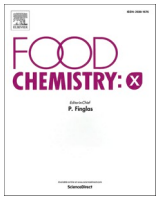
Food Chemistry: X 20 (2023) 100918

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



A combination of commercial and traditional food-source-derived enzymatic treatment acts as a potential tool to produce functional yuzu (*Citrus junos*) powder

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ARTICLE INFO

Keywords:
Enzyme treatment
Yuzu
Dietary fiber
Bitter flavonoid
Anti-obesity

ABSTRACT

Enzymatic modifications have been applied in citrus to enhance their physicochemical and biological properties and reduce their bitterness. Notwithstanding, research on the combination of enzyme treatment of yuzu is lacking. In this study, yuzu was treated with a combination of isolated cellulase NY203, pectinase UF, and cellulase KN, and this enzymatic treatment was found to increase monosaccharide, naringenin, and hesperetin levels. In contrast, dietary fiber, cellulose, hemicellulose, lignin, and pectin levels were decreased. Moreover, the enzymes disintegrated the inner and outer surface structures and chemical bonding of yuzu, thus improving its solubility rate, water-holding capacity, oil-adsorption capacity, cholesterol-binding capacity, and water-swelling capacity. Furthermore, NY203 + UF + KN combination treatment reduced the bitterness of treated yuzu by 50 % compared with the control. Additionally, NY203 + UF + KN treatment yielded a 28 % decrease in lipid accumulation and two-fold higher lipolytic activity in 3T3L-1 adipocytes. These findings are potentially beneficial to the food/nutraceutical industries regarding functional yuzu powder production.

1. Introduction

Yuzu (*Citrus junos*) is a Northeast Asian citrus crop that originated in China and was introduced to Japan and other countries around the 4th to 8th centuries. Yuzu is a peel-eatable fruit with a potent distinctive aroma and fragrant outer peel. It contains dietary fiber (DF), flavonoids, and other high-added-value compounds including vitamins, pigments, and fats that contribute to its biological activities (Hirota et al., 2010; Yoo & Moon, 2016). Yuzu peel primarily comprises cellulose, polysaccharides, lignin, and proteins and is mainly used as a source of dietary fiber (DF) (Nam et al., 2021). Owing to its tart flavor, raw yuzu fruit is rarely consumed. Therefore, yuzu is industrially used to make sugar-pickled tea, obtain juice to produce beverages and various other food products, extract its flavonoids and essential oils, and the volatile compounds from the peel that are used as aroma and flavor additives (Lan-Phi, Shimamura, Ukeda, & Sawamura, 2009; Phi & Sawamura, 2008). In addition to food applications, the peel can also be used as a natural ingredient in biodegradable polymers and packaging materials, cosmetics, and biofertilizers (Nam et al., 2021). Comprising several bioactive flavonoids, yuzu has traditionally been utilized to treat the common cold and amend blood circulation in China, Korea, and Japan (Yoo, Hwang, Park, & Moon, 2009; Nam et al., 2021). Its numerous health functions include anti-inflammatory, antioxidant, antidiabetic, anticarcinogenic, neuroprotective, and antihypertensive activities (Kim et al., 2013; Lee et al., 2022; Nam et al., 2021). However, its constituent flavonoids, especially naringin, narirutin, hesperidin, and neohesperidin, impart bitterness/tartness to yuzu, often reducing consumer preference, and deteriorating quality (Kore & Chakraborty, 2015; Lee et al., 2022). Therefore, yuzu's bitterness needs to be eliminated.

Enzymatic modification of dietary fiber (DF) is an eco-friendly

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https://doi.org/10.1016/j.fochx.2023.100918
Received 6 July 2023; Received in revised form 5 September 2023; Accepted 1 October 2023
Available online 2 October 2023

technology possessing the advantages of simplicity, the possibility of high yields of target compounds, few undesirable side reactions, and high-quality recovery of products (Das & Eun, 2020). Enzymatic hydrolysis has been reported to improve the functional properties of DF due to the effective degradation of lignin, cellulose, and hemicellulose, resulting in component changes (Song, Qi, Liao, & Yang, 2021). Cellulase and xylanase have been reported to degrade insoluble dietary fiber (IDF), causing the entire structure to become bulky and the water-swelling capacity (WSC) to increase. Cellulase acts on the β-1,4 glycosidic bonds of cellulose (Cao & Tan, 2005), thus degrading crystalline cellulose into amorphous cellulose and soluble cellulose degradation products. Cellulase treatment has been found to improvethe physicochemical and functional properties of rice bran DF (Liu, Zhang, Yi, Quan, & Lin, 2021), and the modification of IDF from carrot pomace by combined cellulase and xylanase treatment methods increased the content of soluble dietary fiber (SDF) (Yu, Bei, Zhao, Li, & Cheng, 2018). However, the proper combination of enzyme treatment and hydrolysis method is crucial to improve the functional properties of target products (Das & Eun, 2020). Although several enzymatic modifications have been applied to citrus fibers, research on hemicellulose- and cellulose-rich yuzu fruit, especially regarding enzymatic hydrolysis-based treatment, remains lacking. Therefore, investigating the combination treatment with hemicellulose- and cellulose-degrading enzymes and naringinase to enhance the functional and organoleptic properties of yuzu powder is imperative. Therefore, this study explored the potential of combination treatment with commercial enzymes and enzymes isolated lactic acid bacteria (LAB) obtained from Korean traditional food to improve the physicochemical properties of yuzu powder obtained from the lyophilization of yuzu fruit. Moreover, we investigate the effects of enzyme-treated yuzu on bitterness via human taste 2 receptor member 16 (TAS2R16) and its biological activities via anti-lipid accumulation and glycerol release in 3T3-L1 cells. We hypothesized that treating yuzu with a combination of enzymes improves its physicochemical properties and biological activities and reduces its bitterness, thereby extending its application in the food, nutraceutical, and cosmetic industries.

2. Materials and methods

2.1. Materials and microorganisms

Yuzu (*C. junos*) fruit was collected from the Fruit Research Center of the Jeonnam Agricultural Research and Extension Services (Jeollanam-do, Korea). Pectinex Ultra SP-L and Viscozyme L were obtained from Novozyme (Bagsværd, Denmark). Rohament® CL, Tannase (TAH), Laminex® BG2, and Sumizyme AC were acquired from AB Enzymes (Darmstadt, Germany), Kikkoman (Tokyo, Japan), Danisco (Copenhagen, Denmark), and Shin Nihon Chemical (Aichi, Japan), respectively. Peclyve CP and Pecylve PR were obtained from Soufflet Biotechnologies (Colombelles, France), respectively. Plantase UF, Pluszyme 2000P, and Cellulase KN were purchased from Bision Corporation (Seoul, Korea). Korean traditional fermented foods, including red pepper paste, soybean paste, kimchi, and Makgeolli (Korean commercial rice wine) were obtained from a local grocery store (Hanaro Market, Jeollanam, Korea). De man-Rogosa-Sharpe (MRS) and yeast and malt (YM) media were acquired from Difco (Detroit, MI, USA) and Kisanbio (Seoul, Korea), respectively. Sodium carboxymethyl cellulose (CMC), Congo red, ethylenediaminetetraacetic acid (EDTA), sodium dodecyl sulfate (SDS), fructose (>97 %), arabinose (>97 %), glucose (>97 %), galacturonic acid (>97 %), 3-isobutyl-1-methylxanthine (IBMX), insulin, dimethyl sulfoxide (DMSO), and Oil Red O were purchased from Sigma-Aldrich (MO, USA). The total dietery fiber assay kit (K-TDFR-200A) and pectine identification assay kit (K-PECID05/16) were purchased from Megazyme (Wicklow, Ireland). Enzyme-based lipolysis and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) kits were acquired from Alfa Aesar (Ward Hill, MA, USA) and BioVision (Milpitas, CA, USA), respectively. Six kinds of flavonoids (naringin, narirutin, hesperidin, neohesperidin, naringenin, and hesperetin) with over 95 % purity were obtained from ChromaDex (Irvine, CA, USA). Dulbecco's modified Eagle's medium (DMEM), bovine calf serum (BCS), fetal bovine serum (FBS), and 1 % penicillin and streptomycin were purchased from Gibco BRL (Grand Island, NY, USA). All other chemical reagents used were of analytic grade. The 3T3-L1 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA).

2.1. Yuzu powder preparation

The fruit was sliced in half and squeezed to obtain the juice, which was lyophilized by freeze-drying (FD8512 freeze dryer, IlshinBioBase, Gyeonggi-do, Korea). Lyophilized yuzu powder was ground using a pulverizer (FM-681C, Hanil Electric, Gyeonggi-do, Korea) and subsequently sieved through a 60-mesh sieve for further use.

