Influence of autoclave treatment and enzymatic hydrolysis on the antioxidant activity of *Opuntia ficus-indica* fruit extract

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Abstract The objectives of this study were to obtain *Opuntia ficus-indica* fruit (OFIF) extract by autoclave treatment, to convert the flavonoid glycosides in the autoclave extract (AE) to aglycones by enzymatic hydrolysis, and to compare the antioxidant activity of AE and OFIF extracts obtained by other conventional methods. It was revealed that the total polyphenol and flavonoid content and antioxidant activity of AE were higher than those of water extract but were a slightly lower than those of ethanol extract, which indicates that autoclave treatment might be an efficient extraction method for OFIF. Moreover, it was confirmed that the conversion of various flavonoid glycosides to aglycones in all the OFIF extracts does not significantly affect the antioxidant activities. Therefore, it is extrapolated that the antioxidant activity might be correlated to the intestinal absorption rates and metabolic pathway induction upon oral administration rather than the structure of compound itself.

Keywords *Opuntia ficus-indica* fruit (OFIF) · Autoclave treatment · Enzymatic hydrolysis · Aglycone · Antioxidant activity

Introduction

Opuntia ficus-indica is a species of cactus that harbors cladodes and fruits, which can be used to prepare food products such as jam or juice [1]. The *Opuntia ficus-indica*

fruit (OFIF) is known to be responsible for a wide range of biological and pharmacological activities, including decreasing cholesterol levels as well as enhancing antioxidant, antimicrobial, anti-inflammatory, and hypoglycemic effects [2]. In general, the pharmacological benefits of OFIF arise from polyphenols that are produced during photosynthesis of green plants. In particular, the glycosides of isorhamnetin, quercetin, and kaempferol have been considered as the major polyphenols in OFIF [3–5].

Most bioactive substances in fruits and vegetables such as polyphenols have been well known for their instability under specific environment such as high temperature [6]. Therefore, bioactive substances in OFIF must be extracted and stored using suitable methods for preserving their pharmacological benefits. To determine the appropriate extraction method, multiple parameters such as the type of solvent, solvent–solute ratio, total extraction time, and temperature must be considered, which can affect the yield, composition, and bioactivity of the extract [7]. Among various extraction methods, autoclaving has been recently introduced and has been proven to extract bioactive substances more efficiently since it utilizes the higher pressure and temperature than other methods do, resulting in an increase in extraction yield [8]. In other words, by applying pressure at high temperature using an autoclave possibly enhances the contact between the solvent and raw material, resulting in an increase of extraction yield. In this study, we hypothesized that an autoclave treatment could facilitate the efficient extraction of bioactive substances, such as isorhamnetin, quercetin, and kaempferol, from the OFIF. Therefore, autoclave extraction was compared to ethanol and water extraction methods, which have been considered as general extraction methods in terms of extraction yield, as well as bioactive composition and antioxidant activity of the compounds obtained.

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Although bioactive substances in fruits and vegetables can be used for health benefits, it has been known that there is an absorption limit of these substances in the gastrointestinal (GI) tract. The degree of hydroxylation and molecular structure of flavonoids are important factors affecting membrane affinity and permeability [9]; moreover, other factors can influence the absorption rate of bioactive substances. In particular, it has been known that flavonoid glycosides are rarely absorbed by the GI tract [4], whereas their aglycones are more efficiently absorbed [10, 11] and some studies suggested that quercetin and kaempferol aglycone have a higher antioxidant activity than that of their glycoside form [12–14]. Therefore, it has been of particular interest to improve the bioavailability of these compounds by deglycosylation using enzymatic treatment. In this study, the influence of deglycosylation of flavonoid glycosides on their bioactivity represented by the antioxidant activity was determined.

OFIF extract was produced using different methods (ethanol, water, and autoclave extraction) and the effectiveness of the autoclave treatment was evaluated in terms of extraction yield as well as the total polyphenol and flavonoid content by comparing the results of different methods. In addition, the enzymatic treatment was applied to obtain the flavonoid aglycones from OFIF extract, and the influence of deglycosylation on their antioxidant activity was investigated.

