

## Antihypertensive effects of abalone viscera fermented with *Lactiplantibacillus pentosus* SN001 via angiotensin-converting enzyme inhibition

Mayu Yamanushi<sup>a</sup>, Mariko Shimura<sup>b</sup>, Hiroshi Nagai<sup>c</sup>, Naoko Hamada-Sato<sup>a,d,\*</sup>

<sup>a</sup> Course of Safety Management in Food Supply Chain, Tokyo University of Marine Science and Technology, Konan-4, Minato-ku, Tokyo 108-8477, Japan

<sup>b</sup> Research and Development Department, Bull-Dog Sauce Co., Ltd., 3-6-1, Mitsuwa, Kawaguchi-shi, Saitama 334-0011, Japan

<sup>c</sup> Department of Ocean Sciences, Tokyo University of Marine Science and Technology, Konan-4, Minato-ku, Tokyo 108-8477, Japan

<sup>d</sup> Department of Food Science and Technology, Tokyo University of Marine Science and Technology, Konan-4, Minato-ku, Tokyo 108-8477, Japan

### ARTICLE INFO

#### Keywords:

Fermentation  
*Lactiplantibacillus pentosus*  
 Abalone viscera  
 Hypertension  
 Spontaneously hypertensive rats

### ABSTRACT

Abalone viscera, which accounts for more than 20% of body weight, is typically discarded. With increases in abalone aquaculture production, novel uses for abalone viscera are needed. Here, we evaluated the effects of abalone viscera fermented with *Lactiplantibacillus pentosus* SN001 on angiotensin-converting enzyme (ACE) activity and blood pressure elevation in spontaneously hypertensive rats. The fermented product significantly reduced systolic blood pressure compared with the control. There were no significant differences in blood glucose, triglyceride, total cholesterol, or high-density lipoprotein cholesterol levels; alanine aminotransferase activity; and aspartate aminotransferase activity between the fermented product and control groups. Uracil was isolated and identified from the fermented product. Uracil may be the active component. Overall, *L. pentosus* SN001-fermented abalone viscera showed sustained inhibitory effects on blood pressure elevation but did not alter blood components after long-term intake. These results provide insights into the safety of *L. pentosus* SN001-fermented abalone viscera as a food product.

### 1. Introduction

Abalone aquaculture production is increasing annually, with aquaculture production in 2019 equivalent to approximately four times that of a decade ago (FAO Fisheries and Aquaculture, 2021). Typically, the viscera, which accounts for 20–30% of the total weight of the abalone, is discarded during industrial processing (Li, Lin, Chen, & Fang, 2015), resulting in significant waste of natural resources and environmental pollution. However, abalone viscera contain useful substances, such as proteins, fatty acids, and polysaccharides, which are beneficial to health (Zhu et al., 2010). Therefore, it is necessary to develop effective methods for the use of discarded abalone viscera.

Fermentation is a means of improving the quality and shelf life of foods. Lactic acid bacteria (LAB) have been used to ferment foods for at least 4000 years (Rotar et al., 2007). Fermented foods and beverages vary dramatically according to the traditions and cultural preferences of

the different geographic regions in which they are produced (Swain, Anandharaj, Ray, & Rani, 2014). Fermentation is the process of decomposition of organic matter induced by microorganisms or enzymes, resulting in conversion of carbohydrates into alcohols or organic acids (Battcock & Azam-Ali, 1998). Fermentation of fish has many advantages and features, making this approach useful for improving the organic quality, nutritional value, and digestibility of the product (Speranza et al., 2017). LAB can easily and reproducibly ferment fish visceral waste (Vázquez et al., 2011), and fermentation by LAB has been shown to have antihypertensive (Garbowska, Pluta, & Berthold-Pluta, 2019) and cholesterol-lowering effects (El-Dein et al., 2021). Additionally, *Lactobacillus pentosus* has been reported to have probiotic properties (Guantario et al., 2018) and angiotensin-converting enzyme (ACE) inhibitory activity (Fujimura, Shimura, Nagai, & Hamada-Sato, 2021). Thus, foods developed through the fermentation of LAB are expected to have various applications.

\* Corresponding author at: Course of Safety Management in Food Supply Chain, Tokyo University of Marine Science and Technology, Konan-4, Minato-ku, Tokyo 108-8477, Japan.

E-mail addresses: [m206008@edu.kaiyodai.ac.jp](mailto:m206008@edu.kaiyodai.ac.jp) (M. Yamanushi), [mariko.shimura@bulldogsauce.com](mailto:mariko.shimura@bulldogsauce.com) (M. Shimura), [nagai@kaiyodai.ac.jp](mailto:nagai@kaiyodai.ac.jp) (H. Nagai), [hsnaoko@kaiyodai.ac.jp](mailto:hsnaoko@kaiyodai.ac.jp) (N. Hamada-Sato).

<https://doi.org/10.1016/j.fochx.2022.100239>

Received 2 June 2021; Received in revised form 21 January 2022; Accepted 1 February 2022

Available online 4 February 2022

2590-1575/© 2022 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Hypertension is an important risk factor for the development of cardiovascular diseases (Unger, 2002). The development of hypertension is influenced by a number of factors, such as genetic disposition, aging, overweightness, and lifestyle (Pihlanto, Virtanen, & Korhonen, 2010). ACE is a multifunctional enzyme related to the renin-angiotensin system that regulates blood pressure and catalyses both the production of the vasoconstrictor angiotensin II and the inactivation of the vasodilator bradykinin (Hernández-Ledesma et al., 2004). ACE inhibitors, such as captopril and enalapril, inhibit ACE but exhibit many side effects, including zinc deficiency, cough, hypotension, and dizziness (Beuf-Gibot et al., 2020). Therefore, food-derived components with ACE inhibitory activity have been studied, and ACE inhibitory components have been reported in goat milk (Shu et al., 2018), mushrooms (Paisansak et al., 2021), fish (Chen et al., 2019), and walnuts (Chen et al., 2020).