2.2. Enzymes

2.2.1. Commercial enzymes

For screening, 11 commercial enzymes (Table S1) were used to treat yuzu powder. For treatment, 1 % enzyme was added to a 10 mL reaction mixture containing yuzu powder (0.5 g) in 50 mmol/L sodium acetate (Na-Ac) buffer (pH 5.2) at 50 °C for 3 h. The reaction mixture without added enzyme was used as the control. Then, the reaction mixture was centrifuged at 7000×*g* (Model 6200, Kubota, Tokyo, Japan) for 15 min, and the supernatant was carefully removed by slowly tilting the centrifuge tube. The solubility was calculated by Eq. (1):

$$\text{Solubility (\%)} = [1 - (\text{wet weight}/\ \text{control wet weight})] \times 100 \quad (1)$$

2.2.2. Isolation and preparation of Korean traditional food-source-derived enzymes

Fermented food samples (20 g) were added to 80 mL of 0.85 % (w/v) sterile normal saline, then spread onto agar plates of lysogeny broth (LB), MRS medium, and YM medium, then incubated at 37 °C for 48–96 h. Afterward, LAB colonies were picked and cultured in LB, MRS, and YM broth until the optical density of 600 nm (OD_{600}) reached 0.5. Then, LB, MRS, and YM agar plates containing 1 % (w/v) CMC or 1 % (w/v) soluble starch were inoculated with 10 μL of the corresponding cultured medium and incubated at 37 °C for 20 h. The 1 % CMC plate was subsequently stained with 0.1 % Congo red for 15 min, followed by decolorization with 1 mol/L NaCl. Congo red indicates the cellulolytic enzyme activity of bacteria; the dye can bind to the polysaccharide substrate of the medium, forming transparent zones around the colony. Therefore, the diameter of the transparent circles (hydrolyzed cellulose) was analyzed to determine the cellulose-degrading ability of strains. The cellulose-degrading bacterial strains were incubated in LB broth containing 1 % CMC at 37 °C for 72 h. The supernatant was obtained by centrifugation at 8000×*g* for 10 min and used as the crude enzyme. The enzyme reaction was performed by adding the enzyme to 1 % (w/v) CMC in 20 mmol/L Na-Ac buffer (pH 5.2) at 37 °C for 24 h and analyzed by thin-layer chromatography (TLC).

The strain with the highest cellulose-degrading ability was identified by 16S rRNA sequencing by Macrogen (Seoul, Korea). Phylogenic analysis was conducted using MEGA software version 11.0 with the Kimura two-parameter method with 1,000 replicated for the bootstrap values. The isolated strain was deposited in the Korean Collection for Type Cultures (KCTC, Jeollabuk-do, Korea) as KCTC 13679BP.

2.3. Enzyme treatment

Yuzu powder was treated with Plantase UF, isolated cellulase NY203, the combination of Plantase UF and isolated cellulase NY203 (NY203 + UF), and the combination of Plantase UF, cellulase KN, and isolated

cellulase NY203 (NY203 + UF + KN) by adding 10 % (v/v) isolated cellulase NY203 to a reaction mixture containing 10 % (w/v) yuzu powder in 50 mmol/L Na-Ac buffer (pH 5.2) and incubating at 37 °C and 60 rpm for 6 h. Then, 1 % Plantase UF was added to the above reaction mixture and incubated at 50 °C and 60 rpm for 3 h. Finally, 1 % cellulase KN was added to the reaction mixture and incubated at 50 °C and 60 rpm for 3 h. Then, the enzymes were inactivated by incubating the reaction mixture at 98 °C for 15 min. Reaction mixture was lyophilized, and stored at −80 °C for further study.

2.4. Analysis of enzyme-treated yuzu

2.4.1. Analysis of DF composition

2.4.1.1. SDF and IDF. SDF and IDF were measured using the Association of Official Analytical Chemists (AOAC) enzymatic–gravimetric method 991.43 (AOAC, 2000) with a total dietary fiber assay kit (K-TDFR-200A). The sample was suspended in MES-Tris buffer and hydrolyzed heat-stable α-amylase at 95 °C, protease at 60 °C, and amyloglucosidase at 60 °C, sequentially. The reaction mixture was filtered through tared fritted glass crucibles. Crucibles containing IDF were rinsed with diluted alcohol, followed by acetone, and oven-dried at 105 °C overnight. Filtrates were mixed with 95 % ethanol to precipitate soluble materials for 1 h. Precipitates were filtered via tared fritted glass crucibles. Soluble and water-insoluble DF contents in the residue were considered SDF and IDF, respectively.

2.4.1.2. Analysis of acid detergent fiber (ADF) and neutral detergent fiber (NDF). ADF and NDF were extracted at 100 °C for 1 h by adding 0.5 g sample to 200 mL of the acetic detergent solution comprising of 20 g cetyl trimethyl ammonium bromide in 1 L of 1 N H_2SO_4 and 200 mL of the neutral detergent solution consisting 6 g SDS, 3.72 g EDTA, 1.36 g sodium borate, 0.91 g disodium hydrogen phosphate, and 2 mL of 2-ethoxy ethanol. Then, crucibles containing detergent fiber were rinsed with dilute alcohol, followed by acetone, and oven-dried at 105 °C overnight.

2.4.1.3. Analysis of lignin, pectin, hemicellulose, and cellulose. Lignin was extracted by adding 0.5 g of the sample to 15 mL of 72 % H_2SO_4 and stirring for 2 h. Then, the suspension was diluted to 3 % H_2SO_4 and digested sequentially for 3 h at 100 °C. Lignin was obtained by rinsing crucibles with 150 mL of distilled water and oven-dried at 105 °C overnight. Lignin content was calculated by dividing the precipitate weight by the initial sample weight. The pectin identification assay kit (K-PECID 05/16) was used to analyze pectin content with low ester pectin from citrus peel (supplied in the detection kit) as standard. Cellulose and hemicellulose contents were calculated using Eq. (2) and (3), respectively. All data are presented in milligrams per gram of dry weight (mg/g DW).

$$\text{Hemicellulose} = \text{NDF} - \text{ADF} \quad (2)$$

$$\text{Cellulose} = \text{ADF} - \text{Lignin} \quad (3)$$

2.4.2. Analysis of monosaccharides and galacturonic acid

Free sugars (fructose, arabinose, glucose, and galacturonic acid) in the water extract of yuzu powder were triply analyzed by injecting of 10 μL sample into an LC-20A high-performance liquid chromatography (HPLC) system (Shimadzu, Kyoto, Japan) equipped with a RID-10A detector (Shimadzu, Kyoto, Japan) and a SUPELCOGEL C-610H column (7.8 × 300 mm; Supelco, Bellefonte, PA, USA). The separation was performed using an isocratic elution system comprising 0.1 % (v/v) phosphoric acid in water with a flow rate of 0.5 mL/min at 40 °C. The areas and retention times of the analyte peaks were compared with those of the standards. The monosaccharide or galacturonic acid content is expressed as a percentage (%).

2.4.3. Analysis of flavonoids

The concentration of flavonoids, including naringin, naritutin, hesperidin, neohesperidin, naringenin, and hesperetin, in the 80 % (v/v) ethanol extract of yuzu were triply determined using HPLC according to our previous study (Nam et al., 2021). Flavonoids were analyzed using an Agilent 1260 Infinity II LC system (Agilent Technologies, Waldbronn, Germany) equipped with an Agilent 1260 diode array detector HS detector and a ZORBAX Eclipse Plus C_{18} column (4.6 × 250 mm, 5 μm; Agilent Technologies, Palo Alto, CA, USA). Flavonoids were separated using 0.1 % formic acid in water (eluent A) and methanol–acetonitrile (eluent B) as the following gradient: 0–5 min, 20 % B; 5–10 min, 40 % B; 10–15 min, 50 % B; 15–20 min 70 % B, 20–25 min 100 % B, post-run 25–30 min 20 % B at a flow rate of 0.5 mL/min. The injection volume, column temperature, and UV detector were set at 10 μL, 35 °C, and 280 nm, respectively. Naringin, naritutin, hesperidin, naringenin, hesperidin, and neohesperidin (0.5–5.0 mg/mL) were used as standards (Table S2). The areas and retention times of the analyte peaks were compared with those standards, and contents were displayed as milligrams per gram of dry weight (mg/g DW).

2.5. Structural characterization

Morphological analysis of yuzu powder was accomplished using a scanning electron microscopy (SEM) (Gemini 500, Zeiss, Oberkochen, Germany), and functional group analysis was achieved by Fourier transform infrared spectroscopy (FT-IR) using a Spectrum 400 spectrometer (PerkinElmer, Waltham, MA, USA). For SEM, samples were placed on a specimen holder, sputter-coated with gold, and observed under the SEM at an accelerating voltage of 15 kV and 400-fold magnification. FT-IR spectra were recorded from 450 to 4,000 cm^{-1}.

