Food Science and Nutrition Studies
ISSN 2573-1661 (Print) ISSN 2573-167X (Online)
Vol. 2, No. 2, 2018
www.scholink.org/ojs/index.php/fsns

Original Paper

Physiological Activities of *Perilla Frutescens Var. Frutescens* Leaf Extracts and Storage Stability in Kneaded Noodles

Yoshiyuki Watanabe[1*], Hiroko Kitayama[1], Mina Yamamoto[1], Yusuke Kono[1], Yasue Yamada[1] & Masato Nomura[1]

[1] Department of Biotechnology and Chemistry, Faculty of Engineering, Kindai University, Higashihiroshima, Japan

[*] Yoshiyuki Watanabe, Department of Biotechnology and Chemistry, Faculty of Engineering, Kindai University, Higashihiroshima, 739-2116, Japan

Received: May 16, 2018 Accepted: May 22, 2018 Online Published: June 4, 2018

doi:10.22158/fsns.v2n2p12 URL: http://dx.doi.org/10.22158/fsns.v2n2p12

Abstract

Perilla (Perilla frutescens var. frutescens) leaves were fractionated based on their chemical properties, and the physiological activities of the fractions were evaluated. The acidic fraction had high radical scavenging ability, whereas the superoxide dismutase-like activities of all fractions were low. A positive correlation was observed between scavenging activity and polyphenol content. The inhibitory effects of the extracts on α-amylase and on α-glucosidase activities were low, indicating a weak suppressive effect of the leaf extracts on diabetes. The acidic and phenolic fractions suppressed pancreatic lipase activity and accelerated lipid hydrolysis in adipocytes differentiated from 3T3-L1 cells. Flour noodles kneaded with leaf powder were prepared, and storage stability was examined. The functional compounds in the leaves were heat-sensitive in the flour noodles. We fractionated perilla leaves to isolate and identify valuable components to provide functionality to processed food and determined that some conditions, such as storage temperature, must be considered to effectively use the compounds.

Keywords

flour noodle, perilla leaves, physiological activity, storage stability

1. Introduction

Perilla frutescens var. frutescens is an annual labiate that exclusively inhabits East and South Asia. Perilla seeds have been used for their extracted oil for a long time. Perilla seed oil is more than 60% comprised of the n-3 polyunsaturated fatty acid α-linolenic acid (Ichikawa, 2006). As α-linolenic acid has various physiological functions, such as lowering blood lipid concentration and preventing diabetes

and hypertension, much attention has been focused on the edible oils as dietary supplements. On the other hand, perilla leaves are rarely used as food except in some countries and regions for its unique flavor and bitter and harsh taste. Some polyphenols are expected to be contained in perilla leaves as in other plants. Polyphenols occur naturally in more than 4,000 plant species, where they are synthesized during photosynthesis and play a role eliminating reactive oxygen species. Hence, polyphenols are effective for maintaining and promoting good health, and preventing disease (Jakobek, 2015; Lu et al., 2016). In the present study, perilla leaves were fractionated and the chemical characteristics and physiological activities of extracts of each fraction were evaluated for functional components and applications to processed food. Moreover, flour noodles kneaded with powdered perilla leaves were prepared as a representative application, and storage stability was investigated to verify its utility.

2. Objective

The objective of this study is evaluation for the several physiological activities of components in perilla leaves and examination about effective utilization of the leaves as a food material.

3. Method

3.1 Materials

Perilla leaves were supplied by Fukutomi Bussan Shakunagekan (Hiroshima, Japan). Caffeic acid, cholic acid, glycine, TRIS, 2-morpholinoethane sulfonic acid monohydrate (MES), maleic acid, Folin-Ciocalteu reagent, dexamethasone, 3-isobutyl-1-methylxanthine (IBM-X), insulin, formalin, lecithin from egg yolk, superoxide dismutase (SOD), lipase from porcine pancreas, the SOD-Test Wako, the Amylase-Test Wako, the Glucose CII-Test Wako, and the NEFA C-Test Wako were all purchased from Wako Pure Chemical Industries (Osaka, Japan). (±) -Tocopherol, (+)-catechin, glyceryl trioleate, α-amylase type VI-B, rat intestinal acetone powder, Oil Red-O, and fetal bovine serum (FBS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Trolox was obtained from Merck-Millipore Co. (Darmstadt, Germany). The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was purchased from Tokyo Chemical Industry (Tokyo, Japan). Dulbecco's modified Eagle medium (DMEM) was purchased from ThermoFisher Scientific Inc. (Waltham, MA, USA) and Boehringer Ingelheim GmbH (Ingelheim, Germany), respectively. 3T3-L1 cells were obtained from the Japan Human Science Foundation (Tokyo, Japan). Strong and weak commercial-grade flour products were purchased from Nisshin Flour Milling, Inc. (Tokyo, Japan). *N*-Tris hydroxymethyl methyl-2-aminoethane sulfonic acid (TES) was purchased from Dojindo Molecular Technology, Inc. (Kumamoto, Japan). All other analytical grade chemicals were purchased from either Wako Pure Chemical Industries or Yoneyama Chemical (Osaka, Japan).

