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Research Article

Influence of amylose and endosperm proteins on air cell distribution in rice cracker

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Abstract

This study investigated the roles of amylose and endosperm proteins in rice flour and waxy rice flour on rapid visco-analyser (RVA) pasting profiles, microstructure and oil uptake of rice crackers. Reducing amylose content in rice sheets by raising waxy rice flour to rice flour ratio increased the volume of cracker, the size of air cell and the oil uptake during frying (P<0.05). Confocal laser scanning microscopy revealed that starch and protein fractions existed in a bi-continuous phase in dried rice sheet prior to frying. Removal of more than 50% rice endosperm protein by bromelain hydrolysis allowed the gelatinised flour matrix in rice sheet to expand and had large air cell size (P<0.05). SDS-PAGE indicated that rice flour and waxy rice flour had different molecular weight profiles of oryzenin proteins. The disappearance of some polypeptides enhanced the expansion of the flour matrix, subsequent large air cell size and increased oil uptake of rice cracker during frying.

Keywords: confectionery, pasting profile, oil uptake, frying, oryzenin protein, starch, Thailand.

Introduction

Rice is one of the world's staple food sources and is recognised as the most important crop in Asia. The functional properties of rice grains, such as cooking characteristics, pasting profiles, gelation and water-holding capacity, are important factors in determining the end usage and markets for rice grain and flour. Although the eating qualities of table rice and the processing qualities of rice flours are mainly governed by the starch component in the grains, rice endosperm proteins also play a role in determining rice qualities [1]. Ramesh *et al.* [1] showed that the protein fractions in rice affected the texture of cooked rice to some degree, particularly the tenderness and cohesiveness.

Protein in rice is mainly located in the endosperm. The functional properties of rice grains and flours are determined by the glutelin fraction, called oryzenin, through disulphide bonds [2, 3, 4, 5, 6, 7, 8, 9]. The formation of disulphide bonds restricts the expansion of the starch granules during gelatinisation [10]. Increasing or decreasing the disulphide bond content was reported to alter the pasting properties and textural structure of rice grain and flour [9, 10, 11]. It was also recently

shown that the endosperm proteins from waxy rice could act as a binder for composite flours during pasting [12].

Gelatinisation, retrogradation and pasting characteristics of starch are mostly explained by the ratio of amylose and amylopectin, the degree of branching of amylopectin and the degree of polymerisation of both amylose and amylopectin [13]. However, little attention has been paid to the roles of protein, in relation to amylose, on the structure-forming process of starch/flour-based formulations during and after heating. This study thus aimed to further investigate the roles of endosperm proteins - after the rice flour was gelatinised, cooled and dried, on the formation of solid foam structure by frying.

The solid foam structure is composed of small globules of air or gas dispersed in a solid continuous phase [14, 15]. This structure is found in snack and breakfast cereal, which can be produced by frying, rapid dry heating and expansion extrusion [15]. The most popular and economical way to prepare solid foam in food is a frying process. During frying, sudden vaporization of water and internal steam is formed rapidly, causing the product to puff, which leads to the formation of a low-density, porous, thin-walled solid foam structure [14, 15]. In South East Asia, this kind of solid foam structure fabricated by frying, which is made from cassava or sago starches mixed with fish, is called '*keropok*' in Indonesia and Malaysia. In Thailand, it is called '*kao krieb*' and usually made from rice flour or cassava starch with and without protein addition.

We hypothesized that the size of air cell in fried rice crackers were controlled by the viscoelastic properties of starch-protein matrix prior to frying. Thus regulating the amylose and protein contents could alter the properties of the gelatinised and dehydrated matrix during water vaporisation. Greater understanding of the role of flour composition on the expansion of gelatinised rice flour matrix and the size distribution of air cell, as well as oil uptake, may help designing the characters of flour-based solid foam structure.