Fermentation of abalone viscera with LAB is expected to have beneficial effects, particularly with regard to suppression of blood pressure elevation. In a previous study, abalone viscera fermented with *L. casei* 001 was shown to have inhibitory effects on blood pressure elevation *in vivo* (Fujimura et al., 2021), suggesting that abalone viscera fermented with LAB may be a promising functional food material for blood pressure control. However, the applicability of this approach to other species of LAB and the effects of ingesting fermented abalone viscera powder on blood components, such as glucose and triglyceride levels, have not been confirmed. Metabolic products and lipids present during fermentation vary depending on the species (Chung et al., 2020). Therefore, it is necessary to examine whether the same effects can be confirmed in other bacterial species.

In this study, we evaluated whether abalone viscera fermented with another LAB, *L. pentosus* SN001, inhibited blood pressure elevation *in vivo* and examined the applicability of this approach. We used ACE inhibitory activity as an indicator of inhibition of blood pressure elevation. We assessed the effects of long-term intake of fermented abalone viscera on blood components and identified components specific to fermented products with ACE inhibitory activity. This will allow us to evaluate whether the fermentation of abalone viscera by LAB can be applied to other LAB as well. In addition, we will evaluate the safety of fermented abalone viscera as a food by checking its effects on blood composition and weight change after long-term intake.

## 2. Materials and methods

### 2.1. Materials

Viscera were obtained from Australian Blacklip abalone (*Haliotis rubra*) cultured in Australia, frozen, and shipped. D(+)-Glucose, Na<sub>2</sub>HPO<sub>4</sub>, KH<sub>2</sub>PO<sub>4</sub>, NaOH, soybean oil, *tert*-butylhydroquinone, NaCl, isoflurane, glucose CII-Test Wako, TG E-Test Wako, cholesterol E-Test Wako, high-density lipoprotein (HDL)-cholesterol E-Test Wako, and transaminase CII-Test Wako were purchased from Fujifilm Wako Pure Chemical Co. (Osaka, Japan). D<sub>2</sub>O was purchased from Kanto Chemical Co. (Tokyo, Japan), and the ACE-kit WST was purchased from Dojin Molecular Technology, Inc. (Kumamoto, Japan). Plate count agar with bromocresol purple was purchased from Nissui Pharmaceutical Co. (Tokyo, Japan), and acetonitrile was purchased from Sigma-Aldrich (St. Louis, MO, USA). β-Corn starch, casein, α-corn starch, sucrose, cellulose, AIN76 mineral mixture, AIN76A vitamin mixture, and L-cysteine were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan).

### 2.2. Fermentation of abalone by *L. pentosus* SN001

*L. pentosus* SN001 was incubated in ILS medium (30 °C, 24 h) for preculture. After freeze-drying, the viscera of Australian blacklip abalone was ground in a household mixer and sieved through a 500-μm sieve to form a powder. Ten grams abalone viscera powder and 2 g glucose were added to 100 mL of 33 mM phosphate buffer solution (pH

5.0), adjusted to pH 6.8 with 1 M NaOH, and sterilised under pressure and heat (121 °C, 15 min). Then, 1 mL of *L. pentosus* SN001 (10<sup>9</sup> CFU/mL) was added and incubated. Viable cell counts and pH were measured at 0, 1, 2, and 4 days. The cultures at 0, 1, 2, and 4 days were lyophilised and prepared as *L. pentosus* SN001-fermented abalone viscera powder.

### 2.3. ACE inhibitory activity during fermentation

*L. pentosus* SN001-fermented abalone viscera powder was extracted with water on days 0, 1, 2, and 4 (10 mg/mL, 50 °C, 125 spm, 1 h) and centrifuged (13,000 × g, 10 min). After centrifugation, the supernatant was lyophilised and used for ACE inhibitory activity measurements. ACE inhibitory activity was measured using an ACE kit-WST (Dojin Molecular Technology, Inc.). Measurements were performed according to the manufacturer's protocol, and a microplate reader was used for absorbance measurements.

### 2.4. Long-term effects of fermented abalone

Fourteen-week-old male spontaneously hypertensive rats (SHR/Izm) were used as experimental animals. The rats were divided into three groups (n = 6/group) and housed in individual cages. The rats were fed a standard diet for 1 week after housing to acclimatise them to the environment. Each group was fed *L. pentosus* SN001-fermented abalone viscera powder mixture (fermented group), unfermented abalone viscera powder mixture (unfermented group), and a standard diet (control group). Abalone viscera powder accounted for 5% of the diet. The rats had free access to food and water. Systolic blood pressure and body weight were measured during rearing. Systolic blood pressure was measured automatically by the tail-cuff method using an unheated, non-invasive Model MK-2000 blood pressure monitor for mice and rats (Muromachi Kikai Co., Ltd., Tokyo, Japan) under non-anaesthetic conditions. After 9 weeks of treatment, the rats were fasted overnight. Under isoflurane anesthesia, blood samples were collected, and the animals were euthanized with blood liberation. Blood glucose, triglyceride, total cholesterol, and HDL-cholesterol levels, as well as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured using glucose CII-Test Wako, TG E-Test Wako, cholesterol E-Test Wako, HDL-cholesterol E-Test Wako, and transaminase CII-Test Wako kits (Fujifilm Wako Pure Chemical Co., Osaka, Japan). The measurement method followed the manufacturer's protocol, and a microplate reader was used for absorbance measurements. All obtained values were expressed as means ± standard errors. Rejection of outliers was performed using Smirnov-Grubbs tests, and significance was evaluated using Steel-Dwass tests. The level of statistical significance was set at *p* < 0.05.