3.2 Extraction and Fractionation of Perilla Leaves Based on Chemical Properties

Fresh perilla leaves (20 g) were added to 300 mL distilled water and heated at 95 ℃ for 10 min. After filtering the mixture and evaporating the filtrate under reduced pressure, the condensate was dried in a desiccator containing silica gel to obtain a hot water extract. The fractionation based on chemical

properties was executed as follows: Fresh perilla leaves (100 g) were mixed with 1 L methanol and left in the dark at ambient temperature for 7 days. After filtration and evaporation, the methanol extract was prepared by drying the condensate in a desiccator. In accordance with the fractionation procedure shown in Figure 1 (Urabe et al., 2003), acidic, alkaline, neutral, and phenolic fractions were obtained in their corresponding organic phases. Each fraction was prepared by concentration in a rotary evaporator, freeze-dried (DC400, Yamato Scientific Co., Ltd., Tokyo, Japan), and applied to various assays for evaluating their physiological activities.

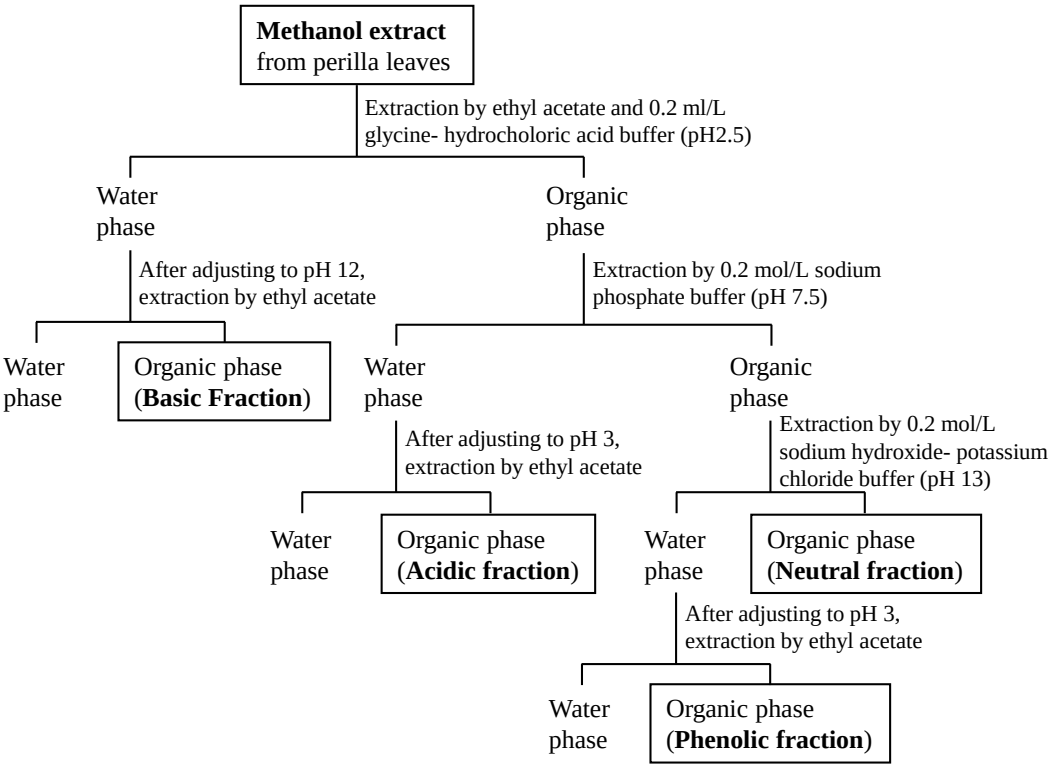

Figure 1. Fractionation Procedure for the Perilla Leaf Methanol Extracts Based on Chemical Properties

3.3 Measurement of Radical Scavenging Activity and Total Polyphenol Content

The radical scavenging activity of the extracts of each perilla leaf fraction was measured according to a previous method (Fujita et al., 2006). A DPPH radical solution (15 mL of 0.4 mM) was mixed with 15 mL of 0.2 M MES buffer (pH 6.0), and the same volume of 3.4 M ethanol solution. The sample solution was prepared at six concentrations by dissolving the powdered fraction in 13.7 M ethanol solution. Then, 600 L of the sample solution and 1.8 mL of the mixture containing the DPPH radicals were added to an amber vial. The headspace in the vial was filled with nitrogen gas to suppress oxidation of the substrate during the reaction, and it was tightly sealed. The vial was shaken and incubated for 20 min at 25℃. Absorption of the sample was measured at 520 nm using a spectrophotometer (DR4000; HACH Co., Loveland, CO, USA), yielding A. A 13.7 M ethanol solution

was used as blank B. The DPPH radical scavenging activity was determined using the following formula:

$$\text{DPPH radical scavenging activity}[\%] = \left(1 - \frac{A}{B}\right) \times 100$$

-Tocopherol and Trolox were used to compare the extracts of the perilla leaf fractions.

