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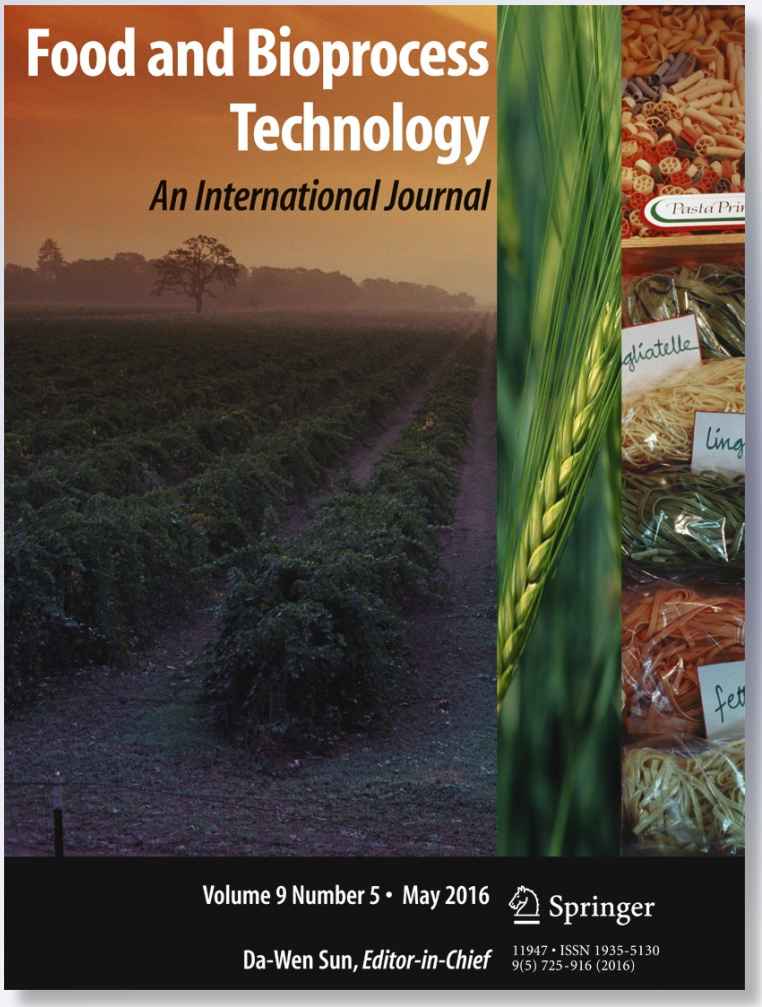

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ORIGINAL PAPER

Emulsifying Properties of Proteins Isolated from Various Rice Cultivars

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Abstract The emulsifying properties of rice proteins isolated from various non-waxy and waxy rice cultivars were studied to evaluate their potential application as oil-in-water (O/W) emulsifier and to compare the emulsifying properties of the rice proteins isolated from various rice cultivars. The solubility of the rice proteins was measured at different pHs (2–10), and O/W emulsions were prepared with the proteins at pH 2, 7, and 10. The rice protein-stabilized O/W emulsions were analyzed by measurement of particle size and zeta-potential, and observation under an optical microscope. The results indicated the potential of the rice proteins as emulsifiers at low and high pH values, and that, in particular, rice proteins isolated from waxy-rice cultivars could form stable emulsions even at neutral pH. The information obtained in this study may be useful for development of emulsion-based food products using rice protein isolate.

Keywords Rice protein · Emulsifying property · Oil-in-water emulsion · Solubility · Waxy-rice cultivar

Introduction

Rice is a staple food in Asian countries and is recognized as the most important crop in the region. However, the consumption of rice grain in these countries is decreasing; thus, there have been efforts to increase its added value. Since the two major components of rice grain are starch and protein, the potential of the effective utilization of rice protein should be considered for adding value. Rice proteins isolated and characterized in terms of their solubility properties using Osborne extraction method (Marshall and Wadsworth 1994; Padhye and Salunkhe 1979) consist of four components: albumin (water-soluble, ~5 %), globulin (salt-soluble, ~12 %), glutelin (alkali-soluble, ~80 %), and prolamin (alcohol-soluble, ~3 %) (Juliano 1994; Romero et al. 2012). Rice proteins are considered valuable because it is highly nutritious, hypoallergenic, colorless, of bland taste, and hypocholesterolemic (Chandi and Sogi 2007; Ju et al. 2001; Pinciroli et al. 2009). The protein content of rice grain varies with rice variety, generally from 4.9 to 15 %, and the composition of the protein varies (Yi et al. 2014). For example, previous studies reported that the difference in globulin content among varieties could be as high as more than fivefold (Teshima et al. 2010). Although rice protein is a healthy protein source for human consumption due to its highly nutritional properties and functionalities (hypoallergenic and hypocholesterolemic), it has limited application as a functional ingredient in food formulation due to its insolubility and high molecular weight (Paraman et al. 2007a, b; Romero et al. 2012). For these reasons, recent studies have been aimed at modifying the physicochemical and functional properties of rice proteins to alter their inherent structural properties which tend to reduce protein molecular flexibility and lead to low solubility and surface-active properties (Paraman et al. 2007a, b).

