ภาคผนวก

บทความวิจัยเรื่องที่ 1

J Polym Environ
DOI 10.1007/s10924-014-0683-6

ORIGINAL PAPER

Characterization of Defatted Rice Bran Properties for Biocomposite Production

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Abstract Commercial defatted rice bran (DRB) was characterized to produce biocomposite. DRB extracted protein plasticized with glycerol presented viscoelastic properties. The whole DRB with different amount and type of plasticizers was extruded into pellets. All extrudates presented pseudoplastic behavior as determined by capillary rheometer. Power-law index (n) and flow behavior consistency (K) of all DRB extrudates were respectively in a range of 0.30–0.32 and 1.2–3.8 $\times$ 10^4 which is closed to agro-polymer due to a modification of DRB structure after extrusion process. In addition, extrusion process promoted protein aggregation. However, no significant effect of plasticizer type and content on DRB protein aggregation for a given temperature processing was observed. The effect of plasticizer content on tensile properties presented the same trend as viscosity results. Extrudate that had a high viscosity presented high mechanical properties.

Keywords Defatted rice bran · Biocomposite · Extrusion · Plasticizer · Viscosity

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Published online: 22 June 2014

Introduction

Currently, the development of biocomposite which mainly consists of biopolymer matrix reinforced by natural fibers from agricultural resources has become an important challenge to substitute synthetic polymer [1, 2]. Thailand is the world's largest exporter of rice. In 2013, Thailand produced about 37 million tons of milled rice [3]. Rice bran, a residue from brown rice, is obtained in the rice milling process [4]. It can be used as raw material for rice bran oil extraction, after which defatted rice bran (DRB) remains as a by-product, used for animal feed because of its low price [4, 5]. DRB contains about approximately 20 % protein, 45 % carbohydrate (mostly starch) and 10 % fiber including DRB cell wall and some natural fibers of rice husks consisting of mostly cellulose, hemicelluloses and lignin [6, 7]. Therefore, DRB could be considered as an interesting raw material for production of biocomposite.

It has been recently shown that extrusion and compression molding of DRB in presence of saw dust and glycerol allowed the development of biodegradable sheets. Optimal properties were obtained for molded sheet prepared with 40 % glycerol, which presented medium water holding capacity (about 53 %) and a hardness of about 50 N. In addition, molded sheet presented eco-friendliness: it was completely degraded in 45 days after planting in the soil [8]. However, in order to have a better understanding of biocomposite production from commercial DRB, properties of DRB should be studied. For example, before producing biocomposite from sunflower cake (SFC) which can be considered as a natural composite (main part of protein matrix reinforced by fiber), Geneau-Sbartai et al. [9] characterized the composition and plastic properties of SFC. In addition, SFC protein was isolated to study thermal and viscoelastic properties [10, 11]. The result showed that the protein matrix of SFC possess a film forming ability similar to

a common polymer [12, 13]. In addition, SFC has similar rheological and physicochemical properties compatible with shaping or molding behaviors of plastic-processing machinery [9].

In biocomposite processing, plasticizer is widely used to improve processability. However, deplastization may occur during processing of biocomposite because fiber may absorb plasticizer [14]. Therefore, optimum plasticizer should be added to have a good processability. Moreover, to produce biocomposite containing protein the processing temperature was limited due to an increase in viscosity associated with extensive aggregation via thiol-disulfide exchange reactions [15]. To produce SFC biocomposite, chemical additive which is a reducing agent was used to reduce protein disulfide bridges as a result of a melt viscosity decrease [16]. In addition, a defibration of husk and the fusion of protein of SFC by extrusion process decreased the viscosity of plasticized SFC, leading to an ease of the injection molding [16].

DRB contains a mixture of carbohydrate, protein and fiber which is quite complicated. Therefore, DRB, plasticized DRB and plasticized DRB proteins should be characterized in terms of processability and properties in order to get an understanding to produce biocomposites from whole DRB.

In this research, the properties of DRB and extracted DRB protein were determined. In addition, the type and amount of plasticizer was studied to optimize the plasticizer composition in order to produce biocomposites from whole DRB.

Firstly, proteins were extracted from DRB in order to conduct their characterization. Then, the plasticized DRB protein was characterized. The DRB pellets with different plasticizers were extruded using a twin screw extruder. Rheological properties of DRB extrudates were determined by capillary rheometry in order to study the viscosity of DRB pellet for further injection processing. Finally, protein aggregation upon extrusion was measured using a size exclusion-high performance liquid chromatography (SE-HPLC).

Experimental Section

Materials

Commercial defatted rice bran (DRB) was provided by Thai Edible Oil Co., Ltd. (Bangkok, Thailand). Anhydrous glycerol was purchased from Roongsub Chemical Ltd. (New South Wales, Australia) in analytical grade. Chemical reagents were obtained from Ajax Finechem (Auckland, New Zealand), Sigma-aldrich (St. Louis, Missouri) and Carlo Erba (Val de Reuil, France) in analytical grade.