Materials and methods

Chemicals and reagents

Cellulase (from *Aspergillus niger*), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), potassium ferricyanide, ferric chloride, and Folin–Ciocalteu's reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA). Commercial isorhamnetin and isorhamnetin-3-o-rutinoside analytical standards were purchased from Extrasynthese (Genay, France) and quercetin-3-o-rutinoside, quercetin, and kaempferol analytical standards were purchased from Sigma-Aldrich. Diethylene glycol was purchased from Junsei Chemical (Tokyo, Japan).

Preparation of OFIF extracts

OFIF was purchased from a local market (Jeju, Korea) and stored in a refrigerator until further treatments. Extraction of OFIF was performed using different methods: 75% (v/v) aqueous ethanol extraction, autoclave extraction, and water extraction. These methods yielded different types of extracts: extracts obtained from 75% (v/v) aqueous ethanol extraction (EE), extracts obtained by an autoclave

extraction (AE) at 121 °C and 0.13 MPa, and extracts obtained by water extraction (WE). An overview of the extraction processes and methods are shown in the flow diagram (Fig. 1). After extraction, all the samples were concentrated by a vacuum rotary evaporator, freeze-dried, ground, and then stored at 4 °C until further analysis.

Determination of the total polyphenol content

Quantification of the total polyphenol content was performed using the Folin–Ciocalteu colorimetric method [15]. The extracted samples were diluted with 50% (v/v) methanol to make its concentration as 2000 mg/L, and the resulting 0.2 mL of sample solutions were mixed with 1.8 mL of deionized (DI) water. Then, 0.2 mL of the Folin–Ciocalteu's phenol reagent was added to the mixtures, followed by resting them at 25 °C for 3 min. Subsequently, 0.4 mL of saturated sodium carbonate solution was added and the final volume was brought up to 4 mL using DI water. The samples were maintained in the dark at 25 °C for 1 h. The absorbance value of the samples was measured at 760 nm using a spectrophotometer (DU 730, Beckman Coulter Inc., Fullerton, CA, USA). The total polyphenol content (TPC) was expressed as the mean value, including standard deviation, in terms of gallic acid equivalent (GAE) per dry weight of the extract (DWE). The standard curve was obtained using 12.5–100 mg/L of gallic acid aqueous solution (gallic acid analytical standard dissolved in DI water).

Determination of total flavonoid content

Quantification of the total flavonoid content was performed using the sodium carbonate colorimetric method [16]. The extracted samples were diluted with 50% (v/v) methanol to make its concentration as 2000 mg/L, and the resulting 0.2 mL of sample solutions were then mixed with 1.8 mL of diethylene glycol. After vortexing for 10 s, 0.02 mL of 1 N NaOH solution was added and the samples were incubated at 37 °C for 1 h. The absorbance values of reaction mixtures were measured at 420 nm. Total flavonoid content (TFC) was expressed as the mean value, including standard deviation, in terms of the quercetin equivalent (QE) per DWE. The standard curve was obtained using 6.25–100 mg/L of quercetin solution (quercetin analytical standard dissolved in methanol).

Water solubility analysis

0.5 g of each extract (EE, AE, and WE) was dissolved in 10 mL DI water followed by sonication for 4 h using an ultrasonic bath (CPX5800H-E, Branson Ultrasonics Corporation, Danbury, CT, USA). Thereafter, centrifugation was applied using a centrifuge (Centrifuge 5810R,

Fig. 1 Flow diagram of the extraction and freeze-dried powderization procedures of *Opuntia ficus-indica* fruits (OFIF)

Eppendorf AG, Hamburg, Germany) with a F-34-6-38 rotor at $12,074.4 \times g$ (10,000 rpm by instrument panel) for 10 min. Subsequently, the sediment was separated and dried at 60 °C until no change in weight was observed. Water solubility was determined as follows:

$$\text{Water solubility} = \left(1 - \frac{\text{Weight of the sediment}}{\text{Weight of the extract}} \right) \times 100(\%)$$

Enzymatic hydrolysis

Each reconstituted extract (100 mg/mL) was treated with 0.065 unit/ μL cellulase and then incubated at 60 °C, pH