Proteins have received much attention in particular as emulsifiers, due to their ability to adsorb at oil-water interfaces and to form interfacial films (Amine et al. 2014). The surface activity of proteins is due to their amphiphilic nature caused by the presence of both hydrophilic and hydrophobic groups

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in their molecular structure (Amine et al. 2014; Singh 2011). Proteins commonly used as emulsifiers include milk proteins, such as whey protein and casein. If rice proteins can form a stable emulsion similar to the emulsion stabilized by milk protein, this would provide a valuable alternative emulsifier obtainable from a natural source, which could potentially enhance the added value of rice. However, as it has been recognized, rice proteins that are not soluble in aqueous systems and have high amount of disulfide bonding are known to be poor emulsifiers. To our knowledge, the comparative study on the emulsifying performance of proteins isolated from different rice cultivars has not been conducted yet.

Therefore, the purposes of this study were to examine and compare the emulsifying properties of rice proteins isolated from various non-waxy and waxy rice cultivars. Through the screening of the emulsifying ability of various rice protein isolates, proper rice cultivars and emulsifying conditions were suggested.

Materials and Methods

Materials

Four varieties of non-waxy rice (Boramchan, Hanareum, Nampyeong, and Goami) and seven of waxy rice (Baekokchal, Hangangchal, Dongjinchal, Hwaseonchal, Boseokchal, Baekseolchal, Sinseonchal) were provided by the National Institute in Crop Science, Rural Development Administration (Iksan, South Korea). The amylose content was as follows: Boramchan, 16.8 %; Hanareum, 18.9 %; Nampyeon, 24.2 %; and Goami, 31.6 %. The amylose content of all waxy rice varieties was less than 1 % (Williams et al. 1971). Whey protein isolate (WPI, Product code: 9500) was obtained from Protient Inc. (St. Paul, MN, USA). HCl and NaOH were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium phosphate (dibasic, anhydrous) and sodium phosphate (monobasic, anhydrous) were purchased from Showa Chemical Co. (Tokyo, Japan). Soybean oil was purchased from a local supermarket and used without further purification.

Methods

Preparation of Protein Isolates

Protein was isolated from white rice grains using 0.2 % NaOH solution. White rice grains were soaked in water for 4 h, and then 0.2 % NaOH solution was added after removing water. After white rice grains were soaked in 0.2 % NaOH for 1 h, rice grains with alkaline solution were ground by Food mixer (Type HR2096, Main Power Industrial Co. Ltd. China) and passed through 100 mesh (Aperture 150 μm, Wire diameter 100 μm, Chung Gye Sang Gong Sa, Seoul, Korea) and 270 mesh sieve (Aperture 53 μm, Wire diameter 36 μm) step by step. The starch slurry in alkaline solution was centrifuged (1646 *g*, 10 min), and all supernatants were collected in beaker and centrifuged again to remove debris. The pH of collected supernatant was adjusted to pH 4.5 to precipitate rice protein, and the precipitate was recovered by centrifugation, washed with deionized water, adjusted to pH 7.0, and freeze-dried. Dried protein was ground and passed through 100 mesh sieve.

Determination of Protein Solubility at Various pH Values

Aqueous protein isolate dispersions (1 wt%) were prepared by dispersing protein isolate in 5-mM phosphate buffer (pH 7.0). To investigate the effect of pH on solubility, the buffer pH was adjusted with 3 N NaOH and 6 N HCl to various pH values (pH 2, pH 3, pH 4, pH 6, pH 7, pH 8, pH 9, and pH 10) before preparation of protein isolate dispersions.

Prior to analysis, the dispersions were stirred at room temperature for 1 h, then centrifuged at 926 *g* for 10 min at room temperature. The protein solubility was taken as the protein content of the supernatants as a percentage of the total protein content of the isolate (Pinciroli et al. 2009). Protein content of the supernatants was determined by the Lowry assay (Lowry et al. 1951).

Emulsion Preparation

A surfactant solution was prepared by dispersing protein isolate (0.6 wt% protein isolate) in 5-mM phosphate buffer containing 0.02 wt% NaN_3 (as an antibacterial agent) at various pH values (pH 2, pH 7, and pH 10). The protein isolate dispersions were stirred at room temperature for 1 h, then centrifuged at 926*g* for 10 min at room temperature to remove the undissolved portion.

An oil-in-water emulsion was prepared by sonicating 3 wt% soybean oil and 97 wt% aqueous protein solution. Before sonication, the oil was dispersed gradually into aqueous protein solutions under agitation using a magnetic stirrer and pre-homogenized using a high speed blender (ULTRA-TURRAX model T25 digital, IKA, Germany) for 2 min. After pre-homogenization, the coarse emulsions were sonicated for 1 min 30 s at a frequency of 20 kHz, amplitude of 40 %, and duty cycle of 0.5 s (VCX 750, Sonics & Materials, Inc., Newtown, CT). Whey protein isolate (WPI) was also used as a standard emulsifier to produce emulsions and to compare its ability to form an emulsion with those of the rice proteins.