Characterization of DRB

Proximate compositions of DRB consisting of moisture, crude fat, crude fiber and ash content were determined according to AOAC (1995) methods. Protein content was determined by the Kjeldahl method using a value of 5.95 as a protein conversion factor. Crude fat content was determined by the Soxhlet method (Soxtec System-Texator, Sweden). Crude fiber was determined by acid and alkali digestion. Carbohydrate was calculated by difference which was the weight of the DRB sample subtracted by the other constituents (moisture, fat, ash and protein). Particle size distribution of DRB was determined by sieving (Retsch, AS200 basic, Haan, Germany).

Thermogravimetric analysis (TGA) was performed with a thermal analyzer (Mettler Toledo, TGA/DSC 1 HT/1600/673/13555) under N_2 flow at 50 ml/min from 25 to 800 °C at 10 °C/min of heating rate. The sample weight was about 7 mg.

Proteins Characterization

Extraction of DRB Protein with the Osborne Method

DRB was first ground and screened to pass through a 70 mesh sieve (270 μm opening) with a hammer mill. Then, proteins were successively extracted at room temperature (25 °C) in distilled water, 5 % NaCl, 0.1 M NaOH and 70 % ethanol according to the classification of Osborne and Campbell, following the procedure of Agboola et al. [17]. Briefly, DRB was extracted with 400 ml distilled water with stirring for 4 h and centrifuged at 3,000*g* for 30 min to obtain the albumin fraction (supernatant). The residue from this step was then similarly extracted with 400 ml of 5 % NaCl to obtain the globulin fraction. The residue after extraction of globulin was extracted with 0.1 M NaOH (1 h) to obtain the glutelin fraction, while the residue after glutelin extraction was extracted with 70 % ethanol to obtain the prolamin fraction. To remove most of the protein each extraction step was repeated twice. The albumin, globulin and glutelin fractions were then purified by isoelectric precipitation at pH 4.1, 4.3, 4.8 and 6.8, respectively and centrifuged at 3,000 g for 30 min. Then, protein fractions were washed with distilled water and freeze-dried using a freeze dryer (Biotech International, Germany). After drying, each protein fraction was weighed to calculate yield, defined as the ratio between the extracted protein weight and the initial weight of protein in DRB. The residual protein amount (proteins which are not extractible with the solvents mentioned above) was calculated as the difference between the initial protein weight of DRB and the total cumulated weight of the four extracted proteins. Each sample was analyzed in 2 replications. The data are presented as mean values with standard deviations.

SE-HPLC Analysis of Extracted DRB Proteins

Molecular size distribution of the DRB proteins was characterized by SE-HPLC. Exhaustive presentation of the method is given in Redl et al. [18]. Briefly, extracted protein (30 mg) was stirred for 80 min at 60 °C in the presence of 20 ml of 0.1 M sodium phosphate buffer (pH 6.9) containing 1 % sodium dodecyl sulfate (SDS). The SDS-soluble protein extract was recovered by centrifugation (30 min at 39,000*g* and 20 °C) and 20 µl were submitted to SE-HPLC fractionation (first extract). The pellet was suspended in 5 ml SDS-phosphate buffer containing 20 mM dithioerythritol (DTE). After shaking for 60 min at 60 °C, the extract was sonicated (Vibra Cell 20 kHz, Bioblock scientific) 3 min at 30 % power setting. Disulfide and weak bonds are disrupted by those chemicals, whose efficiencies are further increased thanks to ultrasonic waves. As a result, these treatments bring insoluble protein from the pellet into solution. After centrifugation (30 min, 39,000×*g*, 20 °C), a part of the supernatant was then mixed volume to volume with SDS-phosphate buffer containing 40 mM iodoacetamide in order to alkylate thiol groups. The reaction was carried out for 1 h in darkness, at room temperature. 20 µl of this solution was submitted to SE-HPLC fractionation (second extract).

The SE-HPLC apparatus is a Waters model (Alliance). A TSK G4000-SWXL (Tosoh Biosep) size exclusion analytical column (7.5 × 300 mm) was used with a TSK-SW (Tosoh Biosep) guard column (7.5 × 75 mm). The columns were eluted at ambient temperature with 0.1 M sodium phosphate buffer (pH 6.9) containing 0.1 % SDS. The flow rate was 0.7 ml/min, and proteins were recorded at 214 nm for UV measurement. Each sample was analyzed in 2 replications. The data are presented as mean values with standard deviations.

Characterization of Plasticized DRB Protein Properties

In order to characterize viscoelastic properties of DRB protein, DRB protein was extracted by alkaline solvent to extract mostly protein in DRB. Briefly, ground DRB was dispersed in distilled water (1:10 w/v) and the pH was adjusted to 9.0 with 2 M NaOH [19]. The suspension was stirred for 60 min and then centrifuged at 9,000*g* for 30 min at 25 °C. The supernatant was adjusted to pH 4 with 2 M HCl and then centrifuged at 9,000*g* for 30 min at 25 °C. The pellet was suspended in water, neutralized with 2 M NaOH, and freeze-dried.