5.0, and 80 rpm for different time periods (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 h) using a shaking water bath (SWB-25, Han Yang Scientific Equipment Co., Ltd, Seoul, Korea). After incubation, thermal treatment was applied to each sample at 100 °C for 5 min to deactivate the enzymatic action, followed by dilution with pure methanol to a final concentration of 80% (w/v). The diluted samples were sonicated at 40 °C for 1 h and filtered using a 0.45 μm syringe filter. During enzymatic hydrolysis, the tendency of enzymatic conversion observed in each extract was expressed as the ratio of the concentration at certain time (C_t) to the initial concentration (C_i) and as the ratio of C_t to the final concentration (C_f), respectively.

Determination of flavonoid glycoside and aglycone

High performance liquid chromatography (Agilent Technologies, Inc., Santa Clara, CA, USA) coupled to a diode array detector was used to identify flavonoid substances in different types of extracts. Analytical separation was performed by reversed phase C₁₈ column (Unisol C18, 4.6 × 250 mm, 5 µm, 100 Å, Bonna-Agela Technologies Inc., Wilmington, DE, USA) and samples were eluted with a mobile phases comprising eluent A (0.1% acetic acid aqueous solution) and eluent B (acetonitrile) at a flow rate of 0.8 mL/min. The gradient elution was used as follows: 0 min (10% B), 0–8 min (26% B), 8–18 min (28% B), 18–35 min (100% B), and 35–40 min (10% B). The temperature of the column was maintained at 35 °C and the injection volume was 20 µL. The detection of flavonoid substance was monitored at 365 nm and expressed as µg/g DWE.

Radical scavenging activity assay with 2,2-diphenyl-1-picrylhydrazyl (DPPH)

DPPH free radical scavenging activity was measured by combining the extracts with DPPH solution at a ratio of 1:1 for 30 min at 25 °C in the dark. In this process, DPPH was diluted to 400 mM with pure methanol and stirred for 30 min. Absorbance of the reaction mixture was measured at 517 nm of wavelength using a spectrophotometer [17]. The absorbance values were expressed as the mean value, including standard deviation, in terms of trolox content [trolox equivalent (TE) per DWE]. The standard curve was prepared using 1–20 mg/L trolox standard solution (trolox dissolved in ethanol).

Radical scavenging activity assay with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)

Before analysis, active ABTS solution was prepared by mixing 7 mM ABTS solution and 2.45 mM potassium persulfate solution at a ratio of 1:1 for 16 h. ABTS solution was then diluted with phosphate buffered saline (PBS, pH 7.4) to an absorbance value of 0.70 at 732 nm. ABTS free radical scavenging activity was measured by mixing the extracted sample with the ABTS solution at a ratio of 5:95 at 25 °C in the dark for 6 min [18]. The absorbance was then measured (at 732 nm) using a spectrophotometer. The standard curve was prepared using 5–100 mg/L of trolox standard solution (trolox dissolved in ethanol).

Statistical analysis

All the experiments were performed in triplicate and the data were statistically analyzed by Duncan's multiple range

tests with 0.05 significance level using the SAS software (Statistical Analytical System, SAS Institute Inc., Cary, NC, USA).

Results and discussion

Solid extraction yield

It was revealed that the solid extraction yield was the highest for the autoclave extraction and was 1.3 and 1.5 times higher than those of the ethanol and water extractions, respectively (Table 1). It can be noted that various bioactive substances, including phenolic compounds, were efficiently extracted due to the harsh environment in the autoclave, characterized by a high pressure and temperature. Specifically, large amounts of bioactive substances were obtained from the OFIF due to the increase of membrane permeability caused by the collapse of cell membranes and protein denaturation. In addition, it is assumed that the increase of soluble fiber caused by the disruption of the insoluble cell wall under high pressure enhanced the solid extraction yield in the AE. These results are in close agreement with the previous study [8], which indicated that autoclave extraction was more efficient than water extraction with increasing extraction yield from *Phellinus linteus* mycelium. In fact, there are some examples in which localized pressure application has proven to be sufficient to the collapse cell wall structures such as in the ultrasonic extraction method [19]. Moreover, several studies have demonstrated that high temperatures lead to an increase in the capacity of water to extract compounds by enhancing their solubility through decreasing the surface tension [20].