Emulsion Characterization

The mean particle diameter and particle size distribution of the emulsions were determined by laser diffraction using a particle size analyzer (Mastersizer 3000, Malvern instruments Ltd.,

UK) equipped with a helium-neon (He-Ne) laser. The samples were diluted in 5-mM phosphate buffer to avoid multiple scattering effects. The particle size was reported as either the surface-weight mean diameter ($d_{4,3}$) or the volume-weighted mean diameter ($d_{3,2}$) (McClements 2005). The electrical charge (ζ-potential) of samples was measured by Electrophoretic Light Scattering Spectrophotometer (ELS-8000, Otsuka Electronics Co., Ltd.) to determine the surface charge at the interface of the droplets. Samples were diluted with buffer solution and placed in a cell equipped with two electrodes to assess the electrophoretic mobility of the particles. The microstructures of the emulsions were observed under a Carl Zeiss microscope (Axio Imager A1, Göttingen, Germany). The emulsions were gently agitated in a glass test tube before analysis to ensure their homogeneity. One drop of emulsion was placed on a microscope slide and covered with a cover slip.

Storage Stability

Ten grams of each emulsion sample in a test tube (internal diameter 15 mm, height 125 mm) tightly sealed with a plastic cap was stored for 1 month at room temperature. After storage, the general appearances of emulsions were observed, and particle size distributions of emulsions prepared at pH 2 were examined using a particle size analyzer (Mastersizer 3000, Malvern instruments Ltd., UK) equipped with a helium-neon (He-Ne) laser.

Statistical Analysis

All data were recorded as mean ± standard deviation and analyzed by SPSS for Windows (version 21.0; SPSS Inc., Chicago, IL, USA). The one-way ANOVA test followed by a Duncan's multiple range tests was performed to identify statistical significances. A p value <0.05 was considered to be statistically significant.

Results and Discussion

pH Solubility

Figure 1 shows the solubility of rice proteins from various rice cultivars as a function of pH. The rice proteins exhibited very low solubility across a wide to moderate pH range, but the solubility was higher in acidic (pH <3.0) and alkaline (pH >8.0) solutions. The minimum solubility, which corresponded to their isoelectric point, was detected around pH 6. Below pH 4, the solubility of the rice proteins increased rapidly and was the highest at pH 2. At pH values >7, solubility increased slowly; however, the increase was small compared to that measured at pH 2 and 3.

It has been reported that the dependence on pH of rice protein solubility is governed by the properties of glutelin, being its most abundant component (Agboola et al. 2005; Hamada 2000). Glutelin has poor solubility due to marked aggregation mediated mainly through its extensive disulfide cross-linking (Agboola et al. 2005; Hamada 2000).

Although the trend of change in solubility with pH was similar in all rice proteins, the proteins exhibited some differences in their solubility at acidic and alkaline pH values. There were also differences between cultivars. Among the non-waxy rice cultivars, the protein isolated from Goami rice displayed the highest solubility at pH 2 and that from Nampyeongbyeo rice the lowest solubility. At alkaline pH, proteins isolated from waxy rice cultivars generally exhibited higher solubility compared to those from non-waxy rice cultivars. Difference in rice protein solubility might be attributed to the inherent variation in protein composition and molecular structure of different rice cultivars, especially for glutelin polypeptides that might have varying degrees of polymerization (Katsube-Tanaka et al. 2004; Pinciroli et al. 2009).

Characteristics of Emulsions Stabilized by Different Rice Proteins

Particle Size The emulsifying properties of 11 types of rice proteins were investigated under different pH conditions. Soybean oil in water emulsions were prepared with rice proteins extracted from four non-waxy and seven waxy rice cultivars at pH 2, 7, and 10, and the mean oil droplet diameter ($d_{3,2}$ and $d_{4,3}$) of emulsions was measured (Fig. 2). The $d_{3,2}$ values of the rice protein-coated emulsions prepared at pH 2 ranged from ~1.7–2.3 μm, and the $d_{3,2}$ values of emulsions prepared at pH 10 were similar to those of emulsions prepared at pH 2.

The most commonly used sources of proteins in preparing food emulsions are derived from milk, such as whey proteins and caseins. To compare the emulsifying properties of rice proteins with those of milk proteins commonly used in emulsion production, a WPI-coated emulsion was prepared, and the particle sizes at different pH values were compared to those of the rice protein-coated emulsions. The $d_{3,2}$ of the WPI-coated emulsion at pH 2 and 10 was 1.71 and 1.79 μm, respectively. These values were similar to those of the rice protein-coated emulsions. This result indicated that rice proteins could form emulsion droplets of a similarly small size to that of WPI-coated emulsion droplets at pH 2 and 10, at which protein solubility was relatively high. Higher solubility indicated more soluble protein molecules existing in an aqueous phase, which consequently implied that there might be sufficient protein molecules to saturate the surface of oil droplets of emulsions. In addition to higher solubility, the charge of protein molecules at pH 2 and 10 is bigger than that measured at pH 7 because pH 2 and 10 are far from isoelectric point of