2.6. Physicochemical properties

2.6.1. Water-holding capacity (WHC)

WHC was analyzed using the method described by Wang et al. (2015) with minor modifications. The sample (0.5 g) was mixed with 10 mL of water, kept at 22 °C for 24 h, and then centrifuged at 7,000 × *g* for 15 min. The supernatant was carefully removed by slowly tilting the centrifuge tube. WHC was calculated using Eq. (4):

$$\text{WHC (g/g)} = (\text{wet weight - dry weight}) / \text{dry weight} \quad (4)$$

2.6.2. Water swelling capacity (WSC)

WSC was analyzed using the method described by Wang et al. (2015). The sample (0.5 g) was mixed with 10 mL of distilled water and left to hydrate at 4 °C for 18 h. The final volume of the swollen sample was recorded to enable calculation of the WSC using Eq. (5):

$$\text{WSC (mL/g)} = (\text{wet volume - dry volume}) / \text{dry weight} \quad (5)$$

2.6.3. Oil adsorption capacity (OAC)

OAC was carried out using the method described by Wang et al. (2015). The sample (0.5 g) was mixed with 10 mL of olive oil, kept at 22 °C for 24 h, and then centrifuged at 7000×*g* for 15 min. The OAC was calculated using Eq. (6):

$$\text{OAC (g/g)} = (\text{pellet weight - dry weight}) / \text{dry weight} \quad (6)$$

2.6.4. Cholesterol-binding capacity (CBC)

Fresh egg yolks were diluted with distilled water (9-fold) at pH 2.0 and 7.0. Yuzu powder (0.2 g) was mixed with 5 mL of the diluted yolk, shaken at 120 rpm for 2 h at 37 ℃, precipitated with four volumes of 95 % ethanol, and centrifuged at 3,800 × *g* for 20 min. The ethanol was discarded using a vacuum evaporator. The collected sample was diluted with four volumes of glacial acetic acid. Next, 50 μL of sample solution was added to a mixture containing 50 μL of glacial acetic acid, 50 μL *o*-phthalaldehyde, and 250 μL H_2SO_4 and mixed for 20 min. The reaction was read at 550 nm using a microplate reader (Synergy HTX, Biotek Epoch, Agilent, Santa Clara, CA, USA). The data are presented as milligrams per gram (mg/g).

2.7. Bitterness analysis

The mature and healthy *Xenopus* oocytes were surgically harvested from the ovary and then treated with collagenase. Oocytes were injected with approximately 5 to 20 ng of *TAS2R* + *GIRK1/4* RNA using a nanoliter injector (Drummond Scientific, Vernon Hills, IL, USA). The injected oocytes were cultured in ND96 solution (96 mmol/L NaCl, 2 mmol/L KCl, 1 mmol/L $MgCl_2$, 5 mmol/L HEPES, 1.8 mmol/L $CaCl_2$, 2.5 mmol/L Na-pyruvate, 50 μg/mL gentamycin, pH 7.4) for 2–3 days according to the method described by Quick and Lester (1994). The two-electrode voltage-clamp (TEVC) method was performed as described (Takashi et al., 1995). Membrane potentials of oocytes were measured by the peak area response to the bitter taste of the sample when high-concentration potassium was added to confirm the activity of the bitter taste receptor according to the sample treatment.

2.8. Cell viability

The 3 T3-L1 cells were cultured in DMEM containing 10 % BCS, 1 % penicillin and streptomycin in a 37 °C 5 % CO_2 incubator until 80–90 % confluent. Then, the cells were dispensed in a 96-well plate at 1×10^5 cells/mL for 24 h, followed by treatment with different concentrations of yuzu powder (10–300 μg/mL) for 24 h. Cell viability was determined using MTT assay kit. MTT solution (5 mg/mL, dissolved in phosphate buffered saline) was added to attain a final 0.45 mg/mL and incubated for 4 h at 37 °C. DMSO was added to remove the remaining solution in the plate. Absorbance at 570 nm was measured using an ELISA reader (Epoch; BioTek Instruments, Winooski, VT). The cell viability (%) was calculated using Eq. (7):

$$\text{Cell viability (\%)} = (\text{treated cells - blank cells}) / (\text{control cells - blank cells}) \times 100 \quad (7)$$

2.9. Glycerol release

The lipolytic activity of enzyme-treated and untreated yuzu samples in 3T3-L1 cells was determined by measuring the dissolved glycerol in the medium. After completely inducing differentiation in a 24-well plate using 3T3-L1 preadipocytes, the cells were treated with the sample for 48 h. Then, lipolysis was induced, and the glycerol content secreted by the cells was quantified using an enzyme-based lipolysis assay kit. The reagent was pre-warmed to 37 °C, the collected culture medium was added and incubated at 37 °C for 1–3 h, and then the absorbance was measured at 570 nm.

2.10. Oil red O staining

The 3T3-L1 cells were dispensed in a 6-well plate at 5×10^4 cells/mL. The medium was changed on the day 2. When the cells were post-confluent on the day 4, they were treated with 0.5 mmol/L IBME, 1 μmol/L dexamethasone, 10 μg/mL insulin, and sample (10 and 50 μg/mL) to induce differentiation. After 2 days, DMEM medium containing 10 μg/mL insulin and 10 % FBS was altered every 2 days up to day 8. On the day 8 from the initiation of differentiation, the medium was discarded, washed with phosphate-buffered saline, and then fixed with 10 % formaldehyde for 1 h. After removing formaldehyde and washing the medium three times with distilled water, 1 mL of Oil Red O working solution was added, stained for 30 min at 22 °C, and washed three times with distilled water. Stained fat globules were photographed, and 100 % isopropanol was added to elute the lipids accumulated in the cells. After transferring to a 96-well plate, the absorbance was read at 500 nm to evaluate the degree of lipid accumulation.

2.11. Statistical analysis

Data were presented as mean ± standard deviation (SD) (n = 3). Significant differences between experimental groups were determined by analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. A significance level was established at $p < 0.05$. Statistical analyses were performed using SPSS 23.0 for Windows program (SPSS, Inc, Chicago, IL, USA). All figures were constructed using GraphPad Prism 9.5.1 software (La Jolla, CA, USA).

3. Results and discussion

3.1. Screening

3.1.1. Screening of commercial enzymes

Detailed information on the 11 commercial enzymes employed in this study and the hydrolysis of yuzu powder by these enzymes are shown in Table S1 and Fig. 1A, respectively. Yuzu powder solubility improved from 13.21 % to 51.90 %, depending on the enzyme (Fig. 1A). The lowest and highest solubility were recorded for hydrolysis by tannase from *Aspergillus oryzae* and Plantase UF from *Aspergillus niger*, respectively. Of the 11 enzymes, cellulase KN contained naringinase, one of the enzymes that hydrolyze naringin to reduce the bitterness of citrus fruits. Therefore, cellulase KN and Plantase UF were selected for yuzu treatment.

3.1.2. Screening of high-producing cellulase microorganism

To screen high-producing cellulase microorganisms from Korean traditional fermented foods, seven strains were selected based on the diameter of the transparent circle (hydrolyzed cellulose) in 1 % CMC agar plates from 519 LAB colonies (Fig. 1B). Five of these strains hydrolyzed CMC, according to TLC analysis (Fig. 1B). NY203 (cellulase) was selected for identification based on 16S rRNA and subsequent yuzu powder treatment because of its cellulose-degrading ability; it had the widest diameter of the cellulose hydrolysis zone (4 mm). Phylogenetic analysis of isolated LAB based on 16S rRNA revealed that the NY203 strain and *Leuconostoc mesenteroides* were clustered together (Fig. 1C). Thus, the isolated NY203 strain was identified as *L. mesenteroides*.

3.2. Analysis of the chemical composition of enzyme-treated yuzu

3.2.1. DF composition of enzyme-treated yuzu

The DF composition of untreated and enzyme-treated yuzu powder is shown in Fig. 2A. The total DF (SDF and IDF) content was decreased significantly after enzyme treatment compared with that of untreated yuzu (Fig. 2A). Following enzyme treatment, SDF content was decreased from 17.39 ± 0.73 % to 2.38 ± 0.30 %, and IDF was decreased from 18.50 ± 0.65 % to 5.20 ± 0.89 %. The decrease in the SDF and IDF of yuzu was 1.52 and 3.15 times by cellulase NY203 treatment and 3.70 and 1.44 times by Plantase UF treatment compared to the control. When yuzu was treated with NY203 + UF or NY203 + UF + KN, the SDF and IDF were 7.32 and 3.56 times lower than those of the control, respectively, without significant difference between the two treatment groups (Fig. 2A).

