 0.02^{BC,a}$	$0.22 \pm 0.03^{BC,b}$	$0.25 \pm 0.03^{AB,a}$
A+B	$0.21 \pm 0.03^{CD,a}$	$0.19 \pm 0.02^{C,a}$	$0.21 \pm 0.02^{C,a}$
A+C	$0.19 \pm 0.03^{EF,a}$	$0.16 \pm 0.01^{D,a}$	$0.18 \pm 0.02^{C,a}$
B+C	$0.28 \pm 0.02^{AB,a}$	$0.19 \pm 0.02^{C,c}$	$0.22 \pm 0.01B^{C,b}$
A+B+C	$0.22 \pm 0.01^{DE,b}$	$0.15 \pm 0.01^{D,c}$	$0.19 \pm 0.02^{C,a}$
BHA (butylated hydroxyanisole)	0.52 ± 0.03		
α-Tocopherol	0.85 ± 0.02		

Contents of BHA and α-tocopherol are 40 mg/l. Reported values are the mean ± standard deviation ($n = 3$). Data bearing different superscript letters in the same column (uppercase) and in the same row (lowercase) are significantly different ($P < 0.05$).

Table IV. Scavenging effects of lactic-fermentation of gingers against DPPH radicals.

	Scavenging effect (%)		
	C. domestica Valet	*H. coronarium* Koenig	*V. sasakiana* H. Funak. & H. Ohashi
L. acidophilus (A)	$62.5 \pm 2.7^{B,ab}$	$65.7 \pm 2.5^{B,a}$	$60.3 \pm 1.5^{B,b}$
B. longum (B)	$70.4 \pm 3.1^{A,a}$	$71.8 \pm 2.2^{A,a}$	$72.3 \pm 2.7^{A,a}$
L. casei (C)	$69.1 \pm 2.2^{A,a}$	$66.6 \pm 1.9^{B,ab}$	$65.5 \pm 1.8^{B,a}$
A+B	$63.8 \pm 1.9^{B,a}$	$66.7 \pm 3.8^{B,a}$	$64.8 \pm 1.4^{B,a}$
A+C	$60.8 \pm 3.6^{B,a}$	$58.6 \pm 2.5^{C,a}$	$63.4 \pm 2.7^{B,a}$
B+C	$68.5 \pm 4.3^{A,a}$	$67.4 \pm 1.7^{AB,a}$	$70.5 \pm 2.4^{A,a}$
A+B+C	$58.3 \pm 2.2^{B,a}$	$60.2 \pm 3.2^{C,a}$	$55.9 \pm 3.1^{C,a}$
BHA (butylated hydroxyanisole)	85.4 ± 1.8		
α-Tocopherol	84.4 ± 1.3		

Contents of BHA and α-tocopherol are 40 mg/l. Reported values are the mean ± standard deviation ($n = 3$). Data bearing different superscript letters in the same column (uppercase) and in the same row (lowercase) are significantly different ($P < 0.05$).

The antioxidant activity of three samples using different combination of starters ranged from 55% to 75%. The performances were best observed using *B. longum* as the starter in three ginger samples (Table II)—gingers fermented with *B. longum* showed higher antioxidant activity. The results obtained from the mixture were 55.5–66.9%.

In reducing power, three gingers using *B. longum* as starter exhibited higher activity (0.30 absorbance) than those of a mixture, which averaged 0.22 (Table III). A similar result was also found in DPPH scavenging activity, *B. longum* used as the starter in three ginger substrate remains highest in scavenging activity (>70%) (Table IV), with the mixture groups showing an average of merely 63%. In superoxide radical and hydrogen peroxide scavenging properties, *B. longum* displayed higher levels in anion and hydrogen peroxide radical removal effects using three substrates, averaging 29% and 40%, respectively (Tables V and VI). The mixture groups merely gave an average of 18% and 25%, respectively.

Table V. Scavenging effects of lactic-fermentation of gingers against superoxide anion radicals.