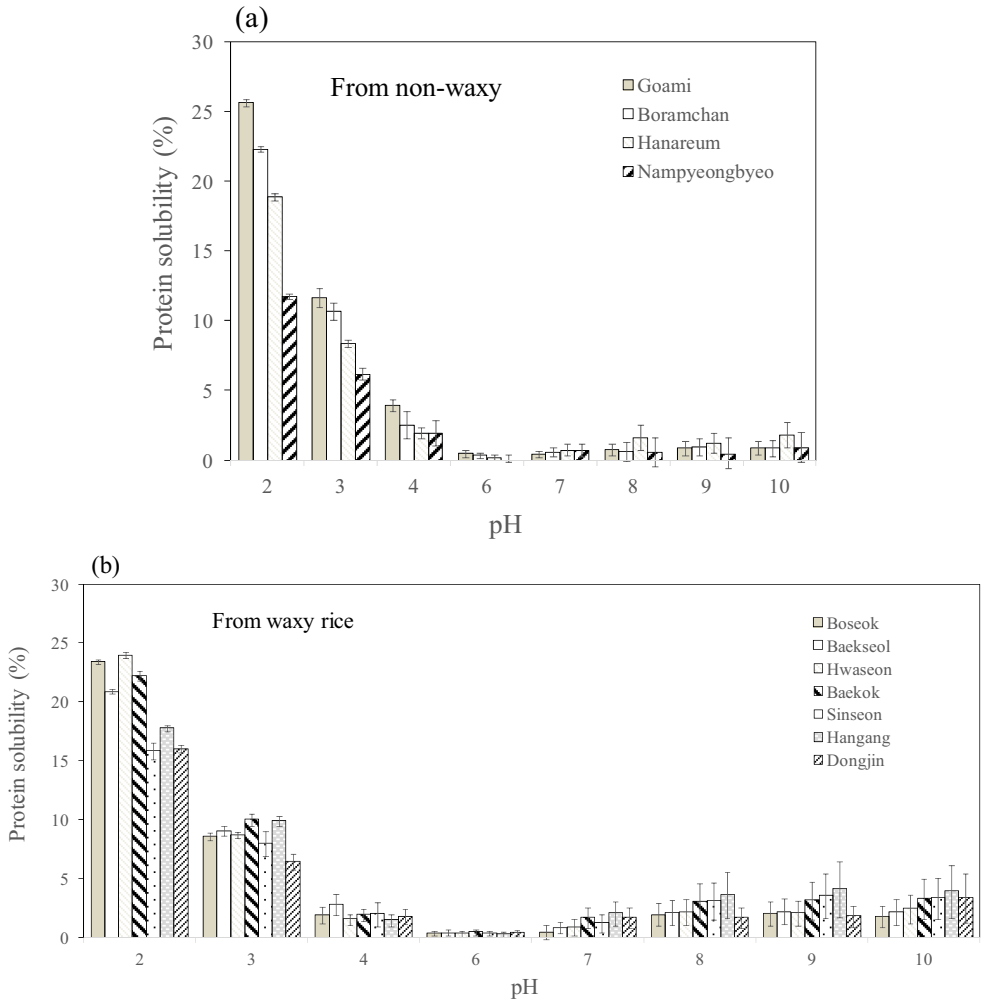

Fig. 1 Solubility of rice proteins isolated from various rice cultivars **a** non-waxy rice, **b** waxy rice at different pH values

rice protein. Hence, the increase of the electrostatic repulsion between the oil droplets would be expected.

However, emulsions prepared with proteins isolated from non-waxy rice at pH 7 had a larger $d_{3,2}$ than those obtained from emulsions prepared at pH 2 and 10 as expected. This result was attributed to the fact that the isoelectric point of rice protein was near pH 7. At pH 7, the charge of protein molecules surrounding the oil droplets was not sufficient to make a strong electrostatic repulsion between oil droplets, resulting that unstable emulsion (having big droplets) formed.

In the case of emulsions stabilized with rice proteins extracted from waxy rice cultivars, (Bakokchal, Hangangchal, Sinseonchal), the $d_{3,2}$ values of some of the emulsions did not differ from those obtained from emulsions prepared at pH 2 and 10, while the $d_{3,2}$ values of the remaining emulsions (Hwaseonchal, Boseokchal, Baekseolchal, Dongjinchal) were higher than those of emulsions prepared at other pH values. However, the difference in these $d_{3,2}$ values was small compared with the difference in $d_{3,2}$ of emulsions prepared with non-waxy rice protein between pH 2 and pH 7.

A positive correlation between the solubility of a protein and its ability to form an emulsion has been reported in previous studies (Cao et al. 2009; Pinciroli et al. 2009; Voutsinas et al. 1983). Our results also indicated that the solubility of a protein is related to its emulsifying properties. Nevertheless, the ability of the rice proteins to form an emulsion could not be due only to their solubility, and other authors have reported that emulsifying properties and solubility are not always well correlated (Voutsinas et al. 1983).

Despite the fact that the solubility of proteins extracted from waxy cultivars at pH 7 was higher than that of proteins extracted from non-waxy rice and the solubility at pH 7 was lower than that at pH 2 and 10, the emulsions stabilized by the Baekokchal, Hangangchal, and Sinseonchal rice proteins showed similar $d_{3,2}$ values regardless of pH, which indicates that other factors affect the emulsifying properties. One possible factor related to the ability of rice proteins to form an emulsion is the properties of the individual protein, such as the ζ-potential.