### 2.5. Purification of ACE inhibitory components

Fermented abalone viscera powder and unfermented abalone viscera powder were extracted with water (10 mg/mL, 50 °C, 125 spm, 1 h) and centrifuged (13,000 × g, 10 min). After centrifugation, the supernatants were lyophilised and used as aqueous extracts of fermented and unfermented products. The aqueous extracts of fermented and unfermented products were separated according to molecular weight (>30 kDa, 3–30 kDa, and < 3 kDa) using Vivaspin 20 columns (Sartorius Stedim Lab Ltd., Goettingen, Germany). The separated fractions were concentrated in a rotary evaporator and lyophilised. Fractions with high ACE inhibition (aqueous extracts of fermented products [*<*3 kDa] and unfermented products [*<*3 kDa]) were dissolved in distilled water, filtered through a 0.22 μm polyvinylidene difluoride membrane, and subjected to high-performance liquid chromatography analysis. The column used for the separation was TSKgel ODS-120 T (4.6 × 250 mm; Tosoh, Tokyo, Japan), and the mobile phase consisted of distilled water (eluent A) and acetonitrile (eluent B). The elution conditions were as follows: eluent B, 5%; flow rate, 1.0 mL/min. The collected peaks for fraction 1

(characteristic of the aqueous extract of fermentation products) were concentrated using a rotary evaporator and centrifugal concentrator. The concentrated fraction 1 was separated under the same elution conditions and purified by concentration in a rotary evaporator and centrifugal concentrator. The ACE inhibitory activity of the purified fraction was measured using an ACE kit-WST (Dojin Molecular Technology, Inc.). The measurements were performed according to the manufacturer's protocol, and a microplate reader was used for the absorbance measurements.

## 2.6. Identification of ACE inhibitory components

Nuclear magnetic resonance (NMR) and mass spectroscopy (MS) were used to determine the structure of the purified fraction 1.  $^1\text{H}$  NMR was performed by dissolving 3.7 mg fraction 1 in 0.55 mL  $\text{D}_2\text{O}$  and then evaluating the sample using a Bruker ASEND<sup>TM</sup> 600 MHz (Billerica, MA, USA). MS was performed by dissolving 10  $\mu\text{g}$  fraction 1 in  $\text{H}_2\text{O}$  and then evaluating the sample using a Bruker micrOTFO-QII (Billerica, MA, USA).

## 2.7. Ethical statement

All experiments using animals were conducted in accordance with the Tokyo University of Marine Science and Technology Guidelines for Animal Experiments (approval no.: H30-5). This study was conducted in accordance with the ARRIVE guidelines and in accordance with the UK Animals (Chemical Procedures) Act 1986 and related guidelines, the 2010/63/EU Directive on Animal Experiments, or the UK National Institute for Health Research Guide for the Care and Use of Laboratory Animals (NIH Publication no. 8023, revised 1978).

## 3. Results

### 3.1. ACE inhibitory activity during fermentation

As shown in Fig. 1, in *L. pentosus* SN001-fermented abalone viscera, the viable cell counts increased to  $10^8$  CFU/mL after the first day of incubation, and the pH was below 4 after the first day. The ACE inhibition rate at a final concentration of 0.83 mg/mL increased to approximately 80% on day 1 and was maintained thereafter. Thus, *L. pentosus* SN001-fermented abalone viscera on 1 day of culture was used in subsequent experiments.

### 3.2. Long-term effects of fermented abalone

Trends in systolic blood pressure for each group are shown in Fig. 2, body weight trends are shown in Table 1, and serum parameters are shown in Table 2.

As shown in Fig. 2, the systolic blood pressure in the control group fed a standard diet was consistently high from the beginning of the experiment, and changes were minimal. In contrast, the systolic blood pressure in the fermented group was lowest among the three groups after week 4, except for two points, and the values in the fermented group were significantly lower than those in the control group at four points ( $p < 0.05$ ). Body weight gradually increased (Table 1), and there were no significant differences among groups. The unfermented group showed significantly higher total cholesterol levels than the control group (Table 2). However, there were no significant differences in blood glucose, triglyceride, total cholesterol, and HDL cholesterol levels or in ALT and AST activities between the fermented and control groups.