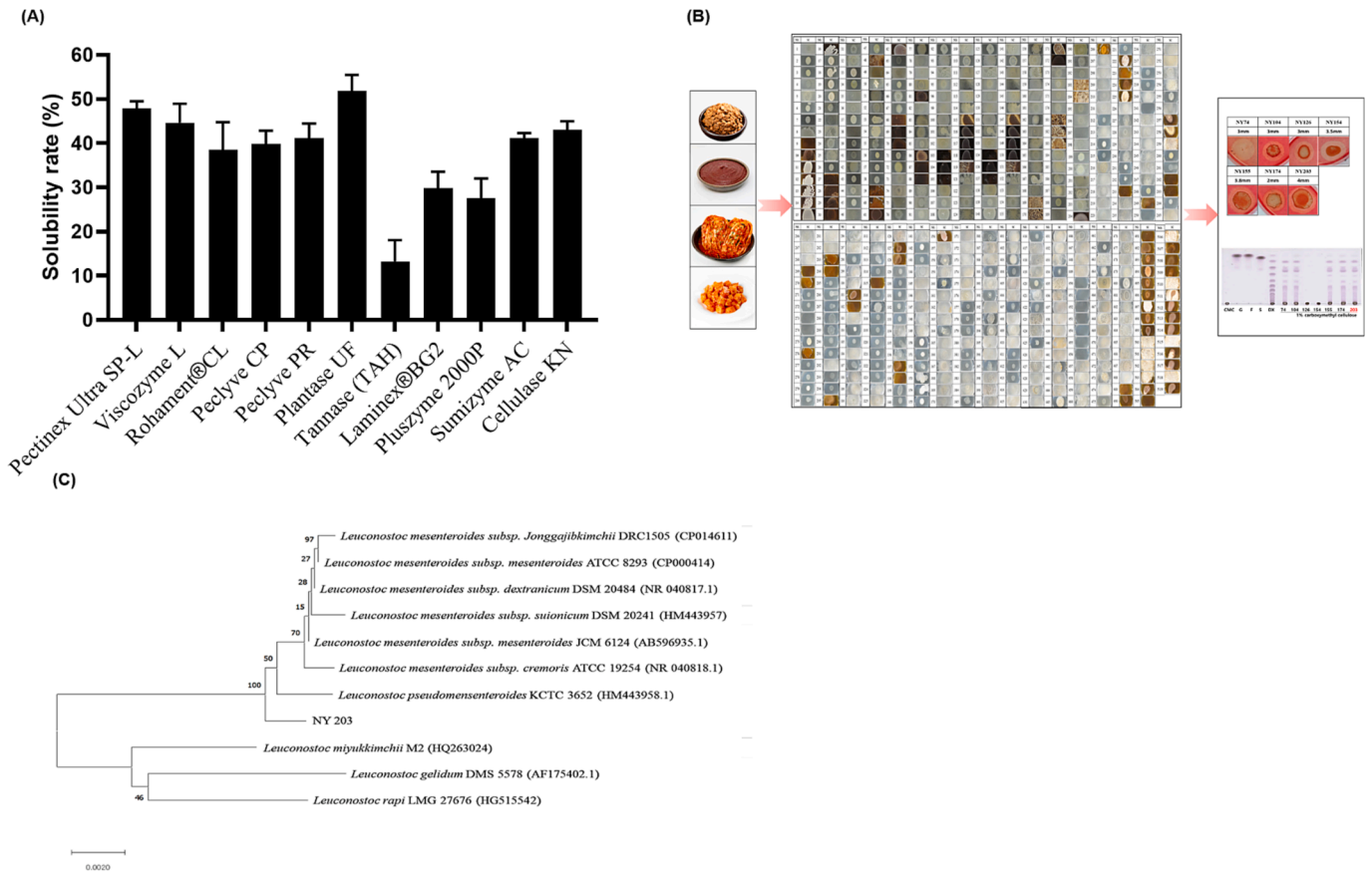

Fig. 1. Water solubility of yuzu powder after hydrolysis with commercial enzymes (**A**), graphical representation of single colony isolation of lactic acid bacteria and screening of potential strains from Korean traditional fermented food sources (**B**), and phylogenetic tree analysis of traditional-food-source-derived NY203 strain based on 16S rRNA. Different lowercase letters represent the significant differences among enzymes by Dunnett's multiple comparison test ($p > 0.05$).

3.2.2. Lignin, pectin, hemicellulose, and cellulose contents of enzyme-treated yuzu

The hemicellulose, cellulose, pectin, and lignin contents of yuzu and enzyme-treated yuzu are shown in Fig. 2B − E. The cellulose content of yuzu treated with NY203, UF, NY203 + UF, and NY203 + UF + KN was 4.54, 2.09, 3.82, and 3.10 times lower compared to the control (Fig. 2B). The hemicellulose content of yuzu was 1.15 times higher after treatment with UF, and 1.11, 1.15, and 3.82 times lower after treatment with NY203, NY203 + UF, and NY203 + UF + KN than that of the control, respectively (Fig. 2C). The lignin and pectin contents of NY203-treated yuzu were insignificantly different from those of the control. Although the lignin content of UF-treated yuzu was not significantly different from the control, the pectin content was 3.50 times lower (Fig. 2D, E). Compared with that of the control, the lignin content of yuzu treated with NY203 + UF and NY203 + UF + KN was decreased by 1.25 and 1.5 times, and the pectin content was decreased by 3.11 and 4.29 times, respectively. Our results were exhibit consistency with a previous study by (Song et al., 2021)) that enzymatic hydrolysis of citrus fiber by xylanase significantly decreased the cellulose and hemicellulose contents because of partial removal of some of the hemicelluloses between the fibrils by the enzyme and enzyme-induced hydrolysis of bonds between hemicelluloses and cellulose or lignin.

3.2.3. Monosaccharide and galacturonic content

The monosaccharides, including glucose, fructose, arabinose, and galacturonic acid, of untreated and enzyme-treated yuzu powder, are shown in Fig. 2F–I. The glucose content of enzyme-treated yuzu was increased from 1.17 times to 1.94 times compared to the control. The highest glucose content in yuzu was obtained after treatment with NY203 or NY203 + UF + KN (Fig. 2F). No significant difference in the fructose content was noted between UF or NY203 + UF-treated yuzu. However, it was 1.13 and 1.09 times higher in yuzu treated with NY203 and NY203 + UF + KN, respectively (Fig. 2G). Furthermore, arabinose and galacturonic acid, which were not detected in the untreated yuzu, were significantly increased by enzyme treatments (Fig. 2H, I). Arabinose is one of the main monosaccharides found in pectin and hemicellulose but is rarely found in cellulose (Salvador, Suganuma, Kitahara, Tanoue, & Ichiki, 2000), whereas galacturonic acid is the main component of pectin (Zou, Xi, Hu, Nie, & Zhou, 2016). When yuzu was hydrolyzed by UF alone or in combination with NY203, KN, or NY203 + KN, the arabinose and galacturonic acid contents were higher than those found in yuzu treated with NY203. Grohmann & Baldwin (1992) reported that combination treatment with cellulase and pectinase increased the concentrations of glucose, galactose, arabinose, and galacturonic acid in orange peel. Therefore, the increased fructose, glucose, arabinose, and galacturonic acid contents after enzyme treatment of yuzu might be due to the cell-wall destruction by enzymatic hydrolysis. The significantly decreased pectin content (Fig. 2E) positively correlated with the increased galacturonic acid content after enzyme treatment (Fig. 2I).

3.2.4. Flavonoid content

Previous studies have reported that the main flavonoids in yuzu are naringin, narirutin, hesperidin, neohesperidin, naringenin, and hesperetin (Nam et al., 2021; Yoo et al., 2009). Therefore, the variety of these flavonoids in untreated and enzyme-treated yuzu was analyzed. As shown in Fig. 2J–O, no significant differences in the naringin, naritutin, and hesperidin contents of NY203-treated yuzu were observed with those of the control. However, in yuzu treated with UF, NY203 + UF, and NY203 + UF + KN, the naringin, narirutin, hesperidin, and