The electrical charges of two proteins extracted from Boseokchal (waxy) rice and Hanareum (non-waxy) rice at various pH values are shown in Fig. 3. At pH 2 and 3, the ζ-potentials of Boseokchal protein were highly positive, and the isoelectric point (pI) was measured at around pH 4, whereas

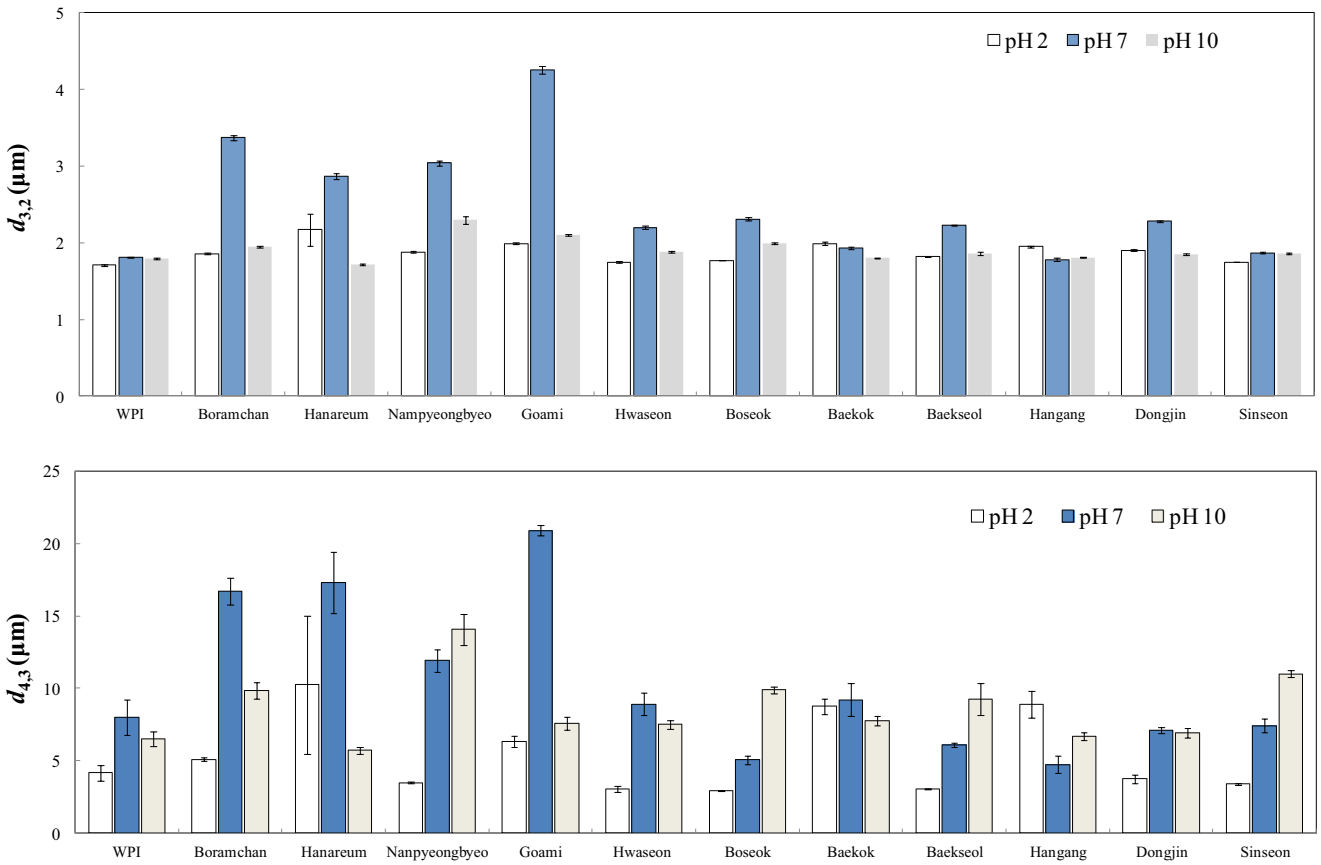

Fig. 2 Effect of pH on mean particle diameter ($d_{3,2}$ and $d_{4,3}$) of emulsion stabilized by rice proteins isolated from various rice cultivars

Hanareum protein had a lower ζ-potential at pH 2 and 3 than Boseokchal protein, and the pI was detected at around pH6. At pH 7, the ζ-potential of Boseokchal protein was highly negative because this pH lies significantly above the pI. However, the ζ-potential of Hanareum protein was low (= −2.5 mV) at pH 7. The proteins isolated from waxy rice had greater electrical charge than those isolated from non-waxy rice cultivars at pH 7 and had similar electrical charge to that at pH 10 (Fig. 3), indicating that the electrostatic repulsions among protein chains were stronger in waxy rice than non-waxy rice. Proteins are complex molecules comprising various amino acids, which have different pKa values, and are responsible for the charge at different pH values. Hence, the difference in charge between two rice cultivars at pH 7 might be due to the different amino acid compositions of proteins from the two cultivars.