The SOD-like activity assay was carried out using the SOD-Test Wako (Yamashoji et al., 1979). SOD and the extract solution were prepared by dissolving them in 0.1 M phosphate buffer (pH 8.0) at 352.9 unit/mL and 2 mg/mL, respectively. The extract solution (50 L), SOD solution (500 L), and coloring reagent solution (500 L) were mixed and incubated at 37 ℃. After sampling periodically and stopping the reaction, absorbance was measured at 560 nm with the spectrophotometer.

The total quantity of polyphenolic compounds in the perilla leaf extracts was determined according to the Folin-Ciocalteu procedure (Kähkönen et al., 1999). Folin-Ciocalteu reagent (1 mL) was added to 1 mL of the extract solution and allowed to stand for 3 min at room temperature. Then, 1 mL of 10% (w/v) sodium carbonate was added, and mixed for 1 h. The mixture was centrifuged at 3,000 rpm for 10 min, and absorbance of the supernatant was measured at 760 nm using the spectrophotometer. Caffeic acid was used as the standard to express the total amount of polyphenolic compounds as caffeic acid equivalents/mg extract.

3.4 Measurement of Inhibitory Effects on α-Amylase, α-Glucosidase, and Pancreatic Lipase Activities

The inhibitory effect of the perilla leaf extracts on α-amylase activity was tested by the Amylase-Test Wako (Anno et al., 2004; Hara & Honda, 1990; McDougall et al., 2005; Saito et al., 2007). First, 5.35 unit/mL α-amylase solution was prepared in 250 mM phosphate buffer (pH 7.0). Starch solution (1 mL) as the substrate and 100 L of each extract were mixed at specific concentrations and incubated at 37 ℃ for 5 min. Then, 20 L of α-amylase solution was added to the mixture and incubated again for 7.5 min. The absorbance of the mixture was measured at 660 nm in a spectrophotometer, following the addition of 1 mL of coloring reagent solution and distilled water. (+)-Catechin was used as the standard for comparison with each extract.

Inhibition of α-glucosidase activity was tested as follows (Anno et al., 2004; McDougall et al., 2005; Saito et al., 2007): Rat intestinal acetone powder (2 g) was added to 45 mL of 56 mM Tris-maleic acid buffer (pH 6.0), and the mixture was homogenized in ice. The supernatant was obtained as a coarse enzyme solution by centrifugation at 3,000 rpm and 4 ℃ for 10 min. A 50 L aliquot of 2% (w/v) maltose, the extract at the given concentrations, the coarse enzyme solution, and the Tris-maleic acid buffer were mixed, and the reaction was carried out at 37 ℃ for 1 h. After stopping the reaction by heat shock at 60 ℃ for 10 min, the concentration of glucose that formed was measured using the Glucose CII-Test Wako. The reaction solution (20 μL) was incubated with 3 mL of coloring reagent solution at 37 ℃ for 5 min, and the absorbance of the mixture was measured at 505 nm using the spectrophotometer.

A substrate emulsion was prepared by ultrasonic treatment of 80 mg glyceryl trioleate, 10 mg lecithin,

and 5 mg cholic acid in 9 mL of 0.1 M TES buffer (pH 7.0) containing 0.1 M sodium chloride to measure pancreatic lipase activity (Edashige et al., 2008; Kwon et al., 2003). The porcine pancreas lipase solution was prepared in buffer. The extracted sample (50 L), 10 units of lipase solution (25 L), and the emulsion (125 L) were mixed at 37 ℃ for 30 min. The amount of free fatty acids in the sample solution was determined by the NEFA C-Test Wako. The sample and coloring reagent solutions were mixed at 37 ℃ for 20 min, and absorbance was measured at 550 nm.