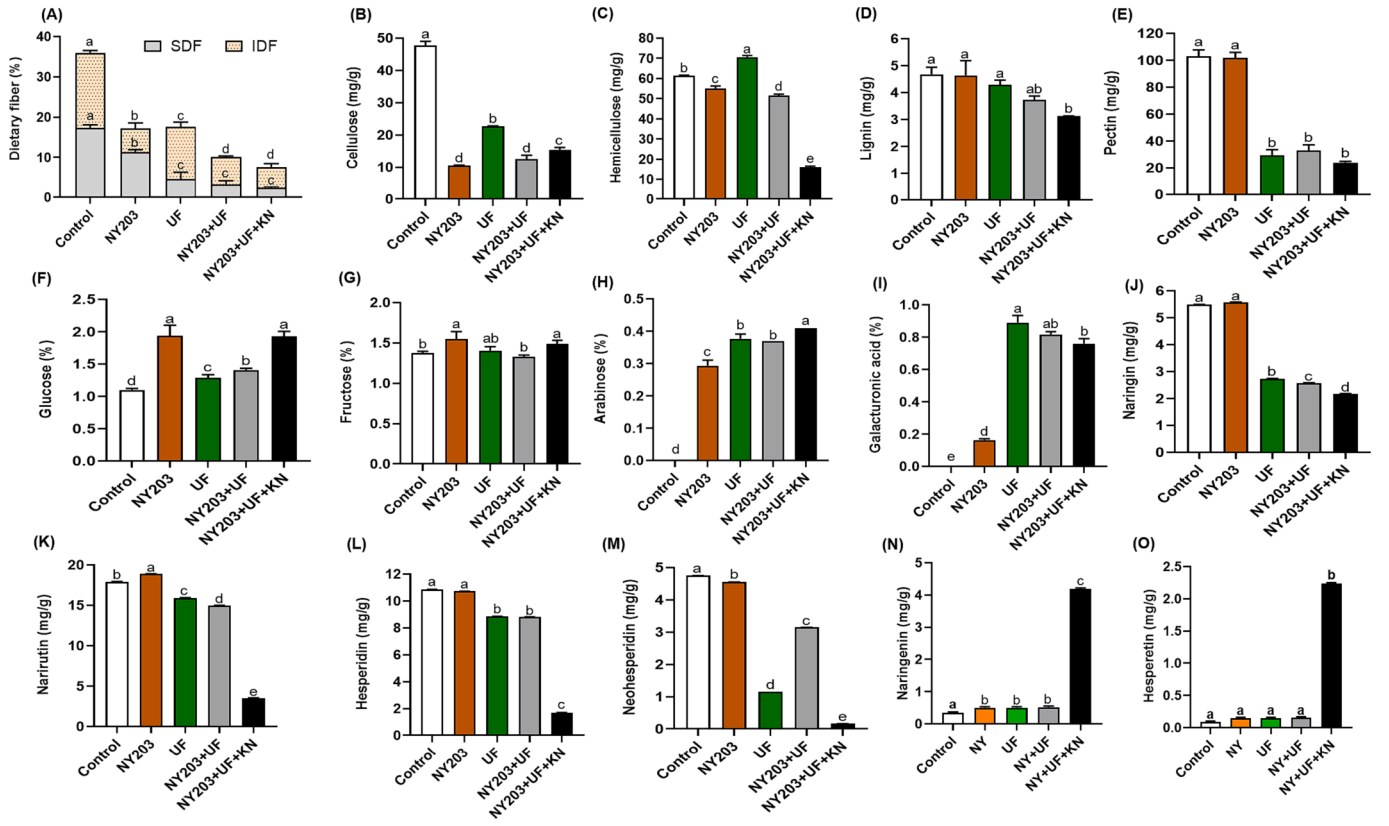

Fig. 2. Insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) content (**A**), cellulose (**B**), hemicellulose (**C**), lignin (**D**), pectin (**E**), glucose (**F**), fructose (**G**), arabinose (**H**), galacturonic acid (**I**), naringin (**J**), narirutin (**K**), hesperidin (**L**), neohesperidin (**M**), naringenin (**N**), and hesperetin (**O**) contents of enzyme-treated and untreated yuzu powder. Different lowercase letters represent the significant differences among treatment and control groups by Dunnett's multiple comparison test ($p > 0.05$). Control: untreated yuzu, NY203: food-source-derived cellulase, UF: commercial pectinase, KN: commercial naringinase.

neohesperidin contents were decreased, whereas naringenin and hesperetin in all enzyme-treated yuzu were increased. Among these treatments, NY203 + UF + KN led to the highest in reducing these detected flavonoids in this study. Following treatment with NY203 + UF + KN, naringin, narirutin, hesperidin, and neohesperidin concentrations decreased by 60.47 %, 80.32 %, 84.39 %, and 96.63 %, respectively, whereas the naringenin and hesperetin were increased by 1,102.37 % and 2,389.37 %, respectively. The naringenin and hesperetin content increase observed in NY203 + UF + KN-treated yuzu resulted from naringinase activity in commercial cellulase KN. Naringinase is a multienzyme complex comprising both α-L-rhamnosidase and β-glucosidase activities which can hydrolyze the naringin, hesperidin, and neohesperidin in yuzu to release naringenin and hesperetin (Seong et al., 2023).

3.3. Structural characterization

3.3.1. SEM

Photographs of enzyme-treated and untreated yuzu powder are presented in Fig. 3A. Clear visualization of a deeper color was observed after the enzyme treatments, which intensified in the order of NY203, UF, NY203 + UF, and NY203 + UF + KN (Fig. 3A). In Fig. 3B, the SEM images reveal that the surface of untreated (control) yuzu powder had a more intact structure with minute pores and crevices compared to enzyme-treated yuzu. After enzymatic treatment, the structure of yuzu powder was relatively looser, with numerous apparent holes, cracks, and swelled inner structure. A wrinkled surface and tiny pores were observed with all enzymatic treatments. The results indicate that enzyme treatment could destroy the microstructure and result in the degradation of several fibers, which may cause many holes in the yuzu fibers. Thus, each enzyme treatment showed a more transparent looser inner structure than the control. Fiber composition analysis (Fig. 2) exhibited decreased cellulose, hemicellulose, lignin, and pectin levels, verifying the changes in the morphology of yuzu powder. The different enzyme treatments displayed varying levels of structural changes that finally determined the solubility properties of yuzu powder. The NY203 treatment showed a smooth, swollen surface with tiny pores, whereas the UF treatment showed a dense, rough, and highly porous surface. Among all enzyme treatments, NY203 + UF + KN demonstrated a more expanded balloon-shaped structure, looser microstructure, and more porosity, perhaps because of inner structure and surface area damage or loosening of yuzu by this combination of enzymes. Other studies showed that a porous structure facilitated moisture absorption and retention to improve the solubility index (Song et al., 2021; Zhang et al., 2020). Citrus fiber with a loose structure and wrinkled surface has been found to exhibite more wave drapes, potentially enlarging its surface area (Zhuang et al., 2016). Thus, enzyme treatment of yuzu powder may improve its WHC and WSC.

3.3.2. FT-IR analysis

FT-IR spectroscopy revealed that enzyme-treated and untreated yuzu powders had almost similar characteristic spectra (Fig. 3C). The absorption peak at 3,301 cm^{-1} was derived from the intramolecular hydrogen bonding of –OH groups in the crystalline region of cellulose and hemicellulose (Ma et al., 2016). Compared to the untreated control, the peak intensities of all enzyme treatments decreased, but especially after NY203 + UF + KN treatment, indicating the destruction of hydrogen bonds in the cellulose crystalline domain and hemicellulose chains. All yuzu samples had C—H stretching vibrations from $–CH_3$ and $–CH_2–$ groups of polysaccharides at about 2,927 cm^{-1} (Yan, Ye, & Chen, 2015). There was also a peak at 2,927 cm^{-1} assigned to the intermolecular hydrogen bonds of –OH groups of cellulose (Jia et al., 2019). The