### 3.3. Purification and identification of ACE inhibitory components

As shown in Table 3, fractions below 3 kDa had the highest ACE inhibitory activity for both aqueous extracts of fermented and unfermented products. From the chromatograms of aqueous extract of the

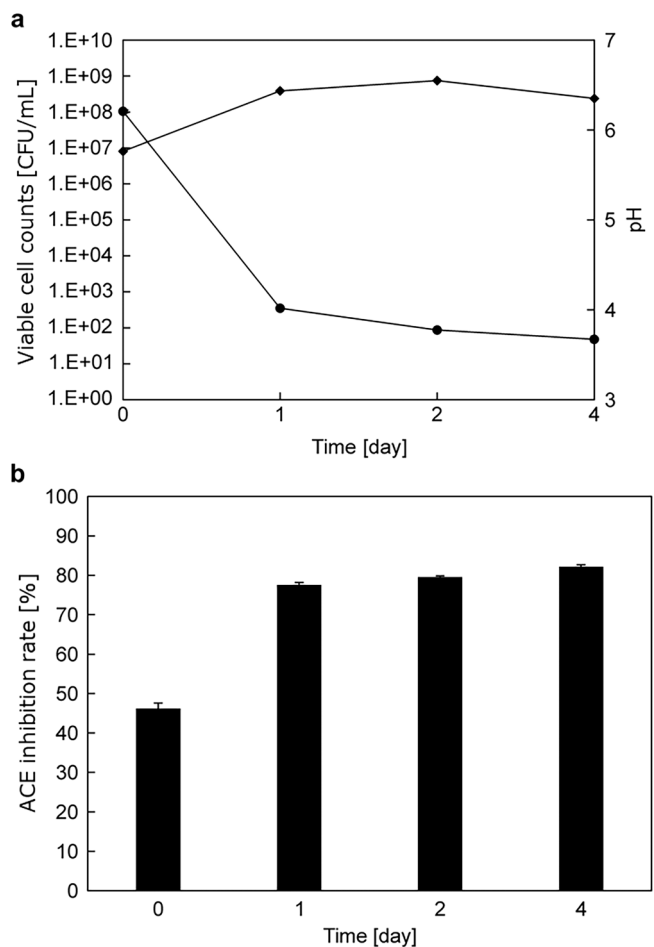


Fig. 1. Viable cell counts (◆), pH (●), and angiotensin-converting enzyme (ACE) inhibition rate (final concentration: 0.83 mg/mL) measured during fermentation of abalone viscera by *Lactobacillus pentosus* SN001. a. Viable cell counts and pH, b. ACE inhibition rate. The data represent means  $\pm$  standard errors ( $n = 4$ ).

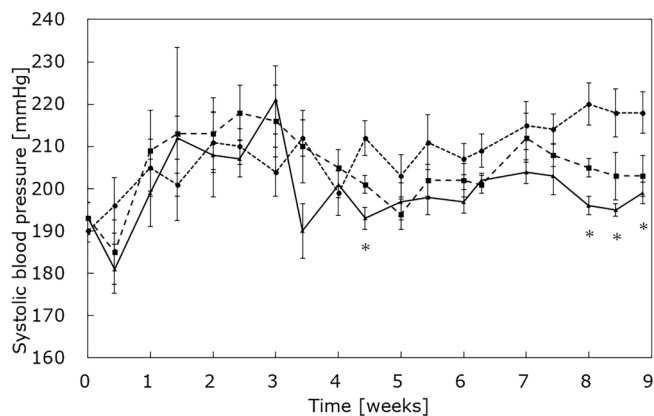


Fig. 2. Systolic blood pressure in spontaneously hypertensive rats treated with different products: standard feed (●), unfermented abalone viscera mixture (■), fermented abalone viscera mixture (▲). Fermented and unfermented abalone viscera mixtures were composed of 5% (w/w) fermented or unfermented abalone viscera mixed with standard feed. The data represent means  $\pm$  standard errors ( $n = 6$ ). \* $p < 0.05$  compared with the control.

fermented product ( $<3$  kDa) and unfermented product ( $<3$  kDa) shown in Fig. 3, a fraction unique to fermented product (fraction 1) was identified at a retention time of 7.22 min. The ACE inhibitory activity of the

**Table 1**

Changes in body weight in spontaneously hypertensive rats during the 9-week treatment period.

Time [weeks]	Body weight [g]		
	Control	Unfermented	Fermented
0	334 ± 2.3	333 ± 3.7	329 ± 2.1
1	346 ± 2.2	349 ± 3.8	341 ± 3.8
2	352 ± 3.0	356 ± 4.5	347 ± 6.1
3	358 ± 3.6	360 ± 4.4	354 ± 6.8
4	364 ± 4.3	364 ± 3.6	360 ± 6.9
5	369 ± 5.0	370 ± 4.3	367 ± 8.1
6	373 ± 5.6	375 ± 3.4	371 ± 8.1
7	376 ± 6.0	376 ± 3.2	372 ± 7.7
8	382 ± 6.3	384 ± 3.8	381 ± 7.3
9	385 ± 6.2	384 ± 3.7	385 ± 7.0

Data are means ± standard errors (n = 6).

**Table 2**

Serum parameters for the three groups of spontaneously hypertensive rats.

	Control	Unfermented	Fermented
Glucose [mg/dL]	168.8 ± 15.4	179.1 ± 21.5	149.5 ± 10.0
Triglycerides [mg/dL]	60.2 ± 5.2	79.9 ± 5.7	69.6 ± 1.9
Total cholesterol [mg/dL]	68.3 ± 2.5	80.2 ± 2.1*	73.7 ± 2.1
HDL-cholesterol [mg/dL]	43.9 ± 2.4	49.4 ± 3.0	47.5 ± 4.0
Alanine aminotransferase activity [IU/L]	23.2 ± 0.1	23.1 ± 0.2	22.4 ± 0.3
Aspartate aminotransferase activity [IU/L]	25.2 ± 0.6	24.6 ± 1.2	25.0 ± 1.1

Data represent means ± standard errors (n = 6). \*p < 0.05 compared with the control.

**Table 3**

Angiotensin-converting enzyme (ACE) inhibition rate. a. Unfermented abalone viscera, b. fermented abalone viscera.