	Scavenging effect (%)		
	C. domestica Valet	*H. coronarium* Koenig	*V. sasakiana* H. Funak. & H. Ohashi
L. acidophilus (A)	$13.6 \pm 2.3^{E,ab}$	$15.6 \pm 1.9^{C,a}$	$11.7 \pm 1.8^{E,b}$
B. longum (B)	$30.6 \pm 2.5^{A,a}$	$29.4 \pm 2.4^{A,a}$	$28.2 \pm 3.1^{A,a}$
L. casei (C)	$18.6 \pm 1.6^{CD,a}$	$16.3 \pm 1.7^{C,a}$	$15.3 \pm 2.3^{CD,a}$
A+B	$16.5 \pm 2.7^{D,a}$	$14.6 \pm 1.3^{C,a}$	$13.8 \pm 1.5^{DE,a}$
A+C	$13.8 \pm 1.5^{E,a}$	$11.4 \pm 1.6^{D,a}$	$14.6 \pm 1.9^{CD,a}$
B+C	$22.9 \pm 1.8^{B,a}$	$19.26 \pm 2.7^{B,a}$	$21.49 \pm 1.8^{B,a}$
A+B+C	$19.2 \pm 2.4^{C,a}$	$15.8 \pm 1.6^{C,b}$	$17.3 \pm 2.3^{C,ab}$
BHA (butylated hydroxyanisole)	46.7 ± 1.6		
α-Tocopherol	55.8 ± 2.2		

Contents of BHA and α-tocopherol are 40 mg/l. Reported values are the mean ± standard deviation ($n = 3$). Data bearing different superscript letters in the same column (uppercase) and in the same row (lowercase) are significantly different ($P < 0.05$).

Table VI. Scavenging effects of lactic-fermentation of gingers against hydrogen peroxide.

	Scavenging effect (%)		
	C. domestica Valet	*H. coronarium* Koenig	*V. sasakiana* H. Funak. & H. Ohashi
L. acidophilus (A)	$32.7 \pm 2.6^{B,a}$	$29.3 \pm 1.8^{B,a}$	$27.5 \pm 3.2^{CD,a}$
B. longum (B)	$40.3 \pm 1.9^{A,ab}$	$36.4 \pm 2.4^{A,b}$	$43.7 \pm 2.6^{A,a}$
L. casei (C)	$30.2 \pm 2.7^{BC,a}$	$25.4 \pm 2.9^{BC,b}$	$33.8 \pm 3.3^{B,a}$
A+B	$27.6 \pm 1.8^{DC,a}$	$24.9 \pm 2.5^{C,a}$	$24.8 \pm 1.9^{DE,a}$
A+C	$21.5 \pm 1.9^{E,a}$	$19.7 \pm 2.8^{D,a}$	$23.6 \pm 2.7^{E,a}$
B+C	$29.2 \pm 2.4^{C,a}$	$25.2 \pm 2.9^{C,a}$	$25.9 \pm 2.2^{CDE,a}$
A+B+C	$26.3 \pm 1.2^{D,ab}$	$23.4 \pm 1.8^{C,b}$	$29.5 \pm 2.5^{BC,a}$
BHA (butylated hydroxyanisole)	21.4 ± 1.3		
α-Tocopherol	33.9 ± 2.2		

Contents of BHA and α-tocopherol are 40 mg/l. Reported values are the mean ± standard deviation ($n = 3$). Data bearing different superscript letters in the same column (uppercase) and in the same row (lowercase) are significantly different ($P < 0.05$).

The product acceptance survey showed that the fermented juice obtained an overall performance of 4.21 points ($op < 0.05$) (Table VII). Ginger juice without any treatment exhibits a pungent and unpleasant flavor and may lead to low acceptability. In color and aroma, all ratio combinations remain similar; however, the sweetness and tartness of product exhibited gradual changes when the ratio of fermented to fresh ginger decreases. It was therefore observed that, after fermentation, decreased tartness of the products may become a major factor for acceptance.