Materials and Methods

Materials

Commercial native-protein rice flour (NPRF, 29.8% amylose content) and native-protein waxy rice flour (NPWF, 4.1% amylose content) (Erawan, Cho Heng Rice Vermicelli Factory Co., Nakorn Pathom, Thailand) and rice bran oil (King, Thai Edible Oil Co., Bangkok, Thailand) were purchased from a local supermarket. The flours were proximately analysed for moisture, protein, lipid and ash contents [16]. Amylose content in NPRF and NPWF were determined by the method described by Chrastil [17] using potato amylose as standard. Bromelain (EC 3.4.22.32) and sodium azide were from Sigma-Aldrich, Inc., Saint Louis, Missouri, USA.

De-proteinisation of rice endosperm protein

Low-protein rice flour (LPRF) and low-protein waxy rice flour (LPWF) were prepared by hydrolysing the proteins in flour using bromelain solution (1% w/v) at 30°C for 19 h in the presence of 0.02% sodium azide. The hydrolysis was carried out using flour suspension having flour to enzyme solution ratio of 1:10 at pH 6.5. After incubation, the suspension was centrifuged at 5000 rpm at 20°C for 15 min. The supernatant was discarded and the samples were washed with distilled water, centrifuged and washed again with distilled water. The washing process was carried out 5 times. The low-protein flours were dried at 40°C for 24 h and proximately analyzed using the methods as described above.

Preparation of rice cracker

Rice sheet samples made from different flours were prepared by dispersing flour in drinking water to obtain 40% w/w slurries, poured onto a tray to acquire the slurries having thickness of 2 mm, steamed for 3 min, cooled down at room temperature (25°C) and frozen at –20°C for 14 h. The retrograded sheets were cut into squares with the dimensions of 10×10×2 mm (width x length x thickness), dried at 45°C for 3 h to obtain moisture content of 11% (wb) in a tray-dryer and deep-fat fried in rice bran oil at 170°C for 15 s to produce a rice cracker with final moisture content of 5%. The oil in rice crackers was drained by placing the fried rice crackers on the screen for 1 min, oil-sorbed using blotting paper for 1 min and centrifuged at 1166 rpm for 5 min to remove excess oil.

Rice crackers were analyzed for moisture and fat contents [16] and calculated for the apparent volume from the dimensions of 10 samples and averaged. The microstructure of crackers was determined by stereomicroscope (Leica S8APO, Leica Microsystems, Wetzlar, Germany) equipped with digital image program (Dewinter Software, New Delhi, India) to calculate size distribution of air cells. The diameter of 10 air cells per field was measured. The data on the size of air cell were collected in 10 fields for each treatment. All data were interpreted into histogram of size distribution of air cell.

Distribution of protein fraction in rice sheet was characterised under a confocal laser scanning microscope (CLSM; Axio Imager MI, Carl Zeiss Pte, Germany). The protein fractions in samples were located using rhodamine B as a fluorescent-labelling dye [9].

Pasting characteristics of rice flour

The pasting characteristics of NPRF, NPWF, LPRF and LPWF were determined by a Rapid Visco-analyser (RVA) (Newport Scientific; Warriewood, Australia) using the AACC Approved Method 61-02 [18]. Amylograms describing pasting characteristics included: pasting temperature (increased temperature of the initial viscosity); peak viscosity (the maximum viscosity developed soon after the heating cycle ended); peak time (time required to reach peak); holding strength (minimum viscosity after peak viscosity obtained during holding at 95°C for 2.5 min); and final viscosity (viscosity after cooling at 50°C for 2 min).

Characterisation of rice storage proteins

The MW of proteins in NPRF, NPWF, LPRF and LPWF were characterized by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) using a Bio-Rad Mini-Protean II cell (Bio-Rad Laboratories; Hercules, CA, USA) using the method described by Laemmli [19]. Proteins were extracted from flours by suspending the flours in sample buffer containing 12.5% of 0.5 M Tris-HCl (pH 6.8), 10% glycerol, 2% SDS, 5% β-mercaptoethanol (β-ME) and 1% (w/v) bromophenol blue. The suspensions were boiled for 20 min with intermittent vortex mixing and centrifuged at 5,000 rpm (Labnet Spectrafuge 16M, Labnet International Inc.; Woodbridge, New Jersey, USA) for 5 min to remove insoluble material. A sample containing 10 μg of protein was loaded into each well.