	<3 kDa	3–30 kDa	>30 kDa
<b>a</b>			
ACE inhibition rate [%]	74.1 ± 0.4	52.7 ± 1.2	28.2 ± 1.9
Final concentration [mg/mL]	1.67	1.67	1.67
<b>b</b>			
ACE inhibition rate [%]	46.5 ± 1.4	30.9 ± 0.7	3.3 ± 0.4
Final concentration [mg/mL]	0.20	0.20	0.20

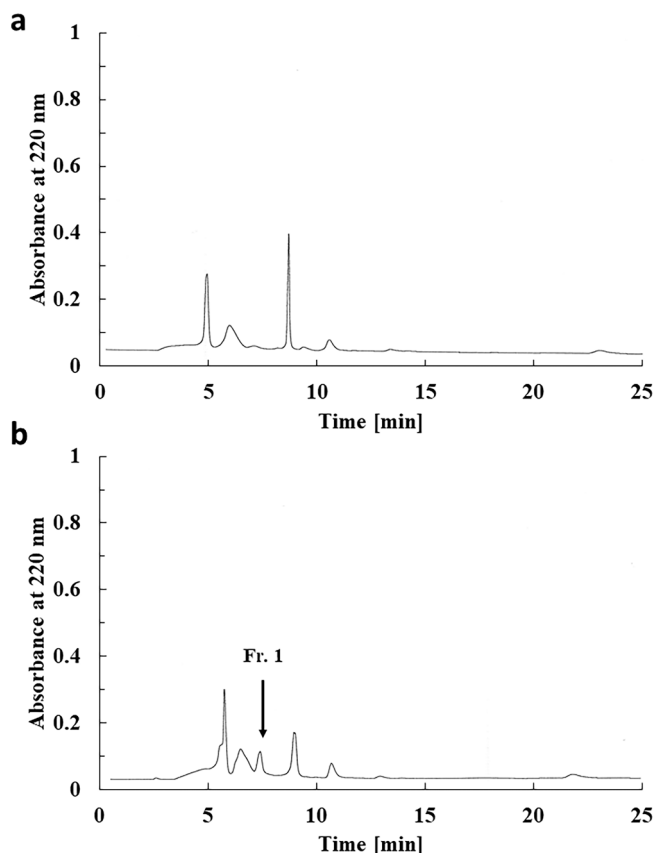
Data are means ± standard errors (n = 3).

purified fraction 1 was higher than that of the fractions below 3 kDa, with a half-maximal inhibitory concentration value of 0.056 mg/mL. Fraction 1 was also identified as uracil (C<sub>4</sub>H<sub>4</sub>N<sub>2</sub>O<sub>2</sub>) by <sup>1</sup>H NMR (Supplementary Fig. 1) and MS analysis (Supplementary Fig. 2).

#### 4. Discussion

Many LAB, including *L. pentosus*, have hydrolytic enzymes that break down proteins in the growth medium into short amino acid chains. These hydrolytic enzymes break down proteins in the growth medium into short amino acid chains, and their degradation products have also been found to have ACE inhibitory activity (Daliri, Lee, Park, Kim, & Oh, 2018). In addition, the production of the ACE inhibitory peptide Ala-Met-Asn by enzymatic hydrolysis in abalone gonads has been reported (Wu et al., 2015), suggesting that components in the abalone viscera are degraded by *L. pentosus* SN001 and contribute to the enhancement of ACE inhibitory activity (Wu et al., 2015). Therefore, the components in abalone viscera may have been degraded by *L. pentosus* SN001, contributing to the enhancement of ACE inhibitory activity.

SHRs, a major model of hypertension, are widely used because of



**Fig. 3.** Comparison of unfermented and fermented abalone viscera by separation on a TSKgel ODS-120 T column (4.6 × 250 mm). a. Unfermented abalone viscera, b. fermented abalone viscera.

their high reliability (Badyal, Dadhich, & Lata, 2003). SHRs have an initial period of clearly elevated arterial blood pressure, with stable values starting at week 10 (Fernández-Vallinas, Miguel, & Alexandre, 2016). In this study, we used SHRs at 15–23 weeks of age. Fermented abalone viscera powder decreased the systolic blood pressure of the SHRs during this period. Additionally, the group that consumed *L. pentosus* SN001-fermented abalone viscera powder showed consistently low blood pressure, suggesting that this treatment had stable, long-term inhibitory effects on blood pressure elevation.

Because there were no significant differences in the weekly trends of food intake among groups (unpublished data), anorexia due to sample mixing was not confirmed, and there were no differences in food intake among the groups. In addition, body weight gradually increased, and there were no significant differences between groups. Significant changes in body weight among test groups have been used as indicators of growth inhibition in rats (Manoharan, Shuib, Abdullah, Ashrafzadeh, & Kabir, 2018). In this study, we found no significant differences between the fermented and control groups, suggesting that administration of fermented abalone viscera did not inhibit the growth of rats. Also, abalone viscera were fed at 2.46 g/day/kg body wt of the rat from the average of body weight and food intake.

Blood glucose levels can be used to assess the presence of diabetes mellitus (Fujiwara et al., 2020). The blood glucose levels in the fermented group were the lowest and were approximately 20 mg/dL lower than those in the control group, although the difference was not significant. LAB consume glucose during metabolism (Cubas-Cano, González-Fernández, Ballesteros, & Tomás-Pejó, 2018). In addition, administration of a closely related species, *L. plantarum*, decreases the amount of glucose in the blood (da Costa et al., 2019). This suggests that glucose consumption by *L. pentosus* SN001 and lowering of blood

glucose concentration by *Lactobacillus* ingestion may explain why the fermented group showed lower blood glucose concentrations than unfermented group and control group.