3.5 3T3-L1 Adipocyte Differentiation Assay

The effect of the perilla leaf extracts on adipocyte differentiation of 3T3-L1 cells was investigated to evaluate suppression of obesity according to previously reported methods with a slight modification (Furuyashiki et al., 2004; Hayashi et al., 1981; Iwashita et al., 2001; Kuri-Harcuch & Green, 1978; Sakuramata & Kusano, 1998). 3T3-L1 cells were cultured in 96-well plates at 1×10^4 cells/well in DMEM containing 20% (w/v) FBS under 5% (v/v) CO_2 and 37 ℃. The culture medium was exchanged with DMEM plus 0.2 M dexamethasone, 0.5 mM IBM-X, and 0.8 M insulin before the cells were confluent, and the mixture was incubated at 37 ℃ for 2 days to begin adipocyte differentiation. Then, differentiation proceeded by continuously exchanging the culture medium with DMEM plus 20% (w/v) FBS and 0.8 M insulin every 2-4 days. The concentration of FBS used in the cultures was decreased from 10% to 4% (w/v) over 2 days. This culture solution (150 L) was added to 150 L/well DMEM with 5-500 g/mL of each of the perilla leaf extract solutions and 4% (w/v) FBS, and the mixture was incubated at overnight 37 ℃. Adipocyte differentiation of 3T3-L1 cells was confirmed by Oil Red-O staining (Kuri-Harcuch & Green, 1978). First, 500 mg of Oil Red-O was dissolved in 100 mL isopropanol. The solution was diluted with distilled water to 60% (v/v) and was left at room temperature for 10 min before filtration. After washing the cells with phosphate buffer, they were fixed for 15 min in buffer with 10% (w/v) formalin. The cells were washed again, and left in 60% (v/v) isopropanol for 1 min. Then, the cells were stained for 15 min with the Oil Red-O solution. The stained cells were observed under a phase-contrast microscope (IX72; Olympus Co., Tokyo, Japan).

Free fatty acid content in the culture was measured using the NEFA C-Test Wako.

3.6 Preparation and Preservation of Flour Noodles Kneaded with Leaf Powder

Two grams of the powder obtained by milling perilla leaves, 50 g of strong and 50 g of weak flour, 200 mg sodium carbonate, and 36 mL distilled water were mixed and fully kneaded to obtain the dough. The dough was rolled out to a *ca*. 3 mm thickness, and 3 mm × 5 cm noodles were prepared by cutting the dough. The noodles were placed in a plastic container that had a glass vial filled with a saturated potassium carbonate solution to regulate relative humidity at 44%. The container was stored in the dark at 75 ℃ for 5 days. Noodles were removed at appropriate intervals and ground with a mortar and pestle. The ground sample (35 g) was added to 84 mL of methanol, and the mixture was stirred for 3 min with a magnetic stirrer. After ultrasonic treatment at 40 ℃ for 10 min, the mixture was centrifuged at 3,000 rpm for 10 min. This treatment was repeated three times, and the three supernatants were mixed. A powdery sample was extracted from the noodles by concentrating and drying the supernatant mixture in

a rotary evaporator. The same storage and extract procedures were applied for the perilla leaf powder, and the extracted sample was used as a reference to the noodle sample in several physiological activity tests.

3.7 Statistical Analysis

All measurements were carried out in triplicate, and mean values were compared by analysis of variance. Significant differences were estimated by *t*-tests at $p < 0.05$.

4. Result and Discussion

4.1 Antioxidant Activities of Extracts of the Perilla Leaf Fractions

The weights of the powders obtained from the hot water extract, alkaline fraction, acidic fraction, neutral fraction, and phenolic fraction after fractionating the 100 g of fresh perilla leaves were 0.958, 0.254, 0.885, 2.66, and 0.325 g, respectively. The neutral fraction was the largest of all extracted samples. The hot water extract results suggest that the effluent of components from the leaves during branching treatment, which is often used for processing and cooking vegetables.

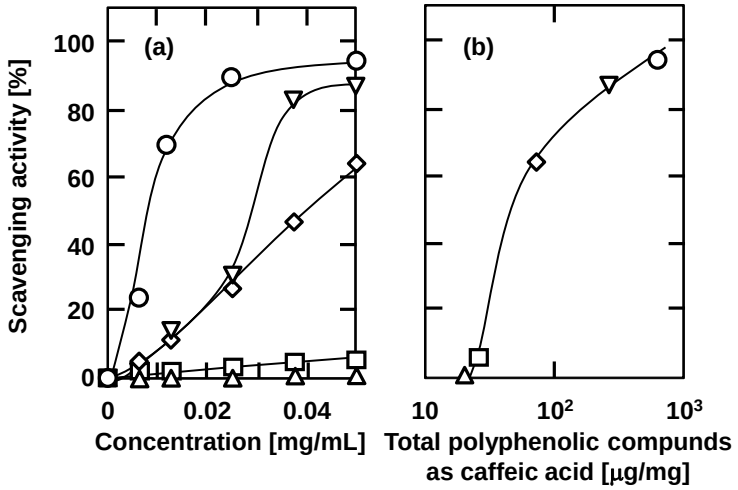

Figure 2. The Relationship between DPPH Radical Scavenging Activity and (a) the Concentrations of the (○) Acidic, (□) Alkaline, (△) Neutral, and (◇) Phenolic Fractions, as Well as (▽) the Hot Water Extract from Perilla Leaves and (b) the Total Amount of Polyphenolic Compounds in Each Extract. The Scavenging Activities in (b) are Plotted for the 50 mg/mL Concentration of Each Extract