Viscoelastic Properties of Plasticized DRB Protein

Fifty grams of extracted protein plasticized with 30 % glycerol were blended in an internal mixer (Plasti-corder W50, Brabender, Duisburg, Germany) at temperature of 80 °C, 100 rpm for 15 min. After mixing, the rheological properties of plasticized DRB protein was investigated by a controlled strain rheometer ARES (Rheometric Scientific, USA). Prior to test, plasticized DRB protein was preconditioned at 25 °C and 0 % relative humidity over P_2O_5. Temperature sweep tests, from 25 to 180 °C, were conducted in a rheometer using a serrated parallel plate geometry, 25 mm diameter and 1–1.2 mm gap. Measurements were performed at constant frequency (6.28 rad/s), 1 % strain (which was always within viscoelastic region) and 2 °C/min temperature ramp. During analysis, storage modulus (G′), loss modulus (G″) and tan δ (G″/G′) were recorded and plotted against temperature [20].

Table 1 Composition of samples for extrusion

Sample	Composition (%wt)		
	Glycerol	Water	DRB
W30:B70	–	30 %	70 %
G30:B70	30 %	–	70 %
W10:G30:B60	30 %	10 %	60 %
G40:B60	40 %	–	60 %

Characterization of DRB Extrudate

Extrusion Processing of Plasticized DRB

DRB was mixed with different types and content of plasticizers (Table 1) in a food mixer (King mixer, Model K-05, USA) at low speed for 10 min and medium speed for 1 min. The extrusion was performed with a co-rotating, self-wiping twin screw extruder (model LTE-20-40, Labtech Engineering Co., Ltd., Thailand), equipped with a circular die with a diameter of 3 mm. The extruder consisted of 10 heating zones, divided into 8 heating zones of barrel and 2 heating zones of die head. The total length of the screw was 800 mm, its diameter being 20 mm [21].

DRB samples were extruded at a constant feed rate (2.5 kg/h) and screw speed (50 rpm). The barrel temperatures in zone 1–2, 3–4, 5–6, 7–8 were respectively 60, 70, 80, 90 °C and a final die temperature in zone 9–10 was 100 °C. Mean residence time of extrudate was determined by introducing 0.5 g of colored feed and estimated by visual evaluation of color change at the die opening [22].

Thermal Stability of DRB Extrudate

Thermogravimetric analysis (TGA) of four plasticized extrudates was performed with a thermal analyzer as the same method described in the section of characterization of DRB. The sample weight was about 30 mg.

Rheological Properties of DRB Extrudate

Rheological properties of the extrudate were measured by using a capillary rheometer (The Rh-2000 Advance Rheometer System, Rosand) with a capillary diameter of 2 mm and a length of 16 mm. Measurements were carried out at 100 and 120 °C under a shear rate ranging from 1 to 1,800 s^{-1}. In the tests, sixty to seventy grams of extrudates were pretreated two times in a rheometer barrel under a pressure of 0.5 MPa with a speed of 1.00 mm/min for 3 min and 6 min of holding heat time for respectively the first and second pretreatment. The shear stress, shear rate and viscosity were recorded. Then, apparent viscosity was plotted against shear rate. A simple mathematical expression describing the relationship between viscosity and shear rate is presented in Eq. 1.

$$\eta = K\dot{\gamma}^{n-1} \quad (1)$$

where the consistency (K) corresponds to the viscosity value for a shear rate ($\dot{\gamma}$) of 1 s^{-1} and the power-law index (n) characterizes the deviation from the Newtonian behavior, for which $n = 1$ [23]. Each sample was analyzed in 2 replications. The data are presented as mean values with standard deviations.

SE-HPLC Analysis of DRB Extrudate

Changes in the molecular size distribution of the DRB proteins were characterized by SE-HPLC. Samples were manually grounded in presence of liquid nitrogen and then blended with soluble wheat starch (1/5 g/g) which allowed the glycerol absorption. The obtained powder (300 mg) was analyzed following the same method as presented in section of SE-HPLC analysis of extracted DRB protein.

Mechanical Properties

Twenty-five grams of extrudates were molded at 100 °C for 15 min. in a Hydraulic Press Machine (20 T., SMC TOYO METAL Co., Ltd., Thailand). A load of 1 ton was directly applied to the sample in the mold. The thickness of the material was approximately 2 mm. The extrudate sheet was cut into a dumbbell shape (length and width of the narrow section were 75 mm and 5 mm respectively) and preconditioned at 25 °C, 53 % RH over a magnesium nitrate salt ($Mg(NO_3)_2$). Tensile tests were performed on a Texture Analyzer (Stable Micro System, TA-XT. plus, Surrey, UK). The initial grip separation was 50 mm and elongation speed was 1 mm/s [24]. Each sample was analyzed in 10 specimens. The data are presented as mean values with standard deviations.