Various studies make use of fruit as a fermentation substrate. Tien et al. (2005) make use of sugar apple (*Annona squamosa* L.) as the fermented substrate. The fermented sugar apple juice exhibited DPPH scavenging efficiency as high of 88% and an iron chelating ability of 49%. The acceptance survey also showed that the fermented juice mix with a fresh juice mixture ratio of 3:7 gained the highest overall performance. This is different to the result shown in this study, as tartness of fresh ginger juice reduced its acceptance. The starter used in this study also was similar to those used by Chen (2002), who found *L. casei* ssp. *casei* to be the optimal fermentation starter in carrot juice. Carrot juice fermentation led by *Lactobacillus* reached pH 3.8 after 60 h, an observation that is rather different from those in this study (35–40 h). Acceptance was higher in fermented gingers without a mixture with fresh ginger.

To compare the antioxidant activity of fermented product with previous studies, fresh *Vanoverberghia* and *Hedychium* methanol extracts, the fermented product, exhibited a decrease of 15–20% of antioxidant, which was previously 89% for both species (Chen et al. 2008); that is, as high as 65–75% of antioxidant and 70% DPPH scavenging activity in the fermented product. This is worthy for promotion due to the additional function for gastrointestinal digestion brought by *Lactobacillus*. This study may suggest a new way of ginger food processing with dual functional property. Also, it would help to popularize growing and processing of endemic Zingiberaceae species.

Table VII. Sensory and consumer test of fermented three ginger substrates.

	Fermented ginger/fresh ginger ratio	Color	Aroma	Acidity	Sweetness	Tartness	Overall acceptance
C. *domestica* Valet	0:1	4.39 ± 1.62^{A}	4.25 ± 2.21^{A}	4.33 ± 2.14^{AB}	0.59 ± 0.23^{C}	3.67 ± 1.14^{C}	3.44 ± 1.12^{C}
	1:1	4.38 ± 1.74^{A}	4.19 ± 1.78^{A}	4.12 ± 1.55^{A}	2.15 ± 0.72^{A}	3.92 ± 1.49^{BC}	3.75 ± 1.02^{C}
	1:0	4.29 ± 1.38^{A}	4.13 ± 1.47^{A}	3.97 ± 1.76^{AB}	3.24 ± 1.28^{A}	4.68 ± 2.15^{AB}	4.02 ± 0.93^{ABC}
H. coronarium Koenig	0:1	4.47 ± 1.53^{A}	4.59 ± 1.33^{A}	3.50 ± 1.52^{AB}	0.87 ± 0.33^{C}	3.21 ± 1.22^{BC}	3.32 ± 1.83^{ABC}
	1:1	4.62 ± 1.86^{A}	4.38 ± 2.04^{A}	3.55 ± 1.35^{B}	1.98 ± 0.92^{B}	4.43 ± 1.68^{AB}	3.79 ± 1.26^{ABC}
	1:0	4.53 ± 2.04^{A}	4.40 ± 1.88^{A}	3.64 ± 1.64^{AB}	3.88 ± 1.58^{A}	4.65 ± 1.62^{AB}	4.22 ± 1.63^{AB}
V. sasakiana H. Funak. & H. Ohashi	0:1	4.35 ± 1.83^{A}	4.25 ± 1.63^{A}	3.35 ± 1.74^{AB}	0.69 ± 0.24^{C}	3.75 ± 1.61^{BC}	3.27 ± 0.84^{C}
	1:1	4.48 ± 2.11^{A}	4.20 ± 1.72^{A}	3.80 ± 1.63^{B}	1.80 ± 0.53^{B}	4.05 ± 1.37^{BC}	3.66 ± 1.32^{BC}
	1:0	4.53 ± 1.87^{A}	4.05 ± 1.68^{A}	4.25 ± 2.25^{A}	3.25 ± 1.47^{A}	5.76 ± 0.85^{A}	4.39 ± 1.41^{A}
	Average	4.44	4.27	3.83	2.05	4.24	

Score of zero indicates the lowest acceptance, seven indicates the highest acceptance. Reported values are the means $\pm$ standard deviation ($n = 3$). Data bearing different uppercase superscript letters in the same column are significantly different ($P < 0.05$)

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