Figure 2 (a) shows the relationship between DPPH radical scavenging activity and the concentration of each perilla leaf extract. The 50% scavenging concentration (SC_{50}) for DPPH radicals on the hot water extract, acidic fraction, and phenolic fraction were estimated to be 35, 7.7, and 41 g/mL, respectively, from these profiles. The SC_{50} values for the alkaline and neutral fractions could not be calculated, as the activities did not reach 50% in the tested concentration range. As the values were ≥ 5.6 and 8.3

g/mL for -tocopherol and Trolox, respectively, the acidic fraction had high antioxidant ability. On the other hand, the alkaline and neutral fractions hardly exhibited any radical scavenging activity. Figure 2 (b) shows the relationships between radical scavenging activity of each 50 g/mL extract and the total amount of polyphenolic compounds in the corresponding extract. A positive correlation was obtained from the plots, suggesting the contribution of polyphenols to the antioxidant activity of the extracts. The high radical scavenging activity and total polyphenolic content in the hot water extract indicates a loss of antioxidant functionality by the leaf branching treatment. Figure 3 shows SOD-like activity of each perilla leaf extract. The activity of the phenolic fraction was the highest, but it was almost half that of SOD. SOD-like activities of the extracts were low.

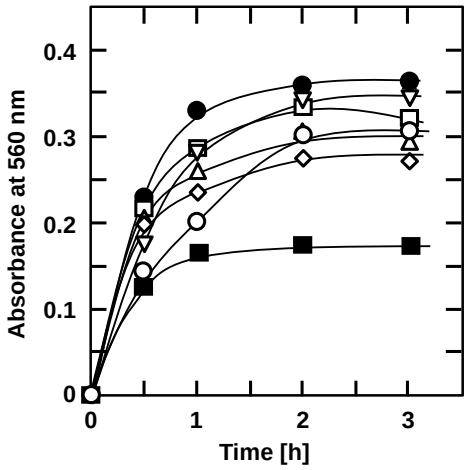

Figure 3. Superoxide Dismutase (SOD)-Like Activities of the (○) Acidic, (□) Alkaline, (△) Neutral, and (◇) Phenolic Fractions, (▽) as Well as the Hot Water Extract from Perilla Leaves. Black Circle, ●, and Square, ■, Represent Control and SOD, Respectively

4.2 Suppressive Effects of the Extracts of the Leaf Fractions on Diabetes and Obesity

The diabetes preventive effects of the perilla leaf extracts were evaluated by testing their inhibitory effects on α-amylase and α-glucosidase activities. Figure 4 (a) shows the inhibitory effects of each extract on α-amylase activity. The phenolic fraction exhibited the highest inhibitory activity, and the 50% inhibitory concentration (IC_{50}) was 1.7 mg/mL. The IC_{50} value of catechin was 55 g/mL, but the inhibitory effects of these extracts on α-amylase were very low. The inhibitory effects of the extracts on α-glucosidase activity are shown in Figure 4 (b). The IC_{50} value of the acidic fraction was 1.8 mg/mL, but the values could not be estimated for other extracts at concentrations < 2.0 mg/mL. Therefore, the inhibitory effects of the leaf extracts on α-glucosidase activity were very low.

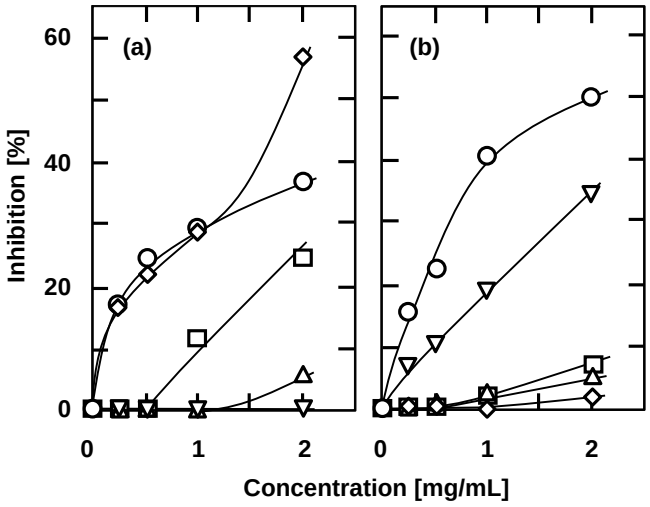

Figure 4. Inhibitory Effects of the Perilla Leaf Extracts on (a) α-Amylase and (b) α-Glucosidase Activities. Symbols are the Same as Shown in Figure 2