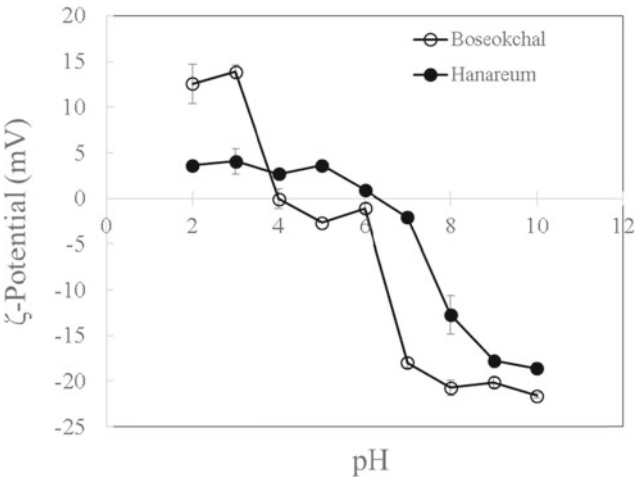

Fig. 3 Dependence of electrical charge of emulsion stabilized by rice proteins isolated from Boseokchal and Hanareum (ζ-potential) on pH

This result suggests that the emulsifying properties depend not only on the solubility but also other properties of the particular protein, for example, the amino acid composition, and the hydrophilic-liphophilic balance which derives from the amino acid composition (Voutsinas et al. 1983; Nakai 1983; Katsube-Tanaka et al. 2004; Pinciroli et al. 2009). Previous study reported that the hydrophilic-liphophilic balance of a protein also affected its emulsifying properties, in addition to the solubility (Nakai 1983).

A similar tendency was observed in the volume-length diameter, $d_{4,3}$, values of the emulsions. The $d_{4,3}$ is the sum of the volume ratio of the droplets in each size class multiplied by the mid-point diameter of the size-class. The $d_{4,3}$ is more sensitive to the presence of large particles in an emulsion than the $d_{3,2}$, and thus the $d_{4,3}$ represents emulsion stability more sensitively. The emulsions prepared with the Boramchan,

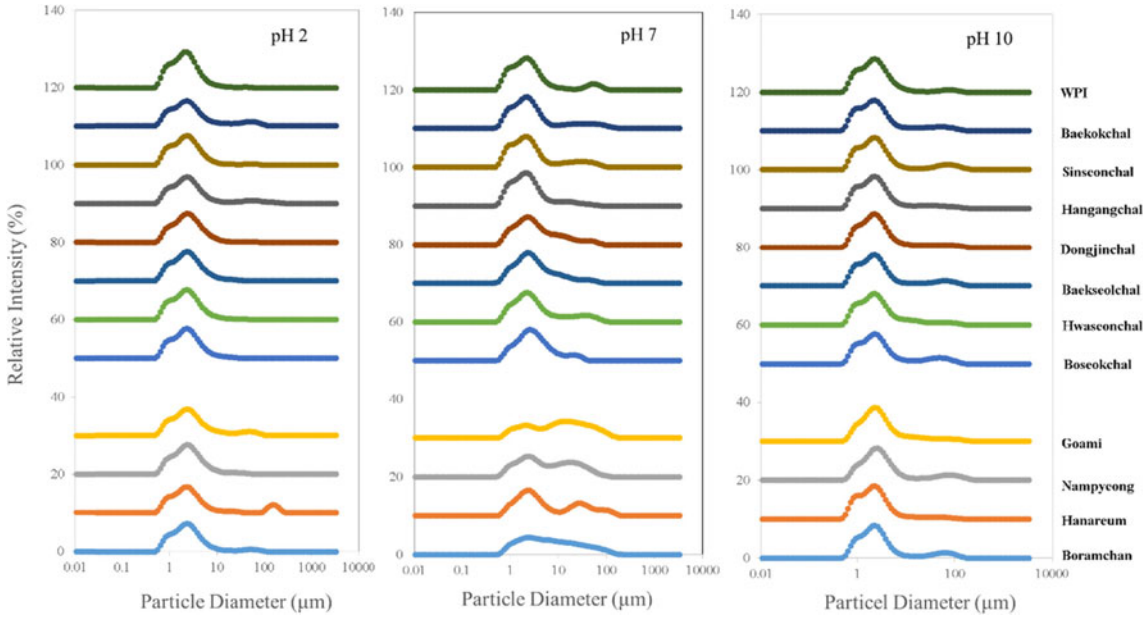

Fig. 4 Effect of pH on particle size distribution of emulsion stabilized by rice proteins extracted from various rice cultivars

Nampyeong Dongjinchal, Hwaseonchal, Boseokchal, Baekseolchal, and Sinseonchal proteins had smaller value of $d_{4,3}$ than those with other rice proteins at pH 2 ($p<0.05$), indicating that proteins isolated from these rice cultivars were better emulsifiers at pH 2. The emulsions prepared with the Hangangchal, Dongjinchal, Boseokchal, Baekseolchal, and Sinseonchal proteins showed smaller value of $d_{4,3}$ at pH 7, and the emulsions prepared with the Hanareum, Hangangchal, Hwaseonchal, and Dongjinchal proteins had smaller value of $d_{4,3}$ at pH 10 than others ($p<0.05$). Generally, the emulsions stabilized with proteins extracted from non-waxy rice cultivars displayed higher $d_{4,3}$ values at pH 7 than those prepared at other pH values and with proteins extracted from waxy rice cultivars, and rice proteins extracted from waxy rice cultivars were better emulsifiers over a wide range of pH values.