The separation was performed at a constant voltage at 150 V for 1 h with a stacking gel containing 4% acrylamide and a running gel containing 15% acrylamide concentration. Gel slabs were fixed and stained simultaneously using Bio-Rad Coomassie blue R-250 stain solution (40% methanol, 10% acetic acid, 0.1% Coomassie blue R-250) for 30 min and de-stained by Bio-Rad Coomassie blue R-250 de-stain solution for 5 h with two or three changes of de-stain solution. The MW of each band was determined by using full-range rainbow molecular weight markers, MW~10-250 kDa (RPN 8000, Amersham Biosciences UK Ltd.; Buckinghamshire, UK), as the MW standards.

Statistical analysis

The experiments were carried out in three separate trials and each trial was analyzed in triplicate. The data were analysed by Analysis of Variance (ANOVA) with significance at P<0.05. Significant differences among mean values were determined by Duncan's multiple range test. All statistical analyses were performed using the SPSS software version 12 (SPSS Inc., Chicago, Illinois, USA) at 95% confidence level.

Results and Discussion

The native-protein rice flour (NPRF) and native-protein waxy rice flour (NPWF) contained 7.69 and 6.39% protein, respectively (Table 1). De-proteinisation of flour using bromelain reduced the protein in NPRF to 2.90% and 2.89% protein, respectively (P<0.05). The de-proteinised flours had lower lipid and ash contents (P<0.05), possibly due to the looser structure of protein matrix and protein bodies in flour and washing processes employed after enzyme hydrolysis.

Table 1. Composition of rice flour and waxy rice flour (% wb).

NPRF = native-protein rice flour; NPWF = native-protein waxy rice flour; LPRF = low-protein rice flour; and LPWF = low-protein waxy rice flour.

Flour type	Moisture	Protein	Lipid	Ash
NPRF	$12.22^{a} \pm 0.16$	$7.69^{a} \pm 0.12$	$0.24^{a} \pm 0.04$	$0.25^{a} \pm 0.01$
LPRF	$12.03^{a} \pm 0.17$	$2.90^{c} \pm 0.19$	$0.09^{b} \pm 0.04$	$0.16^{c} \pm 0.01$
NPWF	$12.38^{a} \pm 0.14$	$6.39^{b} \pm 0.15$	$0.29^{a} \pm 0.03$	$0.20^{b} \pm 0.00$
LPWF	$11.53^{a} \pm 1.07$	$2.89^{c} \pm 0.06$	$0.07^{b} \pm 0.05$	$0.08^{d} \pm 0.01$

Means in the same column followed by different superscript are significantly different (P<0.05).

The low-protein flours had different RVA pasting profiles compared to the native-protein ones (Table 2). Lowering the protein content by bromelain hydrolysis shortened peak time, increased peak viscosity and lowered final viscosity of LPRF compared to those of NPRF (P<0.05). After being hydrolysed by bromelain, LPWF had lower peak viscosity, holding strength and final viscosity compared to NPWF (P<0.05).

Table 2. RVA pasting profiles of rice flour and waxy price flour with different protein content.

NPRF = native-protein rice flour; LPRF = low-protein rice flour; NPWF = native-protein waxy rice flour; and LPWF = low-protein waxy rice flour.