Table 2 Composition of DRB

Component	%wt in DRB
Moisture	10.41 ± 0.55
Crude fat and oil	1.13 ± 0.10
Crude protein	15.17 ± 0.12
Ash	9.66 ± 0.003
Crude fiber	8.11 ± 0.17
Carbohydrate (by difference)	55.52 ± 0.60

Dynamic Mechanical Properties

Rectangular samples ($10 \times 3 \times 1$ mm^3), prepared by molding ten grams of extrudates 100 °C for 15 min. in a Hydraulic Press Machine (20 T., SMC TOYO METAL Co., Ltd., Thailand), were analyzed with a dynamic mechanical thermal analyzer (Mettler Toledo DMA/SDTA 861e) equipped with a cryogenic system fed with liquid nitrogen. A tension test was performed with a temperature ramp from −100 to 150 °C at a heating rate of 4 °C min^{-1}. A variable sinusoidal mechanical stress was applied to the sample (frequency = 1 Hz, maximum force 1 N, displacement amplitude 40 μm, offset 120 %). During analysis, the storage modulus (E′), the loss modulus (E″) and tan δ (=E″/E′) were recorded and plotted against temperature for further evaluation of thermal transition. T_g was identified as the temperature of the tan δ maximum. Each sample was analysed in three replications.

Results and Discussion

Characterization of DRB

Chemical compositions resulting from our characterization are given in Table 2. They are confirmed to the specification of the commercial product used here, which guarantees that protein, crude fiber, and crude fat and oil content is respectively 15 % minimum, 10 and 2 % maximum [25]. For crude fiber content, this value included fiber in DRB and also some natural fibers of rice husks that did not separate in dehusk process. These natural fibers were presented in DRB in a range of 212–710 μm (Fig. 1). The presence of particles with a large size (>1.7 mm) may be the result of particles aggregation during the thermal treatment associated with the commercial oil extraction process [26].

In order to determine the maximum processing temperature, we evaluated the thermal stability of DRB powder. As shown in Fig. 2, a first step of weight loss of DRB powder occurred between 30 and 150 °C, it is associated with the evaporation of water from the sample. Then, from

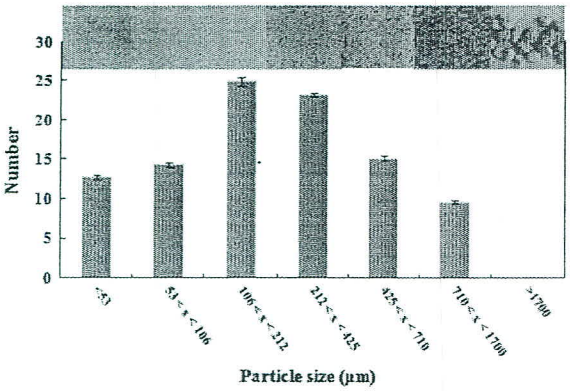

Fig. 1 Particle size distribution of DRB as determined by sieving

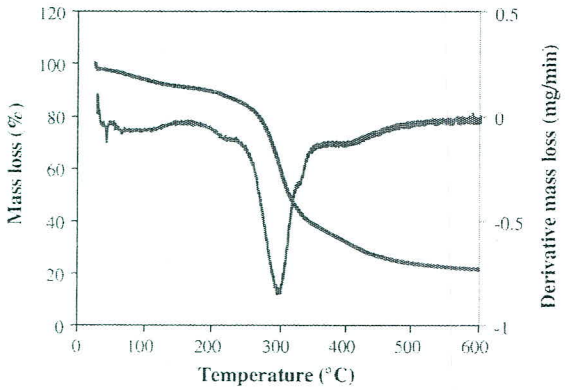

Fig. 2 Thermogravimetric and derivative thermogravimetric curves of DRB powder

150 to 230 °C mass loss is about 8 % of the initial weight, which may be due to reactions such as Maillard's reaction. Finally, the most significant loss of mass, about 55 % of the initial weight, was observed above 230 °C. It can be associated with the thermal decomposition of the product, including starch, cellulose and hemicelluloses. A loss of mass between 350 and 440 °C may be due to a thermal degradation of lignin [9]. Those results suggest that the products should not be processed above 150 °C in order to avoid the product degradation, which is in agreement with the thermal stability of SFC which is reported to be about 190–220 °C [9].