We found that the EE had a slightly higher solid extraction yield than that of the WE, which coincides with the previous study wherein the 75% aqueous ethanol extraction has a higher solid extraction yield than that of the water extraction in bamboo leaves [21]. In this experiment, the AE was visually less viscous than the WE and EE. In nature, OFIF has mucilage gum, which is a high molecular polysaccharide comprising various sugar moieties and is known to affect viscosity [22]. Thus, the mucilage in OFIF was broken down into smaller molecules by exposure to relatively high pressure and temperature in the autoclave treatment, possibly resulted in the lower viscosity of the AE. The abovementioned phenomenon suggests that the autoclave treatment might have efficient extraction abilities for natural substances such as OFIF.

TPC and flavonoid content

Total polyphenol and flavonoid contents of the AE, WE, and EE are shown in Table 1. The amount of polyphenol

Table 1 Extraction yield, total polyphenol content (TPC), total flavonoid content (TFC), and free antioxidant activity (by DPPH and ABTS) of OFIF extracts

Extraction method	Solid extraction yield ¹	TPC ²	TFC ³	Antioxidant activity (DPPH ⁴)	Antioxidant activity (ABTS ⁵)
EE	32.28 ± 1.37 ^b	22.60 ± 0.86 ^a	17.92 ± 1.30 ^a	10.26 ± 0.04 ^a	47.95 ± 1.78 ^a
AE	40.53 ± 2.14 ^a	14.49 ± 0.40 ^b	8.01 ± 0.79 ^c	6.23 ± 0.26 ^b	37.67 ± 1.30 ^b
WE	27.83 ± 1.47 ^c	14.20 ± 0.23 ^b	9.39 ± 1.02 ^b	6.38 ± 1.05 ^b	37.99 ± 4.35 ^b

Results are mean ± SD. Different letters in the same column indicate that the values are significantly different ($p < 0.05$)

¹ Solid extraction yield: %

² Total polyphenol content: mg gallic acid equivalents (GAE)/g DWE

³ Total flavonoid content: mg quercetin equivalents (QE)/g DWE

⁴ 2,2-Diphenyl-1-picrylhydrazyl method: mg trolox equivalent (TE)/g DWE

⁵ 2,2-Azobis(3-ethylbenzothiazoline-6-sulfonate) method: mg trolox equivalent (TE)/g DWE

extracted was higher than that of flavonoid regardless of the extraction method, which is in close agreement with the previous study [23]. Fundamentally, polyphenol belongs to the parent category of flavonoid. In this experiment, the TFC in the OFIF accounted for over half of the total polyphenols, revealing that the majority of polyphenols exist as flavonoids in OFIF.

The EE contained larger amounts of flavonoids than in WE, supporting the previous study that assessed the extraction efficiency of ethanol for relatively low water-soluble compounds represented as flavonoid [24]. In general, flavonoids have lower dipole moments, which lead to a relatively lower solubility in water [25]. Thus, a large concentration of flavonoids in OFIF could be easily extracted by ethanol (ϵ at 293.15 K = 25), which has a lower dielectric constant (ϵ) than water (ϵ at 293.15 K = 80) [26], facilitating the extraction of hydrophobic compounds.

In comparison, considering the solid extraction yield, the AE showed significantly higher total polyphenol and flavonoid content than the WE. This phenomenon suggests that the high pressure used in the autoclave treatment led to a collapse of the cell membranes and subsequently, bioactive substances were eluted more efficiently. Furthermore, the difference in dielectric constant in the extraction solvents also might have led to a difference in extraction ability. The dielectric constant has a general tendency to decrease or increase in response to an increase in the temperature of solvent [27] and the pressure applied [28], respectively. In this experiment, water and ethanol extraction was performed at 70 °C, whereas the autoclave extraction was performed at 121 °C and higher pressure. That is, the dielectric constant of water in the autoclaving condition dramatically decreased to a value lower than 55.7 at 100 °C due to exposure to 121 °C [27]. This result is in agreement with the findings of a previous study [29], which indicated that flavonoid extraction efficiency could be improved by increasing the extraction temperature. Thus,