Flour type	RVA pasting parameters				
	Pasting temperature (°C)	Peak time (min)	Peak viscosity (mPa.s)	Holding strength (mPa.s)	Final viscosity (mPa.s)
NPRF	$50.17^{a} \pm 0.05$	$6.50^{a} \pm 0.00$	$3240.3^{c} \pm 85.2$	$2495.5^{a} \pm 89.8$	$5034.5^{a} \pm 43.8$
LPRF	$50.40^{a} \pm 0.28$	$6.30^{b} \pm 0.04$	$3492.3^{b} \pm 74.6$	$2469.0^{a} \pm 1.4$	$4582.5^{b} \pm 108.2$
NPWF	$53.21^{a} \pm 3.64$	$3.73^{c} \pm 0.00$	$4857.8^{a} \pm 34.3$	$2551.5^{a} \pm 31.8$	$3094.0^{c} \pm 50.9$
LPWF	$50.67^{a} \pm 0.62$	$3.69^{c} \pm 0.02$	$3654.3^{b} \pm 7.4$	$1897.8^{b} \pm 13.1$	$2173.0^{d} \pm 26.9$

Means in the same column followed by different superscript are significantly different (P<0.05).

These results on the effect of bromelain on rice flour corroborated a previous report by Derycke, *et al.* [11] on the effect of protease on RVA pasting parameters of non-waxy rice flour. The increase in peak viscosity of LPRF compared to the NPRF was likely due to the contribution of leached amylose. In addition, the granules in LPRF could also swell to a greater degree than the corresponding NPRF, subsequently resulting in a more viscous paste due to friction observed as higher peak viscosity in LPRF.

However, the swollen granules of LPWF could withstand shear to the least extent, compared to those of NPWF. This resulted in the lower peak viscosity of LPWF paste compared to that of NPWF. Nonetheless, the lower final viscosity of both LPRF and LPWF, compared to their corresponding flours with higher initial protein content, indicated the pastes were less resistant to shear after the temperature was lowered from 95°C to 50°C at the end of RVA testing.

When NPRF and NPWF were mixed at different ratio to lower amylose content in rice flour during rice sheet preparation, it was found that increasing the NPWF ratio significantly increased the average size distribution of air cells to the large size (Figure 1) with broad size distribution (Figure 2). Rice crackers prepared from NPRF had small size air cells of less than 900 μm (Figure 2). However, lowering amylose content by substituting NPRF with NPWF drastically increased the size of air cells up to 2700 μm and increased the oil uptake by almost 1.5 times, although the NPRF was substituted by NPWF for 30%. It should be noted that the moisture content of rice sheet from each treatment was controlled at 11% and the crackers from each treatment retained similar moisture content of 5% after frying (P>0.05).

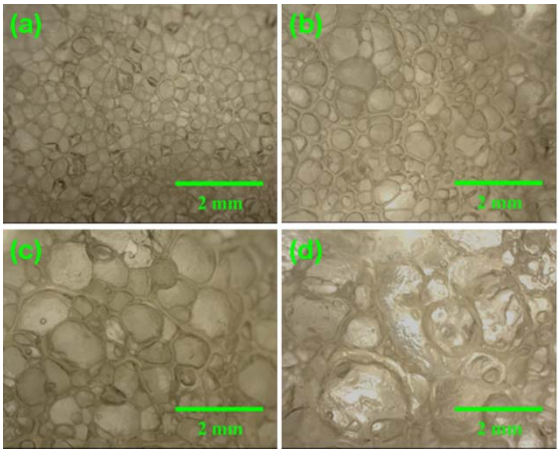

Figure 1. Effect of native-protein rice flour (NPRF) to native-protein waxy rice flour (NPWF) ratios on the microstructure of rice cracker.
NPRF:NPWF ratios were (a) 1.0:0.0; (b) 0.9:0.1; (c) 0.8:0.2 and (d) 0.7:0.3. Bar scale was 2.0 mm.