The effects of the perilla leaf extracts on pancreatic lipase inhibitory activity and 3T3-L1 adipocyte differentiation were examined to evaluate the ability to prevent obesity. Pancreatic lipase inhibitory activities of the extracts at the 500 g/mL concentration are shown in Table 1. The activities were high in the order of the acidic fraction > hot water extract > phenolic > alkaline > neutral fractions. In particular, the activity of the acidic fraction was twice that of the other extracts. However, not all of the values were high. Next, the effect of the extracts on adipocyte differentiation was investigated. The 3T3-L1 cells differentiated and Oil Red-O staining revealed the formation of lipid droplets under a phase-contrast microscope. Table 2 shows the amounts of free fatty acids released from the cells into the culture medium after adding the extracts. The free fatty acid concentration released in response to the 500 g/mL neutral fraction was almost equal to that of differentiated control 2 cells with no extract added. The free fatty acid concentrations released in response to the 500 g/mL acidic fraction, alkaline fraction, and hot water extract were 1.8-1.9-fold that of the control. The free fatty acids released in response to 500 g/mL of the phenolic fraction was 2.6 times that of the control. Thus, the phenolic fraction seemed to be the most effective for suppressing obesity in the tested samples through hydrolysis of triglycerides in the adipocytes. However, concentrations that exhibited an accelerating effect on lipid hydrolysis were too high for the cells. Because the acidic and phenolic fractions suppressed pancreatic lipase activity and accelerated lipid hydrolysis in differentiated adipocytes, respectively, the compounds responsible for these physiological functions must be identified and isolated.

Table 1. Pancreatic Lipase Inhibitory Activities of the Extracts from Perilla Leaves at the Concentration of 500 mg/mL

Extracts	Inhibition [%] (n=3)
Hot water extract	17.4 ± 3.9
Alkaline fraction	5.8 ± 1.3
Acidic fraction	37.5 ± 1.3
Neutral fraction	4.9 ± 3.8
Phenolic fraction	16.0 ± 1.0

Table 2. Effects of the Extracts from Perilla Leaves on the Release of Free Fatty Acids from 3T3-L1 Cells into the Culture

	Added extracts [μg/mL]	Released free fatty acids [μg/mL]
Control 1 (Not differentiated cell)		0.046 ± 0.034
Control 2 (Differentiated cell)		0.070 ± 0.015
Hot water extract	5	0.076 ± 0.011
	50	0.091 ± 0.001
	500	0.130 ± 0.029
Alkaline fraction	500	0.125 ± 0.004
Acidic fraction	5	0.070 ± 0.019
	50	0.076 ± 0.004
	500	0.124 ± 0.023
Neutral fraction	500	0.088 ± 0.004
Phenolic fraction	5	0.085 ± 0.001
	50	0.094 ± 0.026
	500	0.252 ± 0.079

4.3 Storage Stability of Physiological Components in Flour Noodles Kneaded with Perilla Whole Leaf Powder

Flour noodles kneaded with perilla whole leaf powder were prepared and the stability of the physiological activities after storage was verified to explore opportunities for use in processed food. The noodles were preserved under accelerated test conditions at a temperature of 75℃ to evaluate short-term stability. Figure 5 shows the dependence of DPPH radical scavenging activity on the concentration of the extract from noodles kneaded with leaf powder. The whole leaf extract exhibited significantly higher activity, and activity decreased only slightly after 5 days. The immediate DPPH radical scavenging activity after preparing the noodles with the leaf powder was only about 10%. In addition, activity decreased during the preservation and was close to zero. The reason for the large

decrease is unclear. The radical scavenging activity of the leaf extracts was likely due to the polyphenols in the leaves, and the oxidation reaction is generally affected by water activity. Therefore, water sorption onto a matrix, such as starch or gluten, in the noodles may have stabilized the polyphenols during preservation.

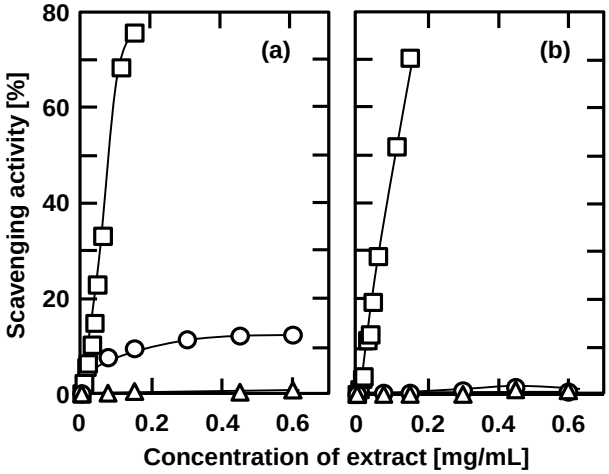

Figure 5. Dependence of DPPH Radical Scavenging Activities on the Concentrations of (○) the Extract from Perilla Leaves, (□) the Extract from Noodles Kneaded with Leaf Powder, and (△) the Extract from Noodles with no Kneading (a) before Preservation and (b) after Preservation for 5 Days at 75 ℃ and 44% Relative Humidity