Characterization of DRB Protein

Proteins can play a key role in the final properties of materials in which they are present due to their ability to cross-link during processing. Protein composition of the commercial DRB used in this study was therefore first characterized. The yields of four extracted proteins and of the residual (unextractable) protein are shown in Table 3. Clearly, glutelin constituted the highest proportion of the extractable protein, representing about 17 % of the proteins. Globlulin, albumin and prolamin were presented in negligible amounts, with respectively about 2, 0.3 and 0.1 % yield. These protein proportions are significantly different than the results of Hamada [27] who reported about 34 % albumin, 15 % globulins, 6 % prolamin and 11 % of acid-soluble glutelins in DRB. This difference could be explained by the different treatment of DRB raw material before use. Indeed, a commercial DRB used in our study was pre-treated with a thermal process to inhibit lipoxygenase enzyme during oil extraction process [26], contrarily to what have been done by Hamada [27] which extracted DRB with a solvent method. Thermal treatments are known to significantly reduce protein solubility. As a result, the commercial sample used in this study showed significantly lower protein extractability, and this might affect the behavior during processing.

Table 3 DRB protein extracted according to the classification of Osborne and Campbell

Protein isolate	Solvent	Yield (%)
Albumin	Distilled water	0.30 ± 0.02
Globulin	5 % NaCl	1.85 ± 0.13
Glutelin	0.1 M NaOH	17.5 ± 1.6
Prolamin	70 % ethanol	0.13 ± 0.02
Residual protein	–	80.3 ± 1.7

To determine size distribution of DRB protein, proteins were solubilized in SDS-buffer to suppress the intermolecular interactions and bring them into solution (SDS soluble fraction). The remaining SDS-insoluble protein fraction can be further extracted in the same SDS-buffer with DTE to cleave the inter disulfide bridges.

Figure 3a presents the elution profiles of SDS-soluble of four extracted proteins (more than 75 % of all extract was SDS-soluble). The SDS-soluble profile was divided into seven fractions according to the observed profile peaks.

Glutelin was characterized by a large peak in fraction 1 (F1) representing large molecules, while albumin fraction was characterized by lower molecular weight which was evidence by a large peak in F6. Globulin has a moderate molecular weight. These results are in an agreement with a result of Agboola [17] who characterized protein isolates from RB flour.

The elution profiles of SDS-soluble and SDS-insoluble protein of the residual protein (after solvent extraction) and of proteins directly extracted in SDS from the whole DRB powder are respectively presented in Fig. 3b, c. The large peak observed between 18 and 21 min in the SDS-insoluble protein is due to DTE. About 50 % of the residual

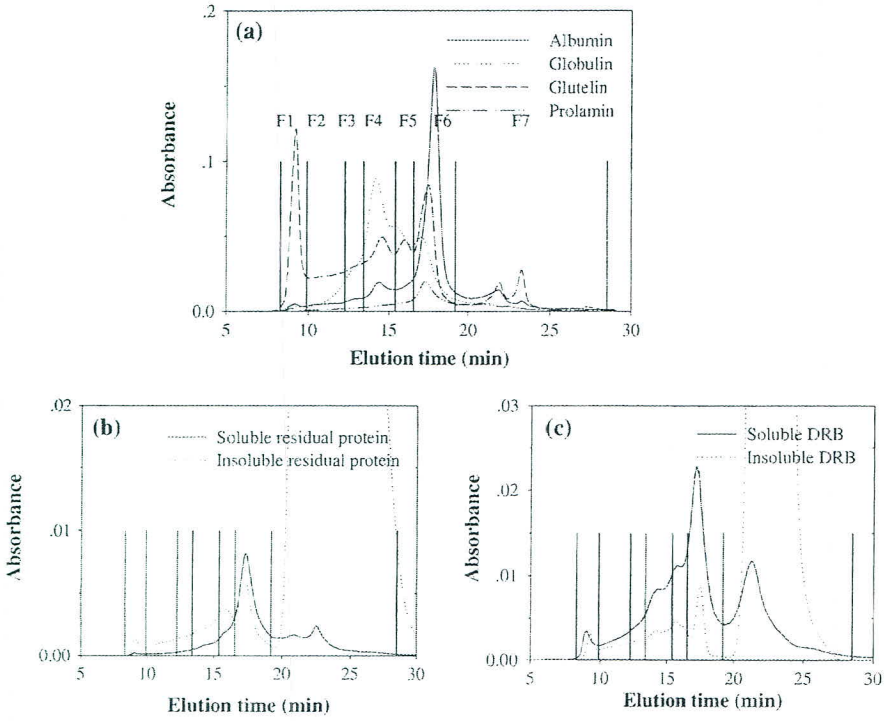

Fig. 3 Size exclusion profiles of extracted proteins (**a**), residual protein (**b**) and protein in DRB powder (**c**)

proteins were SDS-insoluble, while the soluble was characterized by a quite low molecular weight (Fig. 3b). These soluble proteins may link to other components such as starch [17], thus making it difficult to extract.

Characterization of Plasticized DRB Protein

In order to understand the behavior of DRB proteins during extrusion of the whole DRB biocomposite, we characterized the viscoelastic properties of those proteins independently, after their extraction and plasticization. Extraction was conducted using the alkaline method which allows extracting mostly glutelin, the major protein in DRB. Then, these extracted proteins were plasticized with 30 % glycerol.