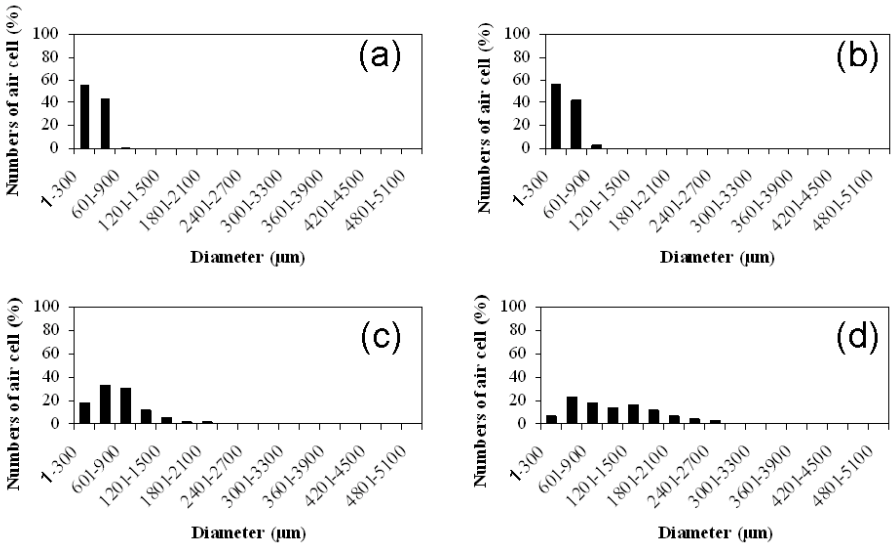

Figure 2. Effect of native-protein rice flour (NPRF) to native-protein waxy rice flour (NPWF) ratios on size distribution of air cell in fried cracker.
NPRF:NPWF ratios were (a) 1.0:0.0; (b) 0.9:0.1; (c) 0.8:0.2 and (d) 0.7:0.3.

The bulk volume, as well as the oil contents of rice cracker, was increased when the amylose was lowered as the ratio of NPWF increased (Figure 3). In this study, rice sheets were prepared in similar fashion as used in the production of dehydrated rice noodle, which included the induction of amylose recrystallisation by freezing the gelatinised flour paste at –20°C for 14 h. This was to enhance the ease of cutting before drying and homogeneous water evaporation of rice sheet during drying. The reduction of amylose content, which resulted in the lower junction zone of amylose recrystallization within the flour matrix in dried rice sheet, likely enhanced water evaporation and expansion of flour matrix in rice cracker during drying.

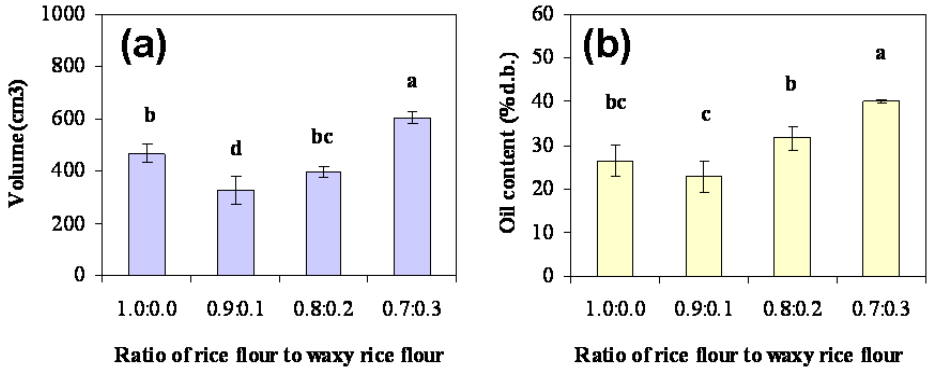

Figure 3. Effect of native-protein rice flour (NPRF) to native-protein waxy rice flour (NPWF) ratios on (a) volume and (b) oil uptake of rice cracker.

NPRF:NPWF ratios were (a) 1.0:0.0; (b) 0.9:0.1; (c) 0.8:0.2 and (d) 0.7:0.3. Bars represent standard deviation.

CLSM was used to better understand the roles of rice endosperm proteins as one of the microstructural elements in rice flour sheet after drying. The rhodamined B binds specifically to the amine group of proteins. Figure 4 illustrates that, apart from a continuous dark area of starch-rich phase, the protein fraction (fluoresced in red) also existed as another continuous phase in rice sheet. The bi-continuous structure of both protein and gelatinized starch fractions are most likely responsible for the viscoelastic properties of the flour matrix in rice sheet prior to frying.