Cholesterol and triglycerides are important parameters in cardiovascular disease (Tekkeşin et al., 2020). The unfermented group, in which rats consumed abalone viscera powder, tended to have higher levels of triglycerides in the blood, and total cholesterol was significantly higher than that in the control group. The lipid content in the abalone hepatopancreas and gonads is high, ranging from 36 to 131 mg/g (dry mass), because the hepatopancreas is used for lipid storage in abalone tissue (Nelson, Leighton, Phleger, & Nichols, 2002). The unfermented group ingested abalone viscera powder with high lipid content, which may have resulted in high levels of triglycerides and total cholesterol in the blood. *L. pentosus* is a plant lactic acid bacterium that shows probiotic effects and is reported to be resistant to gastrointestinal conditions *in vitro* (Yuasa, Shimada, Matsuzaki, Eguchi, & Tominaga, 2021; Zielińska). The cholesterol-lowering mechanisms of *Lactobacillus* strains include the ability to bind to cholesterol in the small intestine and via the bile salt hydrolase (BSH) involved in the deconjugation of bile salts in the enterohepatic circulation (Anandharaj, Sivasankari, & Rani, 2014). Moreover, BSH activity has been detected in closely related *L. plantarum* (De Smet, Van Hoorde, De Saeyer, Vande Woestyne, & Verstraete, 1994). This suggests that the cholesterol-lowering effect of *L. pentosus* SN001 was strong and that triglyceride and total cholesterol levels in the blood of the fermented group were lower than those in the unfermented group.

HDL cholesterol levels in the blood tended to be higher in the fermented and unfermented groups than in the control group. Cholesterol can be classified into HDL-cholesterol and low-density lipoprotein-cholesterol, and it is well known that high levels of HDL-cholesterol can reduce the risk of cardiovascular disease (Messedi et al., 2011).

ALT and AST activities are used to evaluate liver function (Zhang et al., 2020). In this study, significant differences were not observed between groups, suggesting that long-term intake of fermented abalone viscera powder had no effect on liver function.

Overall, long-term administration of *L. pentosus* SN001-fermented abalone viscera powder showed sustained inhibitory effects on blood pressure elevation *in vivo*. Furthermore, there were no significant differences in blood glucose, triglyceride, total cholesterol, and HDL cholesterol levels or in ALT and AST activities between the fermented and control groups; these factors are important blood components for the evaluation of rat toxicity, and our data therefore suggested that the risk of side effects was low.

Uracil was identified as a component unique to fermented products. In studies on protein degradation, many peptides have been reported as bioactive substances. In this study, uracil was identified because of following the peaks specific to fermented products, but since abalone viscera is a protein rich substrate, it is possible that peptides are also produced. Uracil is a metabolite that is correlated with the growth of LAB in the presence of uridine, which has been reported to be hydrolysed to uracil by microorganisms (Chung et al., 2020; Hidalgo et al., 2005). An increase in uracil has also been observed in *L. helveticus* HY7801 fermented vegetable juice (Chung et al., 2020). Uracil and glycerol have been found to exhibit high ACE inhibitory activity in combination (Liu, Zeng, Leu, & Tsai, 2015). Triacylglycerol is a major component of abalone viscera (Nelson et al., 2002), and proteolytic and lipolytic activities have been suggested in the same species, *L. pentosus* 31-1 (Liu et al., 2010). Thus, the triacylglycerols in the abalone viscera may be degraded to glycerol by LAB and exhibit ACE inhibitory activity in combination with uracil.

These results suggest that the *in vivo* inhibition of blood pressure elevation observed in the fermentation of abalone viscera by *L. casei* 001 could be applied to the fermentation of other LAB. In addition, *L. pentosus* SN001-fermented abalone viscera is likely to be safe for use as a food product because long-term intake did not affect the blood composition of animals. These findings can serve as a basis for

evaluating the safety of abalone viscera fermented with LAB for use as a food product. In this study, we have not been able to elucidate all the functional components in the fermented abalone viscera, nor have we been able to evaluate its preference as a food or its safety for human consumption. Further studies are needed for functional components, sensory testing and safety evaluation of the above-mentioned fermented products for use as a food product.

## Funding

This study was conducted in collaboration with the Tokyo University of Marine Science and Technology and Bull-Dog Sauce Co., Ltd. (Tokyo, Japan). The Tokyo University of Marine Science and Technology had roles in the methodology, investigation, validation, formal analysis, conceptualisation, supervision, project administration, funding acquisition, and writing – original draft. Bull-Dog Sauce Co., Ltd. had roles in financial support and editing.

## CRedit authorship contribution statement

**Mayu Yamanushi:** Methodology, Formal analysis, Investigation, Writing – original draft. **Mariko Shimura:** Writing – review & editing. **Hiroshi Nagai:** Methodology, Investigation. **Naoko Hamada-Sato:** Methodology, Project administration, Supervision.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

We thank Dr. Hajime Uchida of the Fisheries Technology Institute, Japan Fisheries Research and Education Agency for MS measurements. We thank Editage (www.editage.com) for English language editing.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochx.2022.100239>.