Viscoelastic properties of plasticized DRB protein was characterized in terms of storage modulus (G′) and loss modulus (G″) as a function of temperature (Fig. 4). The evolution of storage modulus of plasticized DRB protein could be considered into four steps. The first step between 25 and 60 °C is plateau which corresponds to vitreous stage. Then, in the second region, between 60 and 100 °C a decrease in G' with temperature is observed due to thermal agitation and glass transition [20]. The third step between 100 and 130 °C is a plateau region which may be associated with protein cross-linking [20]. Finally, above 130 °C,

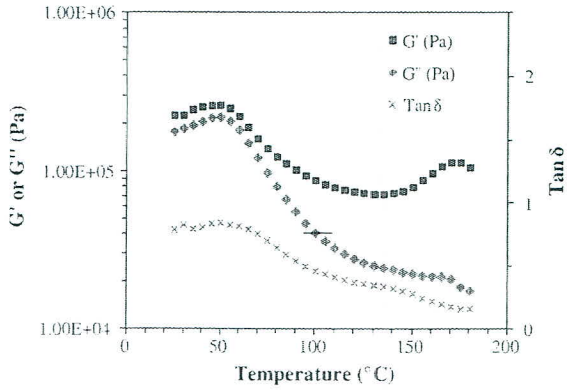

Fig. 4 Viscoelastic functions properties of DRB protein plasticized with 30 % glycerol at a constant frequency of 6.28 rad/s

G′ undergoes a remarkable increase, which may be attributed to protein aggregation [20]. This study showed that DRB protein has a potential to be plasticized and cross-link at processing temperature during the transformation of whole DRB biocomposite.

Properties of the Whole DRB Extrudate

The whole DRB was extruded into pellet to study the rheological properties and protein aggregation in order to

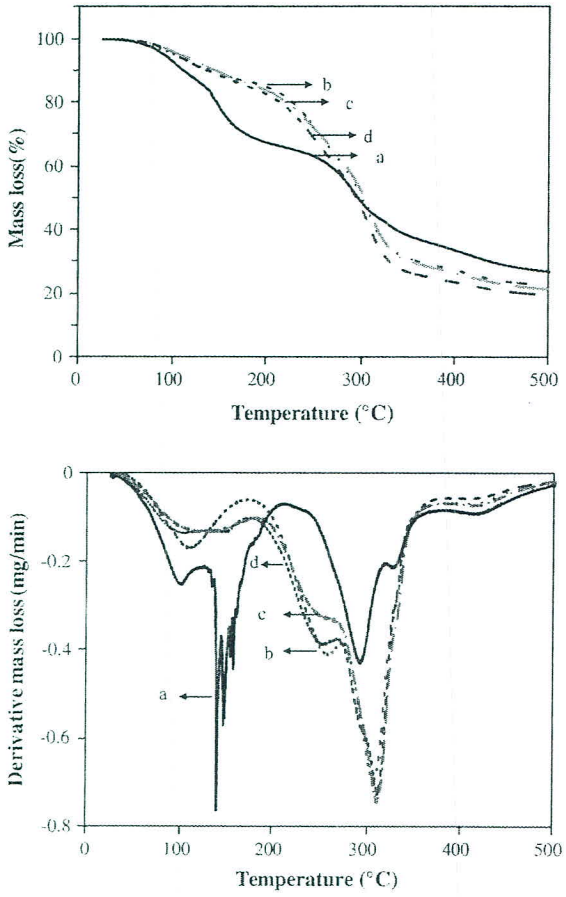

Fig. 5 Thermogravimetric and derivative thermogravimetric curves of DRB extrudates plasticized with 30 % water (*a*), 30 % glycerol (*b*), 10 % water and 30 % glycerol (*c*), and 40 % glycerol (*d*)

understand the viscosity of plasticized DRB pellet after extrusion process for further processing such as injection molding. Plasticized DRB samples were extruded at 100 °C of die temperature at 50 rpm of screw speed and 2.5 kg/feed rate. Different plasticizer type and content were used as shown in Table 1. As DRB contains fibers which may absorb plasticizer [14], therefore different plasticizers at 30 and 40 % contents was used in this study.

Thermal Stability

Thermogravimetric (TG) and derivative thermogravimetric (DTG) curves of extrudates plasticized with different plasticizers were shown in Fig. 5. The initial mass loss below 150 °C was corresponded to the evaporation of moisture in the extrudates. Then, mass loss in the range of 150–270 °C was related to the volatilization glycerol (decomposed temperature of the glycerol is about 213 °C [28]). The little differences of mass loss were related to the