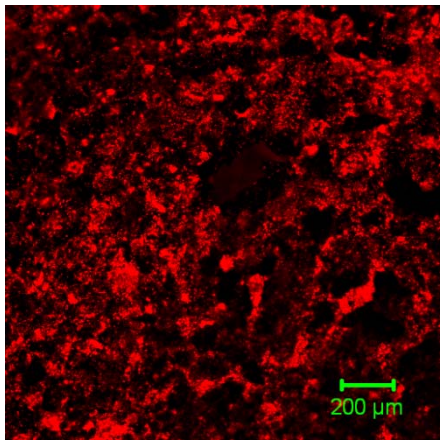

Figure 4. Confocal laser scanning micrographs of dried rice sheet prior to frying.

Protein was stained with rhodamine B and is shown as red fluorescence. Dark area represented starch-rich phase. Bar = 200 µm.

Lowering the protein content by bromelain allowed the rice sheet to expand more, observed as a larger air cell size (Figure 5) and broader size distribution (Figure 6) in rice crackers prepared from low protein flour compared to those prepared from native-protein flour. This was observed in both

rice flour and waxy rice flour. The presence of endosperm proteins may restrict the expansion of the matrix in rice sheet during water evaporation by frying.

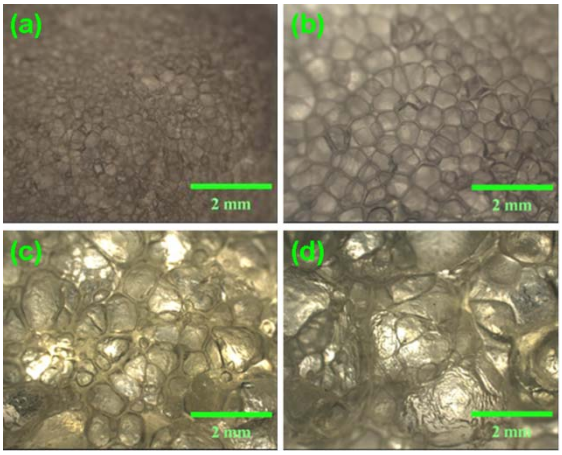

Figure 5. Effect of de-proteinisation of rice flour by bromelain hydrolysis on the microstructure of rice crackers made of (a) NPRF; (b) LPRF; (c) NPWF; and (d) LPWF.
Bar scale was 2.0 mm.

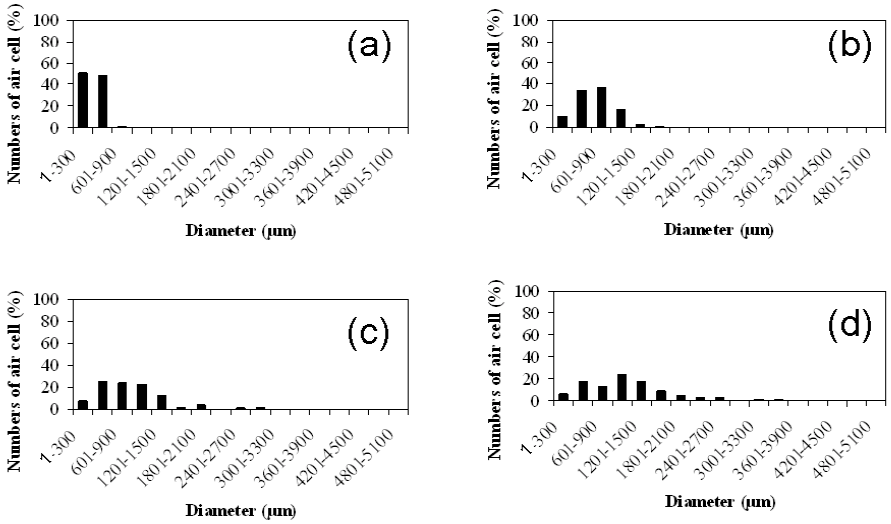

Figure 6. Effect of de-proteinisation of rice flour by bromelain hydrolysis on the size distribution of air cells in rice crackers made of (a) NPRF; (b) LPRF; (c) NPWF; and (d) LPWF.