the most important factor leading to the extraction of large amounts of flavonoids was high temperature since an increase in pressure had relatively little effect on change of the dielectric constant. As a result, the autoclave extraction was more similar to the ethanol extraction than water extraction in respect to flavonoids obtained. Nevertheless, the AE still had lower total polyphenol and flavonoid content than that of the EE, which can be explained by the phenomenon that autoclave treatment partly destroyed phenolic compounds (mostly, pigments) due to the exposure of the sample to high temperatures [30, 31]. In fact, we observed a yellowish aqueous extract after the autoclave treatment, whereas distinctive purple color was maintained when the other two extraction methods were used. In summary, with regard to the particular flavonoids, the autoclave treatment improved the elution efficiency of bioactive compounds by decreasing the dielectric constant. The abovementioned results suggest that the autoclave extraction is able to yield higher amount of bioactive substances than the water extraction. Moreover, the autoclave extraction was performed in less than half of the time compared to the water and ethanol extractions, demonstrating its availability in extracting bioactive compounds from the OFIF. However, these findings also suggest that further studies are required to minimize the destruction of the bioactive substances caused by high temperature and pressure and to optimize the conditions of autoclave treatment for extraction.

Water solubility

The results of water solubility test are shown in Table 2. Water solubility was the highest in the WE, whereas the EE showed the lowest water solubility. From this result, it can be assumed that water-insoluble compounds were extracted more efficiently by the ethanol extraction compared to the autoclave or water extraction methods. This result is

Table 2 Water solubility of OFIF extracts

Extraction method	Solubility (%)
EE	97.01 ± 0.07 ^c
AE	97.68 ± 0.24 ^b
WE	98.32 ± 0.04 ^a

Results are mean ± SD. Different letters in the same column indicate that values are significantly different ($p < 0.05$)

consistent with previous studies [24, 32], which indicated that water extraction methods obtained compounds with higher water solubility in cinnamon than those from ethanol extraction methods, whereas ethanol extraction could extract more water-insoluble compounds from peanut skin. Here the AE and the WE might be expected to obtain considerably higher amounts of water-soluble compounds than that of the EE; however, they actually showed slightly higher solubility than that of the EE. This can be attributed to the 25% water content in the 75% ethanol extraction, which might be sufficient to extract similar amounts of water-soluble substances, including hydrophilic polyphenols. The AE had slightly lower water solubility than the WE, possibly caused by the decrease in the dielectric constant, as mentioned previously. While the AE had a lower dielectric constant than the WE, the high temperature used in the autoclave extraction resulted in the extraction of a certain amount of water-insoluble bioactive substances, represented by flavonoids. Consequently, the high temperature and pressure and subsequent elution of flavonoids affected the water solubility of the AE.

Enzymatic conversion of flavonoid glycoside to aglycone

The initial content of isorhamnetin-3-o-rutinoside was 1520.92, 744.63, and 678.83 µg/g DWE in the EE, AE, and WE, respectively (Table 3). This result is very similar to the tendency of TFC extracted by the different extraction methods. Considering the solid extraction yield, the AE had a larger amount of total isorhamnetin-3-o-rutinoside content than the WE. It is assumed that the harsh environment in the autoclave extraction led to the collapse of cell walls as mentioned above, which could lead to a more efficient extraction of isorhamnetin-3-o-rutinoside. However, the AE had approximately half the initial content of isorhamnetin-3-o-rutinoside as well as total polyphenol and flavonoids found in the EE, indicating that the autoclave extraction was relatively inefficient in comparison to the ethanol extraction since bioactive substances were destroyed by high pressure and temperature (at 121 °C).

Bioactive substances in the ethanol extraction were also destroyed due to the exposure to 70 °C for a long time (4 h); however, the degradation of bioactive substance was more intensive in the autoclave treatment.