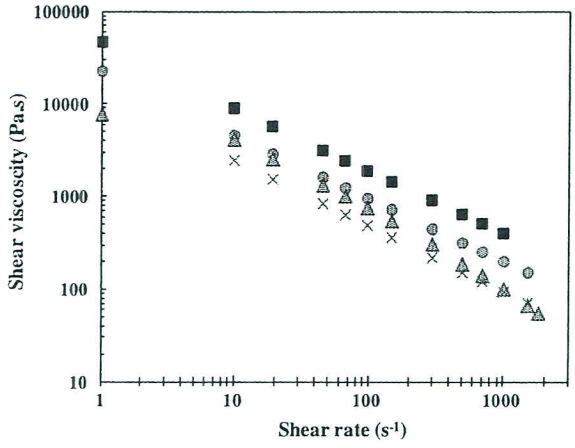

Fig. 6 Shear viscosity of extrudates with different plasticizers: 30 % water (*rounds*), 30 % glycerol (*squares*), 10 % water and 30 % glycerol (*triangles*), and 40 % glycerol (*crosses*), determined at 100 °C of capillary die temperature

different water and glycerol contents in extrudates [29]. From DTG plot, the decomposed temperature (major peak temperature) of all extrudes except sample with 30 %water was about 310 °C which was higher than that of DRB powder (300 °C as shown in Fig. 2). The addition of plasticizer increased thermal stability of DRB. This result may be explained by the plasticizer/polymer interaction [30]. The lower decomposed temperature of sample with 30 %water which is 290 °C may be associated with the depolymerization of DRB during high temperature and shear during extrusion [31].

Rheological Properties

Rheological properties of plasticized DRB pellet were measured by a capillary rheometer at 100 °C of capillary die temperature. However, at higher capillary die temperature, sample with 30 % water (W30:B70) and sample with a combination of 30 % glycerol and 10 % water (W10:G30:B60) cannot flow (data not shown). This may be explained by a loss of moisture which acted as a plasticizer during viscosity measurement at high capillary temperature. Figure 6 presented the viscosity measured at 100 °C of capillary die temperature. Viscosity of extrudates with 40 % plasticizer is lower than those with 30 % plasiticizer, probably due to the plasticizing effect [32].

All DRB samples presented shear thinning behavior as thermoplastic. Power-law index (n) and flow behavior consistency (K) of extrudates with different plasticizers are shown in Table 4. Values of n and K were in a range of 0.30–0.32 and 1.2–3.8 $\times$ 10^4, respectively. This is lower than for SFC biocomposite plasticized with 30 % water

Table 4 Power-law model parameters of DRB extrudates determined at 100 °C of capillary die temperature

Sample	K consistency (Pa s^n)	n
W30:B70	21,863	0.32
G30:B70	38,394	0.31
W10:G30:B60	15,343	0.31
G40:B60	12,052	0.30

($n = 0.04$, $K = 3.2 \times 10^5$) [9]. This can be explained by that DRB was extruded before measuring viscosity. Shear and thermal treatment during extrusion may change the DRB structure such as fiber and starch [16] as shown in Fig. 7. Before extrusion process, raw DRB (Fig. 7a, b) presented some fibers distributed in DRB which may be some rice husks that did not separate in dehusk process. After extrusion, a structural change of DRB may occur due to defibration [16] and starch fragmentation under shear [33]. In addition, compared to plasticized agro-polymer samples [32, 34], n and K values of DRB extrudates were closed to those of agro-polymer samples such as $n = 0.29$, $K = 4.2 \times 10^4$ for soy protein and corn starch blend plasticized with glycerol [34]. This may be due to DRB containing only 10 % of fiber content.

In terms of rheological properties, all DRB extrudates presented flowablity. However, for extrudates plasticized with water, it was difficult to adjust the processing conditions due to the loss of water-plasticizing molecules, so it may be necessary to add some water or other plasticizer for further processing for these samples. In this regard, G40:B60 seemed to be the extrudate with the best properties for further processing.

Protein Aggregation

The molecular size distribution of protein in DRB extrudates with different plasticizers were investigated by SE-HPLC. Protein solubility loss is an indicator of protein aggregation after thermal treatment [35]. The soluble protein content of all extrudates decreased compared to native DRB power because extrusion process promoted protein aggregation as shown in Table 5. Generally, protein aggregation is known to be temperature dependent. Plasticizer did not affect protein aggregation for samples obtained the same temperature processing [36]. Therefore, it seems that there is no major effect of the plasticizer type on protein aggregation. The slightly higher soluble protein content of extrudate with 40 % glycerol may be attributed to a different temperature and a lower residence time (Table 5) during processing.