The relationships between inhibited α-glucosidase activity and the concentrations of the extracts of the noodles kneaded with leaf powder are shown in Figure 6. The immediate inhibition of the extract from the noodles with the leaf powder was *ca*. 35% at 2 mg/mL, but the difference from the extract with no lead kneading was small. The difference also remained small after 5 days, indicating that the inhibitory ability of the noodles with leaf powder was low but that the inhibitory compounds in the noodles were relatively hard to degrade under high temperature storage.

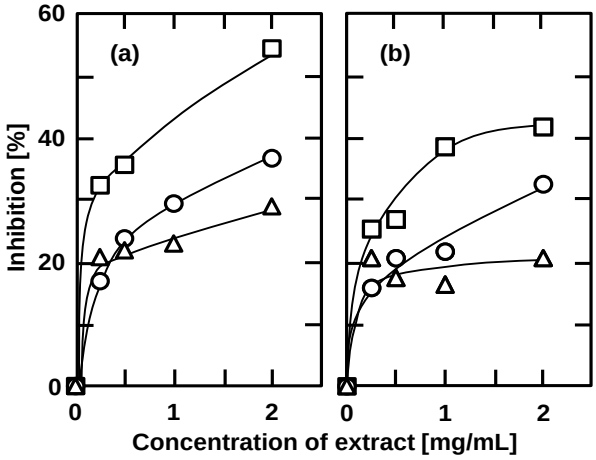

Figure 6. Dependence of the Inhibitory Effect on α-glucosidase Activity on the Concentrations of (○) the Perilla Leaf Extracts, (□) the Noodles Kneaded with Leaf Powder Extract, and (△) the Extract from Noodles with no Kneading (a) before Preservation and (b) after Preservation for 5 Days at 75 ℃ and 44% Relative Humidity

Figure 7 shows the change in the inhibitory effect of the extract from the noodles kneaded with leaf powder on pancreatic lipase activity during preservation at 75 ℃ and 44% relative humidity for 5 days. The inhibition of the noodles with the leaves was no less low, as that of the leaf extract intrinsically was not high. The noodles with no leaves had little inhibitory activity. The inhibition of the noodles with the leaf powder was calculated to be near zero after storage, compared with the result for noodles without kneading. This was similar to the radical scavenging activity results of the extracts. The inhibitory ability of the leaf extracts on pancreatic lipase activity may have also been due to the same polyphenolic compounds as described for radical scavenging activity.

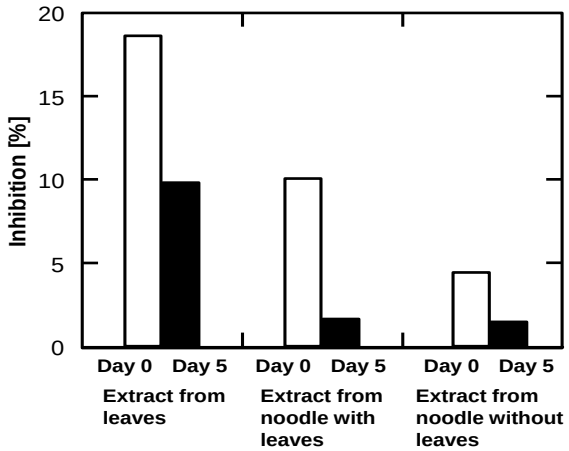

Figure 7. Inhibitory Effect of the Perilla Leaf Extract, the Noodles Kneaded with the Leaf Powder Extract, and the Extract from Noodles with no Kneading on Pancreas Lipase Activity (a) before Preservation and (b) after Preservation at 75 ℃ and 44% Relative Humidity for 5 Days

5. Conclusion

Perilla leaves had some physiological activities, and fractionation based on the chemical properties of the leaf extracts made it possible to clarify the functions of each fraction. For example, the acidic and phenolic fractions effectively suppressed oxidation and obesity. In addition, some functional compounds were detected in the hot water extract. However, the promising components in the leaves were heat-sensitive in flour noodles. Therefore, some conditions, such as storage temperature, must be considered to use these compounds effectively.