In nature, most flavonoids exist in the form of glycoside but they have been known for their low absorption efficiency in the GI tract [4]. Basically, the membrane permeability of flavonoid is correlated with the degree of hydroxylation, molecular structure, [9] and octanol/water partition coefficient related to the number of hydroxyl groups on the molecules [33]. There have been some researches that deglycosylation improve membrane permeability of flavonoid [34] and enzymatic hydrolysis efficiently facilitates the deglycosylation [11]. The tendency of conversion of isorhamnetin-3-o-rutinoside to isorhamnetin during enzymatic hydrolysis is highlighted in Fig. 2(A), (B). By deglycosylation, the isorhamnetin-3-o-rutinoside changed to the isorhamnetin and the conversion rate was similar in all the extracts; however, the rate of decrease in isorhamnetin-3-o-rutinoside content was the lowest in WE (0.563 C_i/C_i at 3.5 h). It is assumed that the high viscosity of the WE caused by the mucilage gum present in the OFIF inhibited the enzyme reaction. On the whole, during deglycosylation process, the amount of converted isorhamnetin was low in comparison to the amount of its substrate (isorhamnetin-3-o-rutinoside), which indicated that isorhamnetin-3-o-rutinoside was not fully converted to isorhamnetin aglycone. In other words, it seemed that isorhamnetin-3-o-rutinoside partially converted to another form of glycoside after enzymatic treatment.

The initial quercetin-3-o-rutinoside content was 56.27, 33.34, and 24.70 µg/g DWE in the EE, AE, and WE, respectively (Table 3). This tendency was similar to that of the total polyphenol and flavonoid content in all extracts. With respect to quercetin-3-o-rutinoside, the autoclave extraction showed higher and lower extraction efficiencies than the water and ethanol extractions, respectively. In all extraction methods, the rate of changes in concentration of quercetin was similar to that of quercetin-3-o-rutinoside decreased (0.709, 0.625, and 0.648 C_i/C_i at 3.5 h for EE, AE, and WE, respectively) during enzymatic hydrolysis [Fig. 2(C), (D)]. Quercetin was observed in all the extracts without enzyme treatment, revealing that OFIF contains quercetin as well as quercetin-3-o-rutinoside in nature. The initial quercetin contents in the AE and WE were 21.84 and 8.34 µg/g DWE, respectively. The quantity of quercetin in the AE and WE showed a big difference considering the solid extraction yield, which indicates that an autoclave treatment can be more efficient in extracting water-insoluble substances than water extraction. These data are in agreement with previous research [29], which revealed that

Table 3 Content¹ of isorhamnetin-3-o-rutinoside (IRR), isorhamnetin (IR), quercetin-3-o-rutinoside (QCR), quercetin (QC), and kaempferol (KF) in OFIF extracts

Extraction method	IRR	IR	QCR	QC	KF
EE	1520.92 ± 23.68 ^a	ND ²	56.27 ± 1.57 ^a	35.18 ± 3.70 ^a	58.86 ± 2.15 ^a
AE	744.63 ± 15.24 ^b	ND ²	33.34 ± 0.70 ^b	21.84 ± 1.11 ^b	24.87 ± 1.11 ^b
WE	678.83 ± 138.03 ^b	24.24 ± 5.71	24.70 ± 4.02 ^c	8.34 ± 0.90 ^c	14.22 ± 2.55 ^c

Results are mean ± SD. Different letters in the same column indicate that the values are significantly different ($p < 0.05$)

¹ Content: µg/g DWE

² ND not detected due to the limit of quantification of instrument

flavonoid aglycones can be extracted more easily than flavonoid glycosides at high temperatures due to the decrease of dielectric constant and weakened hydrogen bonds among water molecules. Moreover, quercetin has a methoxylated group (O-CH₃) that has relatively less polarity, affecting the higher extraction efficiency of the autoclave extraction than that of the water extraction. Moreover, in this experiment, quercetin was produced much more than its substrate (quercetin-3-o-rutinoside) which is decreased by deglycosylation process. This can be attributed to the enzymatic hydrolysis of unidentified quercetin glycosides in OFIF. The rate of change into kaempferol during the enzymatic hydrolysis is shown in Fig. 2(E). In all the extracts, the amounts of kaempferol measured were considerably similar due to the small quantity of kaempferol glycosides in OFIF. The initial kaempferol glycosides content was not measured; however, it is postulated that the majority of the kaempferol glycosides were present as kaempferol-3-o-rutinoside in OFIF, as found previously [4].