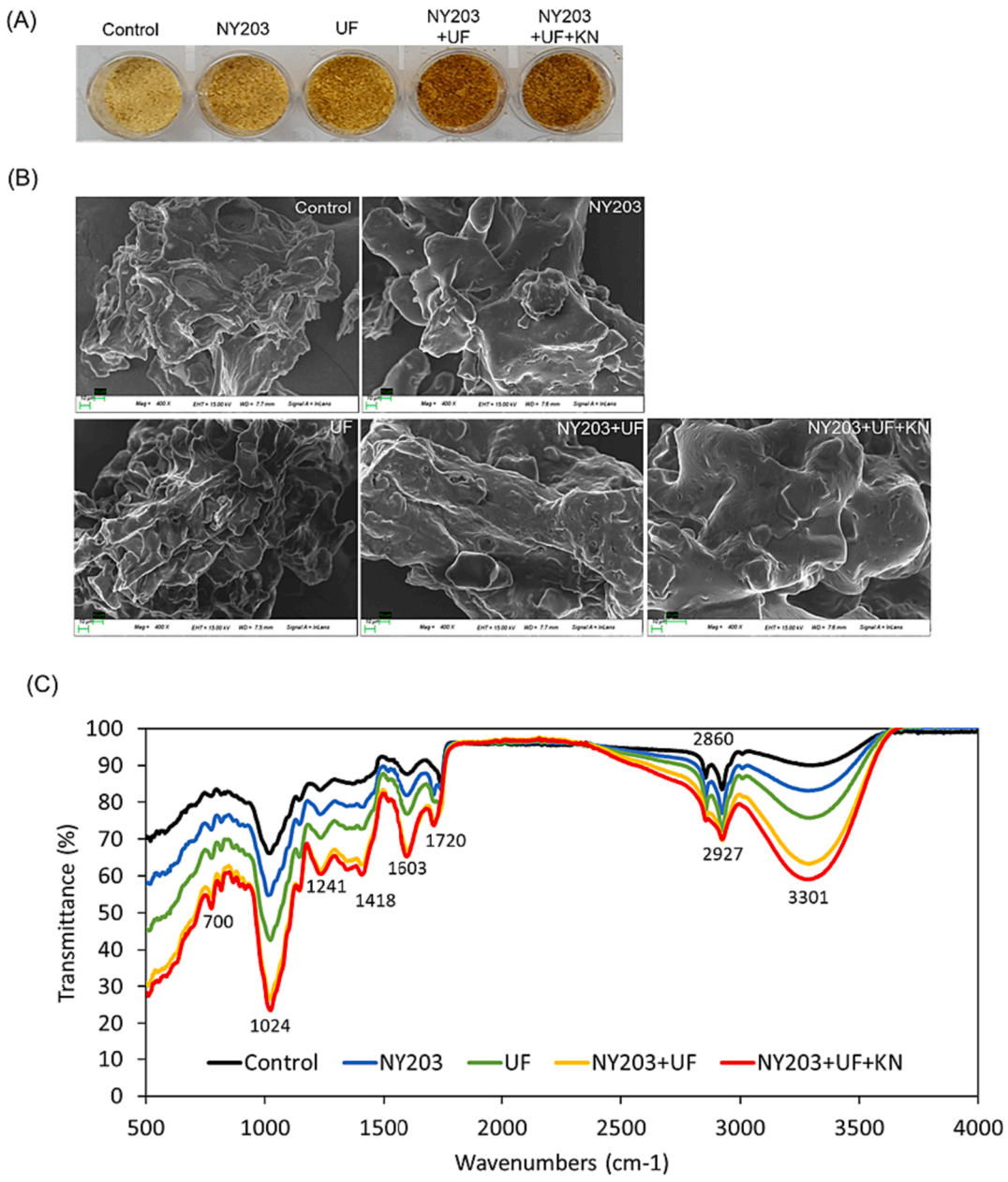

Fig. 3. Photo images (**A**), scanning electron micrographs (**B**), and FT-IR spectra (**C**) of enzyme-treated and untreated yuzu powder. Control: untreated yuzu, NY203: food-source-derived cellulase, UF: commercial pectinase, KN: commercial naringinase.

–OCH_3 group stretching vibrations at around 2,860 cm^{-1}, representing the pectin in yuzu powder (Merci, Urbano, Grossmann, Tischer, & Mali, 2015), displayed a sharp peak in the control but almost disappeared in the NY203 + UF + KN treatment (Fig. 3C). This result concurred with the pectin content result exhibited in Fig. 2E, which further proved the potential of enzyme treatment to destroy the yuzu fiber structure, leading to reduced levels of pectin. The characteristic peak due to acetyl and uronic ester groups of hemicelluloses at around 1720 cm^{-1} was observed in all yuzu samples (Kaushik & Singh, 2011), and the peak intensity decreased after enzymatic hydrolysis, especially after NY203 + UF + KN treatment. Most of the peaks at around 1,603 to 1,418 cm^{-1}, representing aromatic benzene in lignin, were observed in all treated and untreated samples (Ma et al., 2016; Merci et al., 2015), and the absorption decreased in all enzyme treatments, especially after the NY203 + UF + KN treatment. The vibration at around 1,024 cm^{-1} represented the C—O stretching vibration of C—O—C in the pyranose ring of lignin or hemicellulose (Zheng & Li, 2018). The bands at 1,241 cm^{-1} were due to the CH bending vibrations of carbohydrates. The absorption intensity at 1,024 cm^{-1} of the untreated yuzu sample was higher than the enzyme-treated samples, indicating the degradation of lignin and hemicellulose after enzymatic hydrolysis. The absorbance of benzene at 700 cm^{-1} decreased in all enzyme-treated yuzu samples, which implied that enzyme treatment might generate different levels of lignin-derived phenolic compounds (Ma et al., 2016). Almost all the hemicellulose and lignin characteristic peak intensities decreased in all enzyme-treated yuzu samples compared to the untreated control. This decrease was most apparent in the NY203 + UF + KN-treated sample, suggesting that treatment with the combination of enzymes might have a greater modification effect on yuzu powder than a single treatment.

3.4. Physicochemical properties

3.4.1. Solubility and WHC

The solubility (Fig. 4A) and WHC values (Fig. 4B) of enzyme-treated

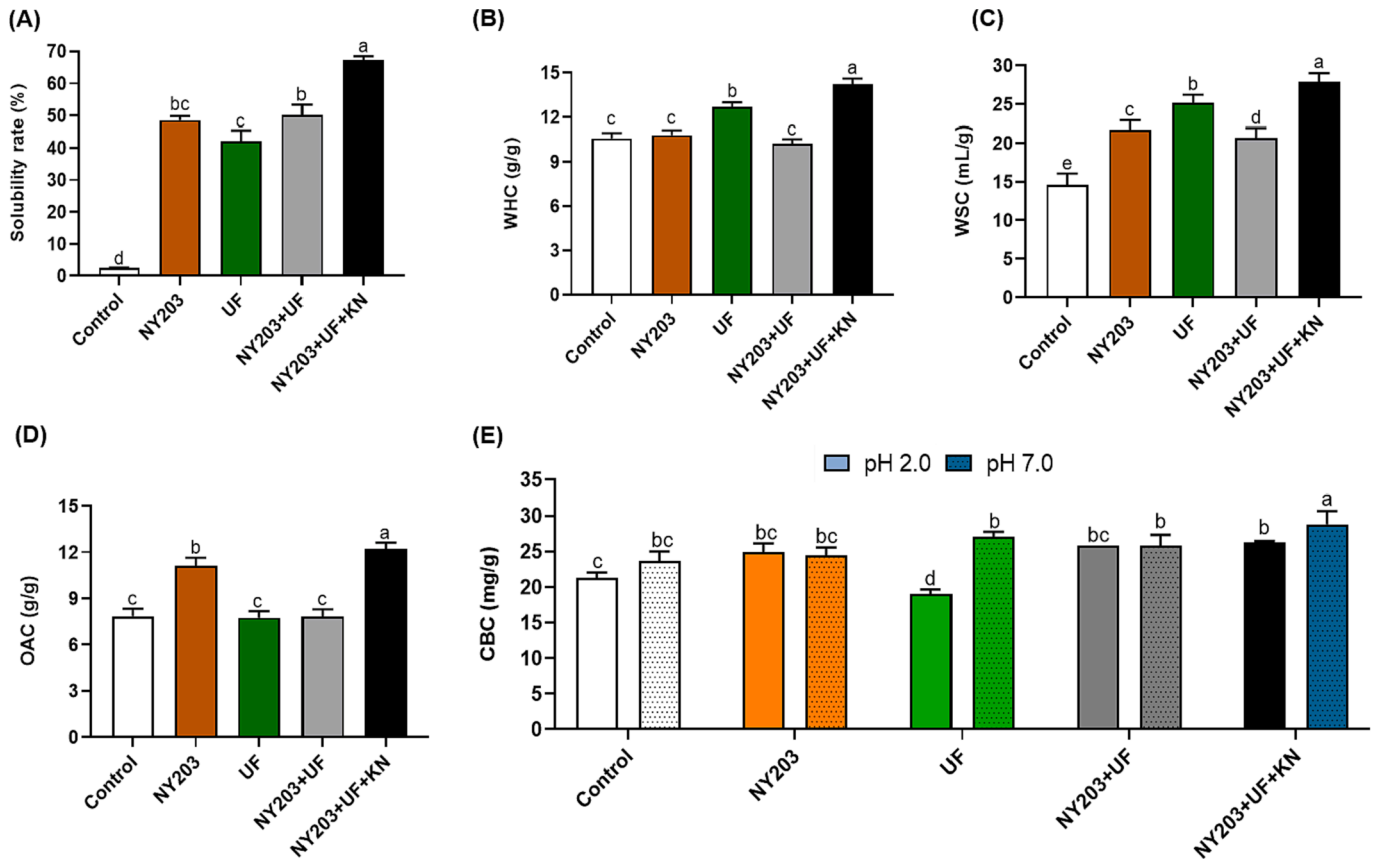

Fig. 4. Solubility (**A**), water-holding capacity (WHC) (**B**), water-swelling capacity (WSC) (**C**), oil-adsorption capacity (OAC) (**D**), cholesterol-binding capacity (CBC) and (**E**) of enzyme-treated and untreated yuzu. Different lowercase letters represent the significant differences among treatment and control groups by Dunnett's multiple comparison test ($p > 0.05$). Control: untreated yuzu, NY203: food-source-derived cellulase, UF: commercial pectinase, KN: commercial naringinase.

and untreated yuzu samples were compared. All enzyme treatments significantly increased the solubility compared to the untreated yuzu (Fig. 4A). The solubility of yuzu treated with NY203, UF, NY203 + UF, and NY203 + UF + KN was 48.5 ± 2.3 %, 42.1 ± 5.7 %, 50.2 ± 5.6 %, and 67.2 ± 2.3 %, respectively, which was 19.4, 16.84, 20.08, and 26.88 times higher than that of untreated yuzu. There was no significant difference in the WHC values among untreated, NY203-treated, and NY203 + UF-treated yuzu (Fig. 4B). However, the WHC of UF-treated and NY203 + UF + KN-treated yuzu was 12.7 ± 0.53 and 14.2 ± 0.68 g/g, which was 1.20 and 1.34 times higher compared to the untreated yuzu, respectively. WHC is affected by the physicochemical properties and environmental conditions of DF. A strong WHC enhances the quality of food by decreasing syneresis. Structural analysis revealed that enzyme treatment could hydrolyze the bonds between hemicellulose and cellulose or lignin, loosen the inner structure and enlarged the surface area of yuzu fiber, and exposed more hydrophilic groups within the fiber structure, all of which can increase the hydration ability and WHC of yuzu powder (Przybysz Buzala, Przybysz, Kalinowska, & Derkowska, 2016).