In conclusion, proteins extracted from waxy rice cultivars might have structural properties and compositions different from those of proteins extracted from non-waxy rice cultivars, which resulted in higher solubility, ζ-potential, and emulsifying capacity for waxy rice proteins over the non-waxy proteins. However, further studies are needed to clarify the

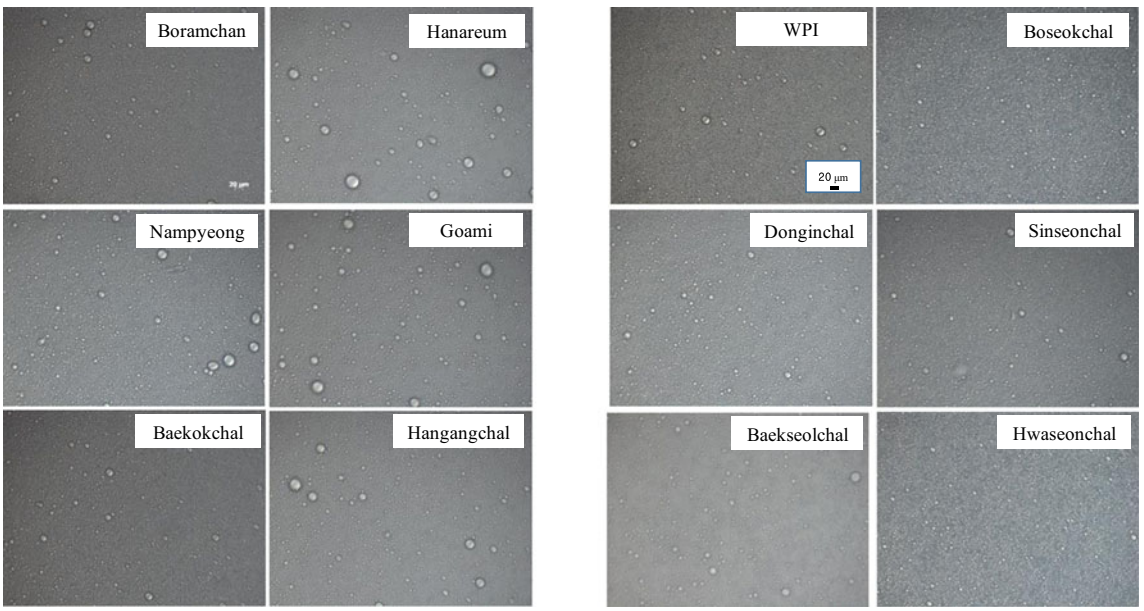

Fig. 5 Optical microscopy image of emulsions stabilized by rice proteins extracted from various rice cultivars (pH 2)