References

Anno, T., Tamura, K., Oono, H., & Tomi, H. (2004). Maltase, sucrase and α-amylase inhibitory activity of morus leaves extract (in Japanese). *Food Preservation Science*, *30*(5), 223-229. https://doi.org/10.5891/jafps.30.223

Edashige, Y., Murakami, N., & Tsujita, T. (2008). Inhibitory effect of pectin from the segment membrane of citrus fruits on lipase activity. *Journal of Nutritional Science and Vitaminology*, *54*, 409-415. https://doi.org/10.3177/jnsv.54.409

Fujita, A., Masumoto, K., Kawakami, K., Mikami, T., & Nomura, M. (2006). Anti-oxidation activity of various rice brans. *Journal of Oleo Science*, *55*(11), 585-591. https://doi.org/10.5650/jos.55.585

Furuyashiki, T., Nagayasu, H., Aoki, Y., Bessho, H., Hashimoto, T., Kanazawa, K., & Ashida, H. (2004). Tea catechin suppresses adipocyte differentiation accompanied by down-regulation of PPAR 2 and C/EBP in 3T3-L1 cells. *Bioscience Biotechnology and Biochemistry*, *68*(11), 2353-2359. https://doi.org/10.1271/bbb.68.2353

Hara, Y., & Honda, M. (1990). The Inhibition of α-amylase by tea polyphenols. *Agricultural and Biological Chemistry*, *54*(8), 1939-1945. https://doi.org/10.1080/00021369.1990.10870239

Hayashi, I., Nixon, T., Morikawa, M., & Green, H. (1981). Adipogenic and anti-adipogenic factors in the pituitary and other organs, *Proceedings of the National Academy of Sciences of the United States of America*, *78*(6), 3969-3972. https://doi.org/10.1073/pnas.78.6.3969

Ichikawa, K. (2006). Nutritional properties and utilization of perilla seed oil (in Japanese). *Journal of Oleo Science*, *6*(5), 257-264. https://doi.org/10.5650/oleoscience.6.257

Iwashita, K., Yamaki, K., & Tsushida, T. (2001). Effect of flavonoids on the differentiation of 3T3-L1 adipocytes. *Food Science and Technology Research*, *7*(2), 154-160. https://doi.org/10.3136/fstr.7.154

Jakobek, L. (2015). Interactions of polyphenols with carbohydrates, lipids and proteins. *Food Chemistry*, *175*, 556-567. https://doi.org/10.1016/j.foodchem.2014.12.013

Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S., & Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry*, *47*, 3954-3962. https://doi.org/10.1021/jf990146l

Kuri-Harcuch, W., & Green, H. (1978). Adipose conversion of 3T3 cells depends on a serum factor.

Proceedings of the National Academy of Sciences of the United States of America, *75*(12), 6107-6109. https://doi.org/10.1073/pnas.75.12.6107

Kwon, C. S., Sohn, H. Y., Kim, S. H., Kim, J. H., Son, K. H., Lee, J. S., … Kim, J. S. (2003). Anti-obesity effect of *Dioscorea nipponica Makino* with lipase-inhibitory activity in rodents. *Bioscience Biotechnology and Biochemistry*, *67*(7), 1451-1456. https://doi.org/10.1271/bbb.67.1451

Lu, W., Kelly, A. L., & Miao, S. (2016). Emulsion-based encapsulation and delivery sytems for polyphenols. *Trends in Food Science & Technology*, *47*, 1-9. https://doi.org/10.1016/j.tifs.2015.10.015

McDougall, G. J., Shpiro, F., Dobson, P., Smith, P., Blake, A., & Stewart, D. (2005). Different polyphenolic components of soft fruits inhibit α-amylase and α-glucosidase. *Journal of Agricultural and Food Chemistry*, *53*, 2760-2766. https://doi.org/10.1021/jf0489926

Saito, Y., Nishi, S., Koaze, H., Hironaka, K., & Kojima, M. (2007). Antioxidant and inhibitory activity on α-amylase and α-glucosidase in legume polyphenols (in Japanese). *Nippon Shokuhin Kagaku Kogaku Kaishi*, *54*(12), 563-567. https://doi.org/10.3136/nskkk.54.563

Sakuramata, Y., & Kusano, S. (1998). Screening of plant extracts with potential to stimulate lipolysis in 3T3-L1 cells (in Japanese). *Nippon Eiyo Shokuryo Gakkaishi*, *51*(6), 361-364. https://doi.org/10.4327/jsnfs.51.361

Urabe, K., Kitao, S., Kouyama, K., Nadamoto, T., Kawamura, M., & Nishikawa, Y. (2003). Growth inhibition of *Escherichia Coli*, *Staphylococcus aureus* and *Bacillus subtilis* by wild grasses extracts (in Japanese). *Nippon Shokuhin Kagaku Kogaku Kaishi*, *50*(8), 350-355. https://doi.org/10.3136/nskkk.50.350

Yamashoji, S., Yoshida, H., & Kajimoto, G. (1979). Photooxidation of linoleic acid by ultraviolet light and effect of superoxide anion quencher. *Agricultural and Biological Chemistry*, *43*(6), 1249-1254.