## References

- Anandharaj, M., Sivasankari, B., & Rani, R. P. (2014). Effects of probiotics, prebiotics, and synbiotics on hypercholesterolemia: A review. *Chinese Journal of Biology*, 2014, 1–7.
- Badyal, D., Dadhich, A., & Lata, H. (2003). Animal models of hypertension and effect of drugs. *Indian Journal of Pharmacology*, 35, 349–362.
- Battcock, M., & Azam-Ali, S. (1998). *Fermented fruits and vegetables—a global perspective*. FAO Agricultural Services Bulletin, No. 134. ISBN: 92-5-104226-8.
- Bœuf-Gibot, S., Pereira, B., Imbert, J., Kerroum, H., Menini, T., Lafarge, E., ... Vaillant-Roussel, H. (2020). Benefits and adverse effects of ACE inhibitors in patients with heart failure with reduced ejection fraction: A systematic review and meta-analysis. *European Journal of Clinical Pharmacology*, 77, 321–329.
- Chen, J., Ryu, B., Zhang, Y. Y., Liang, P., Li, C., Zhou, C., ... Qian, Z. J. (2019). Comparison of an angiotensin-I-converting enzyme inhibitory peptide from tilapia (*Oreochromis niloticus*) with captopril: Inhibition kinetics, *in vivo* effect, simulated gastrointestinal digestion and a molecular docking study. *Journal of the Science of Food and Agriculture*, 100, 315–324.
- Chen, Y., Li, J., Dong, N., Zhang, Y., Lu, X., Hao, Y., & Qi, J. (2020). Separation and identification of ACE inhibitory peptides from defatted walnut meal. *European Food Research and Technology*, 246, 2029–2038.
- Chung, H. J., Lee, H., Na, G., Jung, H., Kim, D. G., Shin, S. I., ... Choi, H. K. (2020). Metabolic and lipidomic profiling of vegetable juices fermented with various probiotics. *Biomolecules*, 10, 725.
- Cubas-Cano, E., González-Fernández, C., Ballesteros, M., & Tomás-Pejó, E. (2018). *Lactobacillus pentosus* CECT 4023 T co-utilizes glucose and xylose to produce lactic acid from wheat straw hydrolysate: Anaerobiosis as a key factor. *Biotechnology Progress*, 35, Article e2739.
- da Costa, W. K. A., Brandão, L. R., Martino, M. E., Garcia, E. F., Alves, A. F., de Souza, E. L., ... Magnani, M. (2019). Qualification of tropical fruit-derived