3.4.2. WSC

WSC properties are crucial for retaining moisture in DF to improve solubility (Baenas et al., 2020). As shown in Fig. 4C, the WSC of all enzyme-treated yuzu increased significantly compared with the untreated yuzu. The WSC of yuzu treated with NY203, UF, NY203 + UF, and NY203 + UF + KN was 21.69 ± 2.15, 25.13 ± 1.86, 20.68 ± 2.16, and 27.87 ± 1.94 mL/g, which was 2.05, 2.37, 1.95, and 2.63 times higher than that of untreated yuzu respectively. NY203 + UF + KN treatment showed the greatest improvement (91 %) in WSC compared with the control group. The WSC property is positively correlated with the changes in microstructure and DF compositions of citrus fibers (Zhang, Qi, Zeng, Huang, & Yang, 2020). The variation of components predicted structural transformation and influenced the change of properties.

3.4.3. OAC

The OAC index predicts the adsorption capacity of DF for lipophilic compounds. Citrus fiber with an increased OAC shows improved efficacy in preventing fat loss during food processing (Navarro-Gonzalez, Garcia-Valverde, Garcia-Alonso, & Periago, 2011). No significant difference in the OAC value were observed among untreated, UF-treated, and NY203 + UF-treated yuzu (Fig. 4D). However, the OAC of NY203- and NY203 + UF + KN-treated yuzu was 11.09 ± 0.95 and 12.21 ± 0.70 g/g, which was 1.05 and 1.15 times higher than the control, respectively. OAC positively correlates with the surface features, charge density, and hydrophilic groups of DF (Zheng and Li, 2018). In this study, the morphology, FT-IR analysis, and OAC variation, which displayed similar tendencies, indicated that the expanded surface and more porous structure of yuzu powder either by NY203 or NY203 + UF + KN treatment could improve oil absorption and retention. Thus, these treatments could effectively improve the OAC of yuzu powder.

The increased solubility of yuzu powder, along with the significantly higher WHC, OAC, and WSC values after enzyme treatment, might be due to the ability of enzymes to degrade the cellular structure, inner and outer surface structure, and chemical bonding, thereby increasing the water and oil adsorption and retention capacities. Moreover, yuzu powder with improved WHC, OAC, and WSC is a potentially used as a food additive in the food industry.

3.4.4. CBC

CBC is reportedly associated with the reduction of blood lipid and glucose levels. Enzyme treatment improved the CBC values of yuzu powder (Fig. 4E). Among all enzyme treatments, NY203 + UF + KN treatment increased the CBC values most efficiently (by 24 % and 22 % at pH 2.0 and 7.0, respectively) compared with control. There are positive correlations between DF and glucose and lipid metabolism (Guillon & Champ, 2000). The CBC may be associated with the structural properties of DFs, including surface area, porosity, and cavities or holes on the surface (Liu et al., 2021). Enzyme treatment increased the CBC value of yuzu powder from 21.20 to 26.24 mg/g at pH 2.0 and 23.67 to 28.80 mg/g at pH 7.0 possibly because the enzyme-induced structural changes of yuzu powder, which loosened the structure and created many obvious holes and cracks as well as a swollen shape that potentially provided more cholesterol-binding sites. Consistent with our findings, a previous study found that the CBC of citrus fiber improved significantly after xylanase treatment (Song et al., 2021). In our study, the CBC value of all yuzu samples was increased more at pH 7.0 (simulated intestinal environment) than at pH 2.0 (simulated gastric environment). This might be because of the partial positive charge carried by the yuzu sample and cholesterol in acidic conditions owing to the higher concentration of hydrogen ions than in neutral conditions. Consequently, the binding force between the yuzu sample and cholesterol was weakened due to the repulsion force produced by the same charge, resulting in decreased CBC values (Song et al., 2021).

3.5. Bitter analysis

Bitterness is a major problem in the citrus industry because it may deteriorate the quality, reduce the consumer acceptability, and lower the economic value of citrus fruit-based products (Kore et al., 2015). The effect of untreated and enzyme-treated yuzu on bitter taste receptor Tas2R16 is shown in Fig. 5. The bitterness values of untreated yuzu and NY203-, UF-, NY203 + UF-, and NY203 + UF + KN-treated yuzu were −9.6, 1.1, −9.6, −10, and −4.8 nA, respectively. Bitter taste receptor analysis based on the TEVC method demonstrated no significant difference in bitterness among untreated, UF-, and NY203 + UF-treated yuzu. In contrast, the bitterness of NY203 + UF + KN-treated yuzu was 50 % lower compared to the control (Fig. 5). Naringin and neohesperidin are major bitter compounds in citrus fruits, whereas the bitterness of naringenin (i.e., deglycosylated naringin) is 300 times lower than that of naringin (Koca et al., 2009; Seong et al., 2023), and hesperidin is tasteless (Drewnowski & Gomez-Carneros, 2000). In this study, the naringin content was significantly reduced by UF (50.09 %), NY203 + UF (53.0 %), and NY203 + UF + KN (60.47 %) treatments, but minimally affected by NY203 treatment (Fig. 1J). Similarly, the neohesperidin content was also significantly decreased by the UF (75.60 %), NY203 + UF (33.80 %), and NY203 + UF + KN (96.63 %) treatments (Fig. 1M). Moreover, the NY203 + UF + KN treatment decreased the narirutin (80.31 %) and hesperidin (84.39 %) contents (Fig. 1K, L). This result indicates that NY203 + UF + KN-treated yuzu powder could improve consumer acceptability by reducing the bitterness of yuzu powder.

3.6. Functional properties

3.6.1. Viability of 3T3-L1 cells

Cell viability data is shown in Fig. 6A. An increased concentration of enzyme-treated yuzu decreased the cell viability, contrary to the control, which showed no significant difference between the 100 and 300 μg/mL treatments and between the 10 and 30 μg/mL treatments (Fig. 6A). In all treatments, cell viability exceeded 90 % at 10- and 50 μg/mL. Therefore, these concentrations were used for the subsequent experiments.

3.6.2. Effects of enzyme-treated yuzu on the glycerol content in 3T3-L1 cells

Lipolysis is the metabolic process by which triacylglycerols are hydrolyzed into their constituent molecules, namely, glycerol and free fatty acids. Thus, glycerol release into the medium of adipocyte cells indicates lipolytic activity. The amount of glycerol in the medium of adipocyte cells exposed to enzyme-treated and untreated yuzu is presented in Fig. 6B. Adipocyte cells treated with 10 μg/mL of enzyme-treated or untreated yuzu did not exhibit any significant differences in glycerol content. However, 50 μg/mL of NY203 + UF + KN-treated yuzu showed a significantly increased glycerol level, which was 2.1 times higher than that of the untreated control and other enzyme-treated samples.

3.6.3. Effects of enzyme-treated yuzu on lipid accumulation in 3T3-L1 cells

Microscopic images of 3T3-L1 cells stained with Oil red O to observe the changes in lipid-droplet accumulation in response to enzyme-treated and untreated yuzu (control) are shown in Fig. 6C. No significant differences were noted among control (10 and 50 μg/mL of), NY203-treated yuzu powder (10 μg/mL), and UF-treated yuzu powder (10 μg/mL) (Fig. 6D). This result agrees with the report by Sharma et al. (2019) wherein up to 100 μg/mL of extracted yuzu (*C. junos*) did not significantly reduce lipid accumulation in 3 T3-L1 cells. However, in the present study, lipid accumulation in 3 T3-L1 cells decreased at 50 μg/mL of NY203- or UF-treated yuzu and at 10 μg/mL of NY203 + UF- or NY203 + UF + KN-treated yuzu (Fig. 6D). The highest suppression of lipid accumulation in 3 T3-L1 cells was observed with 50 μg/mL of NY203 + UF- or NY203 + UF + KN-treated yuzu (Fig. 6D), which reduced lipid accumulation by 27.84 % and 23.98 %, respectively, compared with the control (Fig. 6D). In previous studies, extracted citrus

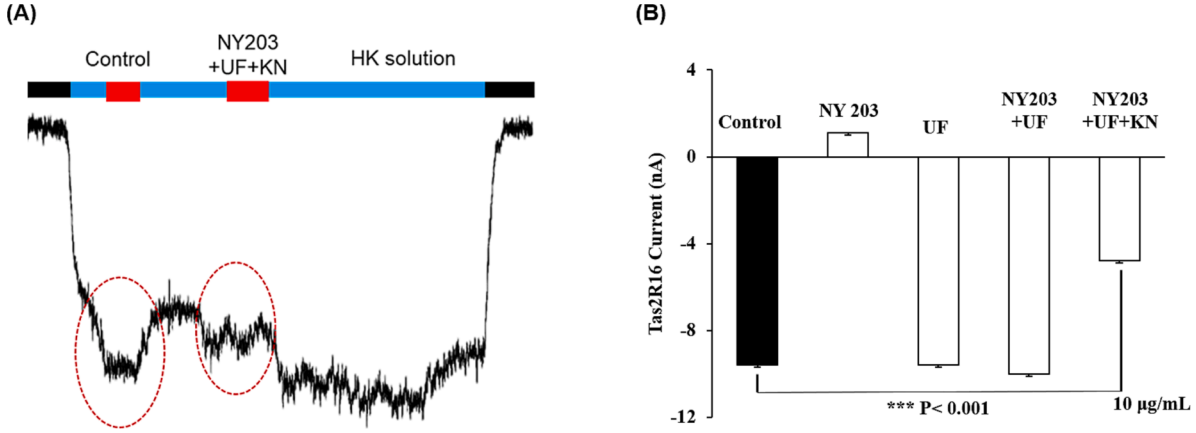

Fig. 5. The membrane potential of oocytes treated with enzyme-treated and untreated yuzu (**A**) and bitterness evaluation of enzyme-treated and untreated yuzu (**B**).