As a result, it was identified that the autoclave extraction was more efficient than the water extraction for extracting bioactive substances from OFIF, whereas it showed relatively low extraction efficiency compared to the ethanol extraction. Moreover, it was revealed that although the autoclave extraction clearly improves extraction efficiency due to the use of high pressure and temperature, it caused undesirable destruction of various phenolic compounds in OFIF. Consequently, it is necessary to determine the optimal extraction temperature and time in order to minimize the destruction of bioactive substances when using the autoclave extraction.

Antioxidant activity

The initial antioxidant activities measured from the extracts are shown in Table 1. The antioxidant activity was the highest in the EE, and no significant difference was

observed between the WE and AE. Moreover, it was revealed that the antioxidant activities were dependent on the TPC rather than flavonoid content, which is in agreement with previous findings [35] wherein antioxidant activity and TPC were positively correlated. However, considering the solid extraction yield, the AE had much higher antioxidant activity than the WE, possibly due to the high pressure of the autoclave extraction method, which led to the collapse of cell walls and enhanced elution of bioactive substances compared to the water extraction. Figure 3 shows the results of antioxidant activity measured by the DPPH and ABTS radical scavenging activity assay after enzymatic hydrolysis. In both analyzes, the EE had the highest antioxidant activity, and it appeared that AE and WE were not significantly different. However, when considering solid extraction yield, AE showed higher antioxidant capacity than WE. Although the deglycosylation of flavonoid glycosides by enzymatic hydrolysis is known to improve antioxidant activities, no significant change was observed in DPPH or ABTS assay during enzymatic hydrolysis periods in this study. From this result, it can be assumed that the conversion to aglycones in extracts did not dramatically affect the increase in antioxidant activity. However, according to the previous studies, it is assumed that the increase in antioxidant activity due to the conversion of flavonoid glycosides to their aglycone forms might be possibly instigated by the digestion processes, including the intestinal absorption and other metabolic pathways.

In summary, the efficiency of different extraction methods was investigated by comparing the extraction yield of polyphenols and flavonoids from OFIF. Autoclave extraction was more efficient in extracting polyphenols and flavonoids than the water extraction; however, it was less efficient than the ethanol extraction. While the autoclave extraction improved extraction yield, finding the proper condition for autoclave treatment is still necessary to minimize undesirable destruction of bioactive substances.

Fig. 2 Change of concentration ratio of the remaining flavonoid glycosides at certain time (C_t) to that of the initial (C_i) and change of concentration ratio of appeared flavonoid aglycones at certain time (C_t) to that of the final (C_f) as a function of reaction time for enzymatic hydrolysis: isorhamnetin-3-o-rutinoside (A), isorhamnetin

(B), quercetin-3-o-rutinoside (C), quercetin (D), and kaempferol (E). Different letters within the same extract sample indicate significant differences ($p < 0.05$). EE extracts from ethanol extraction, AE extracts from autoclave extraction, WE extracts from water extraction

Fig. 3 Change of DPPH (A) and ABTS (B) radical scavenging activity as a function of reaction time for enzymatic hydrolysis. Different letters within the same extract sample indicate significant differences ($p < 0.05$). EE extracts from ethanol extraction, AE extracts from autoclave extraction, WE extracts from water extraction

After enzymatic treatment, isorhamnetin, quercetin, and kaempferol aglycones were obtained as a representative flavonoid aglycone, and during the enzymatic hydrolysis, there was no change in antioxidant activity. The results of this study suggest that improvement of biological activity through deglycosylation of phenolic compounds in OFIF extracts may be more closely correlated to intestinal absorption rates and metabolic pathway induction upon oral administration than the deglycosylation itself. In addition, further investigation is required to optimize the conditions of autoclave extraction and to understand the effects of flavonoid deglycosylation in relation to enhancing antioxidant activity using both in vitro and in vivo animal studies.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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