It is apparent that rice endosperm proteins played a significant role in determining the formation of solid foam structure of rice crackers together with starch fraction. The moisture contents of rice sheet and cracker from each treatment remained at 11% and 5%, respectively. Therefore, the solid foam structure of cracker and the size of air cell were formed based on a similar degree of water evaporation during frying throughout this study.

It was found that the endosperm protein profiles in rice flour and waxy rice flour investigated in this study were slightly different (Figure 7). Under non-reducing condition, both NPRF (lane 1) and NPWF (lane 2) showed similar protein profiles. However, the difference in protein profiles between NPRF and NPWF were more pronounced under reducing condition. In the presence of β-mercaptoethanol, proteins having MW above 250 kDa and between 35 to 50 dissociated into the

intense bands having MWs of around 20 kDa and 25 kDa (lanes 5 and 6). In NPWF (lane 6), there was no band having MW of 50 kDa existing and the band having MW of 48 kDa was less intense than that of NPRF (lane 5). SDS-PAGE revealed that the major rice endosperm proteins existed in subunits linked by disulphide bonds

Oryzenin is the major rice endosperm protein which can be extracted in the presence of 2% SDS and 5% β-mercaptoethanol used during protein extraction in this study. The major subunits of oryzenin have been reported as: α (acidic) subunits (MW around 28-30 kDa) and β (basic) subunits (MW around 20-22 kDa) [7, 9, 20]. The MW of oryzenin subunits depends on rice cultivars, methods of protein extraction and MW characterisation. It is most likely that the oryzenin in NPRF and NPWF had different ratios of the subunits.

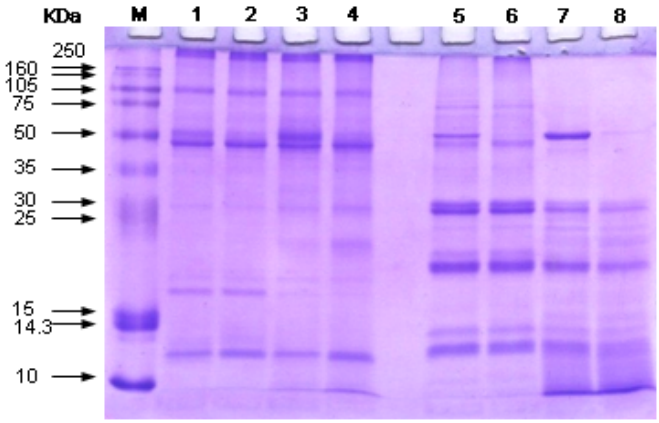

Figure 7. SDS-PAGE profiles of rice endosperm proteins under non-reducing condition (lanes 1-4) and reducing condition (lanes 5-8) by β-mercaptoethanol.
NPRF: lane 1, lane 5; NPWF: lane 2, lane 6; LPRF: lane 3, lane 7; and LPWF: lane 4, lane 8. The molecular weight standards: lane M.

Bromelain hydrolysed the proteins to the lower MW peptides in LPRF (lane 3) and LPWF (lane 4), observed as the disappearance of the bands having MW around 17 kDa in the low protein flours. Although the low MW polypeptides obtained after hydrolysis were washed out during LPRF and LPWF preparation, residual proteins after bromelain hydrolysis were oryzenin fraction with similar MW profile as their original flours and those having MWs observed as smeared bands having MWs lower than 12 KDa under reducing conditions (lanes 7, 8).

Conclusion

It is apparent that it was not only amylose, but also the protein fraction that restricted the matrix of 11% moisture content from expansion. The resulting solid foam structure thus had small air cell size and low oil absorption. This study has addressed the significance of rice endosperm proteins in determining the RVA pasting profiles, subsequent viscosity of rice paste during cooling and their presence in the dehydrated gelatinized flour sheet prior to frying. The significance of rice endosperm proteins on governing the expansion of fried rice crackers, together with amylose, on the expansion of rice flour matrix, was demonstrated for the first time.

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