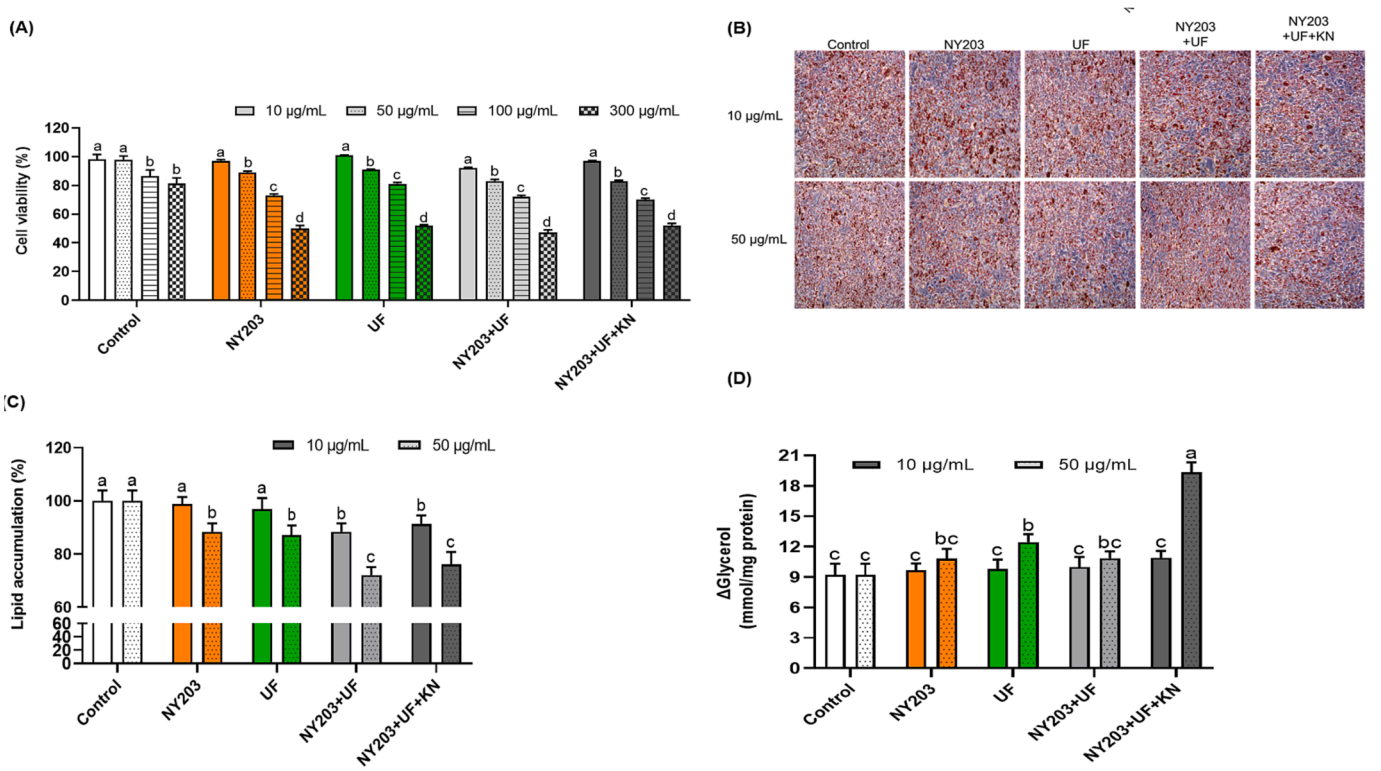

Fig. 6. Cell viability (**A**), Oil red O staining (**B**), lipid accumulation (**C**), and glycerol content (**D**) of enzyme-treated and untreated yuzu on 3T3-L1 cells. Different lowercase letters represent the significant differences among treatment and control groups by Dunnett's multiple comparison test ($p > 0.05$). Control: enzyme untreated, NY203: food-source-derived cellulase, UF: commercial pectinase, KN: commercial naringinase. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

fruit, including *C. junos* (yuzu), *Citrus reticulata* (mandarin peel), and *Citrus aurantium* L. (bitter orange), exhibited anti-lipid accumulation in 3T3-L1 cells at concentrations over 600 μg/mL (Campitelli et al., 2020; Sharma et al., 2019). In contrast, this same property was observed with just 50 μg/mL of enzyme-treated yuzu in the present study. Previous studies have reported that the food matrix affects the absorption of polyphenols (Monfoulet et al., 2020; Ovando-Martinez, Gamez-Meza, Molina-Dominguez, Hayano-Kanashiro, & Medina-Juarez, 2018). Aschoff et al. (2015) showed that despite containing a lower amount of hesperidin, orange juice (*Citrus sinensis* L.) hesperidin was more bioaccessible than that from orange fruit segments (Aschoff et al., 2015). In another study, although the concentration of flavanone in fresh oranges was 4.5 times higher than that in pasteurized orange juice, there was no significant difference in flavanone bioavailability between the two matrices (Aschoff et al., 2016). In addition, citrus flavonoids are not consumed by eating orange pulp because they are located in the inner part of the peel (Ho, Ferruzzi, & Wightman, 2020). Therefore, the difference in food matrix between yuzu and enzyme-treated yuzu might have affected the anti-lipid stimulation and glycerol content in this study. Notwithstanding, further investigation is needed.

4. Conclusions

In this study, enzymatic modification of yuzu powder with commercial enzymes (UF + KN) and cellulase NY203 from isolated *Leuconostoc mesenteroides* improved its physicochemical properties including solubility, WHC, OAC, WSC, and monosaccharides. The naringin, narirutin, hesperidin, and neohesperidin contents in enzyme-treated yuzu decreased by 60.5 %–96.6 %, while their deglycosylation products including naringenin and hesperetin were increased by 1,102.4 %–2,389.4 %, respectively. On evaluating the effect of enzymatic modification on yuzu powder structure, SEM and FT-IR revealed that NY203 + UF + KN treatment potentially enhanced the disruption of surface structural properties by expanding the balloon-shaped structure, loosening the microstructure, and increasing the porosity of the surface structure of yuzu powder. The collapsed structural changes further contributed to the improved physicochemical and functional properties. Moreover, the bitterness and lipid accumulation in 3T3-L1 cells of NY203 + UF + KN-treated yuzu were reduced by 50 % and 23.98 %, respectively, compared to the control. Furthermore, NY203 + UF + KN-treated yuzu exhibited 2.1 times higher glycerol levels than untreated yuzu. Overall, the findings suggest that NY203 + UF + KN treatment is the most potent biological tool for modifying yuzu powder by improving its physicochemical and health functionality properties. Therefore, functional yuzu powder treated with NY203 + UF + KN could be used as an additive in functional beverages and food products.

CRediT authorship contribution statement

Hana Jeong: Investigation, Formal analysis, Writing – original draft. **Protiva Rani Das:** Formal analysis, Writing – review & editing. **Hayeong Kim:** Formal analysis, Writing – review & editing. **Ae Eun Im:** Formal analysis. **Bo-Bae Lee:** Formal analysis, Resources. **Kwang-Yeol Yang:** Conceptualization, Funding acquisition. **Seung-Hee Nam:** Conceptualization, Supervision, Project administration.

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.

Data availability

Data will be made available on request.

Acknowledgments

We gratefully acknowledge Professor Junho H. Lee at the Department of Biotechnology, Chonnam National University, for assistance with the bitterness analysis. This study was financially supported for Technology Development Project for Agricultural Policy Supporting (Project No. RS-2023-00236699) by Rural Development Administration and Open-Lab Fostering Support Project (Project No. RS-2023-00282319) by Ministry of Science and ICT, Republic of Korea.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100918.

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