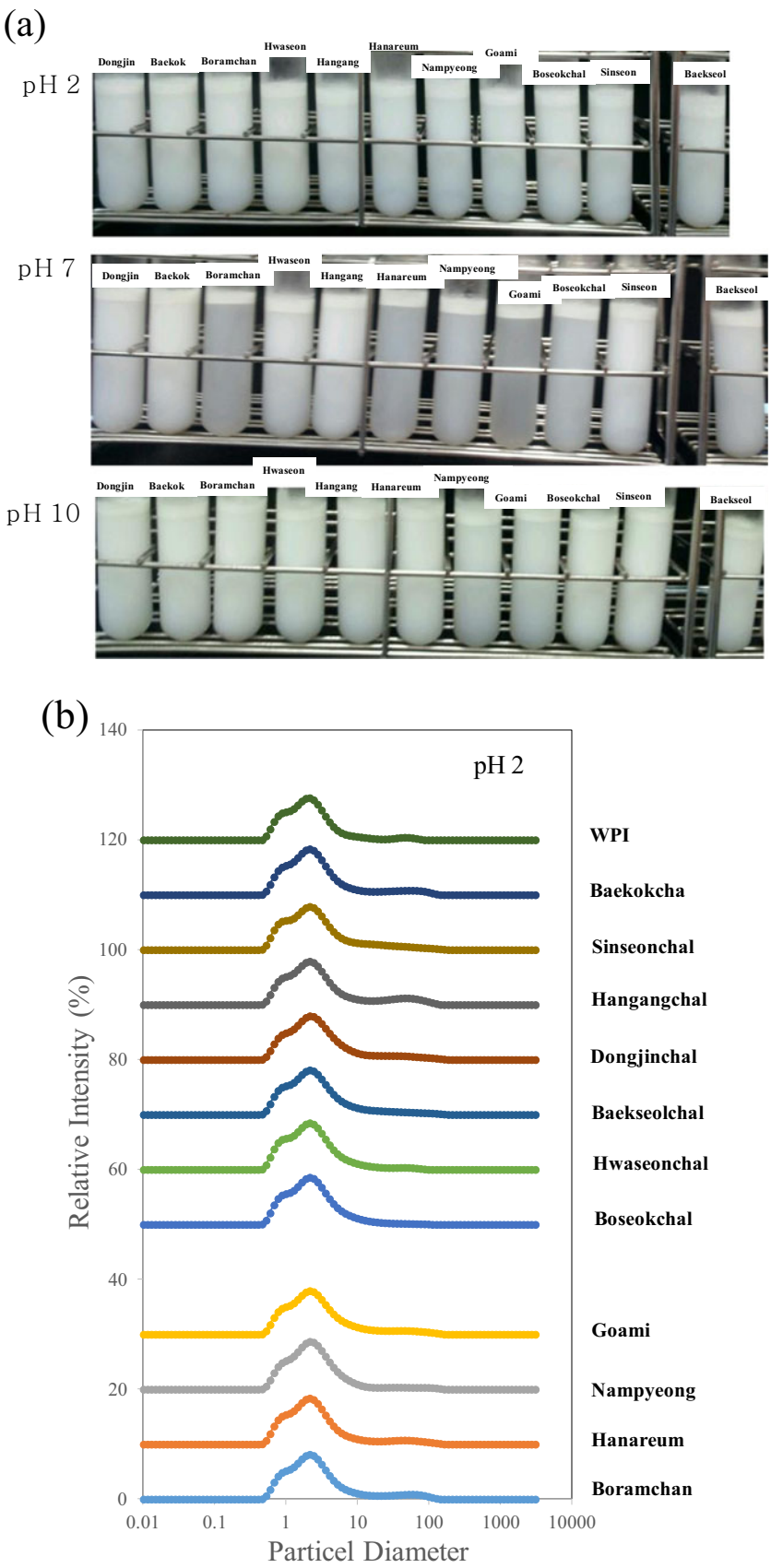

Fig. 6 Appearance of stored rice protein-coated emulsions prepared in different pHs for 1 month (**a**) and particle size distribution of stored rice protein-coated emulsions for 1 month at pH 2 (**b**)

reasons why rice proteins extracted from waxy rice cultivars showed better emulsifying properties than those from non-waxy rice cultivar.

Particle Size Distribution (PSD) Figure 4 shows the particle size distribution (PSD) profiles of emulsions stabilized with rice proteins at pH 2, 7, and 10. Most particles belonged to the peak located at 0.5–10 μm, and a small proportion of relatively large-size particles was observed. The fraction of large-size particles differed depending on the type of rice protein and pH condition; e.g., the fraction of large-size particles was high in emulsions stabilized with proteins obtained from Hanareum and Goami rice at pH 2. Figure 5 shows the microstructures of rice protein-coated emulsions prepared at pH 2. The majority of the particles in these emulsions stabilized using rice proteins were relatively small ($d_{3,2} < 10$ μm), and the microstructure of the emulsions did not differ from that of the WPI-stabilized emulsion. Nevertheless, some large particles were observed in several of the emulsions, such as those stabilized by the Hanarem, Nampyeing, Goami, and Hangangchal rice proteins. These microstructure patterns are in close agreement with the PSD results.

Emulsions freshly prepared in our study displayed a particle size distribution with a small population of relatively large droplets. A sonicator was used to produce the rice protein-coated emulsions, and it proved difficult to prepare emulsions with a monomodal particle distribution. The PSDs obtained from emulsions prepared at pH 7 displayed different peak distributions, especially those stabilized by rice proteins extracted from non-waxy rice cultivars (Fig. 4). The peak in the PSD of the non-waxy rice protein-coated emulsions was broader at pH 7, while the waxy rice protein-coated emulsions produced the first peak at ~0.5–10 μm, and the proportion of larger-size particles was higher compared to those from emulsions prepared at pH 2, indicating that the emulsions became unstable. This result suggests that rice proteins extracted from waxy rice cultivars are better emulsifiers than those extracted from non-waxy rice cultivars, possibly due to differences in their structural properties and compositions.

Storage Effect The general appearance of the emulsions was examined after 1 month of storage at room temperature (Fig. 6). At pH 2 and 10, all emulsions maintained a milky-white liquid appearance and did not exhibit creaming instability after 1 month. However, at pH 7, the emulsion stabilized by non-waxy rice proteins was a transparent liquid, suggestive of instability. The waxy rice protein-coated emulsions were whiter than the non-waxy rice protein-coated emulsions. This is consistent with the particle characteristics and PSD data.

The PSD of emulsions prepared at pH 2, at which the rice proteins displayed their highest solubility, and subsequently stored for 1 month, is presented in Fig. 6. The PSD of emulsions stored for 1 month did not differ from that of freshly prepared emulsions, suggesting that rice protein could be a valuable natural ingredient for use as an emulsifier.

Conclusions

The solubility of rice proteins isolated from various non-waxy and waxy rice cultivars differed depending on the pH, i.e., higher in acid (pH 2 and pH 3) and alkali solutions (pH 10) and lower at around neutral pH (pH 6 and pH 7). The solubility of the rice proteins was related to their ability to form emulsions; however, the solubility was not completely related with the emulsifying property of the rice proteins. The magnitude of charge of the rice proteins at various pH values was also related to their ability to form emulsions. The waxy rice proteins had a more negative charge than the non-waxy rice proteins at pH 7, possibly due to the differences in amino acid composition. The $d_{3,2}$ of the rice protein-coated emulsion at pH 2 and 10 was similar to those of the WPI protein-coated emulsions, and all emulsions did not exhibit creaming instability after storage for 1 month. The PSD of rice protein-coated emulsions prepared at pH 2 and subsequently stored for 1 month did not differ from that of freshly prepared emulsions. The results demonstrated that rice proteins isolated from non-waxy and waxy rice could be used as a suitable emulsifier at pH values at which their solubility was high (e.g., pH 2 and pH 10). Moreover, over a wide range of pH values, the rice proteins isolated from waxy rice cultivars were better emulsifiers than non-waxy rice proteins.

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