- Lactobacillus plantarum* strains as potential probiotics acting on blood glucose and total cholesterol levels in Wistar rats. *Food Research International*, 124, 109–117.
- Daliri, E. B., Lee, B. H., Park, B. J., Kim, S. H., & Oh, D. H. (2018). Antihypertensive peptides from whey proteins fermented by lactic acid bacteria. *Food Science and Biotechnology*, 27, 1781–1789.
- De Smet, I., Van Hoorde, L., De Saeyer, N., Vande Woestyne, M., & Verstraete, W. (1994). *In vitro* study of bile salt hydrolase (BSH) activity of BSH isogenic *Lactobacillus plantarum* 80 strains and estimation of Cholesterol lowering through enhanced BSH activity. *Microbial Ecology in Health and Disease*, 7, 315–329.
- El-Dein, A. N., El-Deen, A. M. N., El-Shatoury, E. H., Awad, G. A., Ibrahim, M. K., Awad, H. M., & Farid, M. A. (2021). Assessment of exopolysaccharides, bacteriocins and *in vitro* and *in vivo* hypocholesterolemic potential of some Egyptian *Lactobacillus* spp. *International Journal of Biological Macromolecules*, 173, 66–78.
- FAO Fisheries & Aquaculture - FAO Yearbook of Fishery and Aquaculture Statistics. Retrieved from Accessed May 11, 2021.
- Fernández-Vallinas, S., Miguel, M., & Alexandre, A. (2016). Long-term antihypertensive effect of a soluble cocoa fiber product in spontaneously hypertensive rats. *Food & Nutrition Research*, 60, 29418.
- Fujimura, Y., Shimura, M., Nagai, H., & Hamada-Sato, N. (2021). Evaluation of angiotensin-converting enzyme-inhibitory activity in abalone viscera fermented by *Lactobacillus casei* 001. *Journal of Functional Foods*, 82, Article 104474.
- Fujiwara, M., Kuwahara, D., Hayashi, M., Zendo, T., Sato, M., Nakayama, J., & Sonomoto, K. (2020). Lowering effect of viable *Pediococcus pentosaceus* QU 19 on the rise in postprandial glucose. *Bioscience of Microbiota, Food and Health*, 39, 57–64.
- Garbowska, M., Pluta, A., & Berthold-Pluta, A. (2019). Antihypertensive peptide activity in Dutch-type cheese models prepared with different additional strains of *Lactobacillus* genus bacteria. *Applied Sciences*, 9, 1674.
- Guantario, B., Zinno, P., Schifano, E., Roselli, M., Perozzi, G., Palleschi, C., ... Devirgiliis, C. (2018). *In vitro* and *in vivo* selection of potentially probiotic *Lactobacilli* from Nocellara del Belice table olives. *Frontiers in Microbiology*, 9, 595.
- Hernández-Ledesma, B., Amigo, L., Ramos, M., & Recio, I. (2004). Angiotensin converting enzyme inhibitory activity in commercial fermented products. Formation of peptides under simulated gastrointestinal digestion. *Journal of Agricultural and Food Chemistry*, 52, 1504–1510.
- Hidalgo, A., Pompei, C., Galli, A., & Cazzola, S. (2005). Uracil as an index of lactic acid bacteria contamination of tomato products. *Journal of Agricultural and Food Chemistry*, 53, 349–355.
- Li, M., Lin, J., Chen, J., & Fang, T. (2015). Pulsed electric field-assisted enzymatic extraction of protein from abalone (*Haliotis discus hannai* ino) viscera. *Journal of Food Process Engineering*, 39, 702–710.
- Liu, G., Griffiths, M. W., Shang, N., Chen, S., & Li, P. (2010). Applicability of bacteriocinogenic *Lactobacillus pentosus* 31–1 as a novel functional starter culture or coculture for fermented sausage manufacture. *Journal of Food Protection*, 73, 292–298.
- Liu, Y. Y., Zeng, S. Y., Leu, Y. L., & Tsai, T. Y. (2015). Antihypertensive effect of a combination of uracil and glycerol derived from *Lactobacillus plantarum* strain TWK10-Fermented soy milk. *Journal of Agricultural and Food Chemistry*, 63, 7333–7342.
- Manoharan, S., Shuib, A. S., Abdullah, N., Ashrafzadeh, A., & Kabir, N. (2018). Gly-Val-Arg, an angiotensin-I-converting enzyme inhibitory tripeptide ameliorates hypertension on spontaneously hypertensive rats. *Process Biochemistry*, 69, 224–232.
- Messedi, M., Jamoussi, K., Frigui, M., Laporte, F., Turki, M., Chaabouni, K., ... Ayedi, F. (2011). Atherogenic lipid profile in Behçet's disease: Evidence of alteration of HDL subclasses. *Archives of Medical Research*, 42, 211–218.
- Nelson, M. M., Leighton, D. L., Phleger, C. F., & Nichols, P. D. (2002). Comparison of growth and lipid composition in the green abalone, *Haliotis fulgens*, provided specific macroalgal diets. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 131, 695–712.
- Paisansak, S., Sangtanoo, P., Srimongkol, P., Saisavoey, T., Reamtong, O., Choo Wongkamon, K., & Karnchanat, A. (2021). Angiotensin-I converting enzyme inhibitory peptide derived from the shiitake mushroom (*Lentinula edodes*). *Journal of Food Science and Technology*, 58, 85–97.
- Pihlanto, A., Virtanen, T., & Korhonen, H. (2010). Angiotensin I converting enzyme (ACE) inhibitory activity and antihypertensive effect of fermented milk. *International Dairy Journal*, 20, 3–10.
- Rotar, M. A., Semeniciu, C., Apostu, S., Suharochi, R., Crina, M., Modoran, C., ... Culea, M. (2007). Researched concerning microbiological evolution of lactic acid bacteria to yoghurt storage during shelf life. *Journal of Agroalimentary Processes and Technologies*, 8, 135–138.
- Shu, G., Huang, J., Bao, C., Meng, J., Chen, H., & Cao, J. (2018). Effect of different proteases on the degree of hydrolysis and angiotensin I-converting enzyme-inhibitory activity in goat and cow milk. *Biomolecules*, 8, 101.
- Speranza, B., Racioppo, A., Beneduce, L., Bevilacqua, A., Sinigaglia, M., & Corbo, M. R. (2017). Autochthonous lactic acid bacteria with probiotic aptitudes as starter cultures for fish-based products. *Food Microbiology*, 65, 244–253.
- Swain, M. R., Anandharaj, M., Ray, R. C., & Rani, R. P. (2014). Fermented fruits and vegetables of Asia: A potential source of probiotics. *Biology Research International*, 2014, Article 250424.
- Tekkeşin, A. I., Hayiroğlu, M. I., Çinier, G., Özdemir, Y. S., İnan, D., Yüksel, G., ... Gürkan, K. (2020). Lifestyle intervention using mobile technology and smart devices in patients with high cardiovascular risk: A pragmatic randomised clinical trial. *Atherosclerosis*, 319, 21–27.
- Unger, T. (2002). The role of the renin-angiotensin system in the development of cardiovascular disease. *American Journal of Cardiology*, 89.
- Vázquez, J. A., Nogueira, M., Duran, A., Pioto, M. A., Rodoriguez-Amado, I., Rial, D., ... Murado, M. A. (2011). Preparation of marine silage of swordfish, ray and shark visceral waste by lactic acid bacteria. *Journal of Food Engineering*, 103, 442–448.
- Wu, Q., Cai, Q. F., Tao, Z. P., Sun, L. C., Shen, J. D., Zhang, L. J., ... Cao, M. J. (2015). Purification and characterization of a novel angiotensin I-converting enzyme inhibitory peptide derived from abalone (*Haliotis discus hannai* ino) gonads. *European Food Research and Technology*, 240, 137–145.
- Yuasa, M., Shimada, A., Matsuzaki, A., Eguchi, A., & Tominaga, M. (2021). Chemical composition and sensory properties of fermented citrus juice using probiotic lactic acid bacteria. *Food Bioscience*, 39, Article 100810.
- Zhang, G., Zhang, J., Wang, B., Zhu, X., Wang, Q., & Qiu, S. (2020). Analysis of clinical characteristics and laboratory findings of 95 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A retrospective analysis. *Respiratory Research*, 21, 74.
- Zhu, B. W., Zhou, D. Y., Li, T., Yah, S., Yang, J. F., Li, D. M., ... Murata, Y. (2010). Chemical composition and free radical scavenging activities of a sulphated polysaccharide extracted from abalone gonad (*Haliotis discus hannai* ino). *Food Chemistry*, 121, 712–718.