Mechanical Properties

Mechanical properties of samples prepared by molding extrudate at 100 °C were determined. Sample with 30 % water presented the highest Young's modulus and tensile

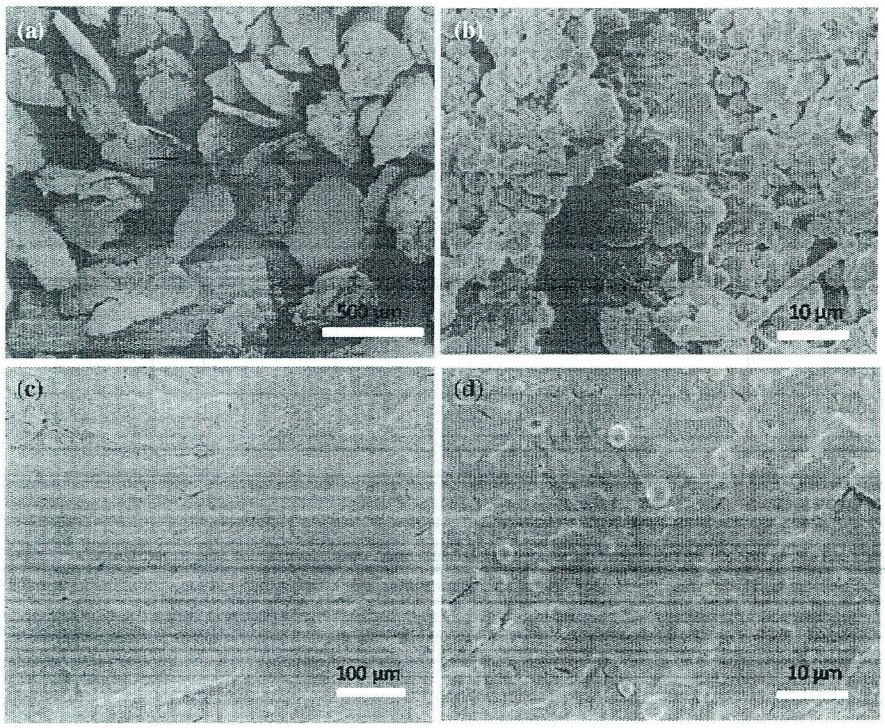

Fig. 7 DRB powder (**a**, **b**) and W10:G30:B6010 extrudate (**c**, **d**) observed by SEM

Table 5 Protein solubility of DRB extrudates extruded at 100 °C of die temperature

Sample	Residence time in extruder (s)	Protein area/total theory protein area (%)		
		Soluble	Insoluble	Total
DRB powder	–	76 ± 1.3	24 ± 1.3	100
W30:B70	196 ± 6	62 ± 2.0	14 ± 0.5	76
G30:B70	198 ± 24	64 ± 5.4	35 ± 6.4	98
W10:G30:B60	196 ± 30	66 ± 1.0	26 ± 6.4	92
G40:B60	187 ± 4	71 ± 1.5	21 ± 1.3	92

Table 6 Mechanical properties of DRB materials with different plasticizers molded at 100 °C

Sample	Young's modulus (MPa)	Tensile strength (MPa)	Elongation at break (%)
W30:B70	1,172 ± 126	8.36 ± 2.37	0.69 ± 0.3
G30:B70	17.4 ± 1.7	0.58 ± 0.17	5.2 ± 1.6
W10:G30:B60	12.5 ± 0.3	0.50 ± 0.12	6.3 ± 1.8
G40:B60	5.1 ± 0.3	0.17 ± 0.04	4.1 ± 1.1

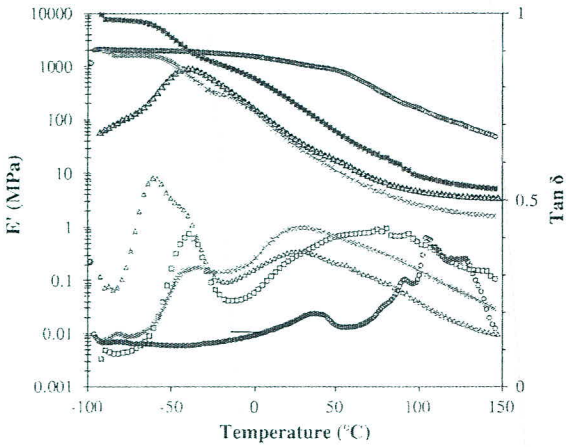

Fig. 8 Storage modulus (*filled symbols*) and tan δ (*empty symbols*) and thermo-molded DRB materials plasticized with 30 % water (*rounds*), 30 % glycerol (*squares*), 10 % water and 30 % glycerol (*triangles*), and 40 % glycerol (*crosses*)

strength, but lowest deformation a break compared to other samples as shown in Table 6. This sample is brittle because water which acted as a plasticizer lost from sample after thermal molding. This result was in an agreement with the storage modulus determined by DMA (Fig. 8). Storage modulus at ambient temperature (25 °C) of this sample was higher than other samples.

Samples with 30 % glycerol presented a higher Young's modulus and tensile strength than 40 % plasticizer. Increasing plasticizer content to 40 % decreased Young's modulus and tensile strength because of a decrease in T_g at high plasticizer content [37] as shown in Fig. 8. T_g, identified as the temperature at a major tan δ peak, of sample with 30 % plasticizer (G30:B70) was approximately 80 °C, while T_g of samples with 40 % plasticizer was about 28–30 °C (G40:B60 and W10:G30:B60). However, elongation at break of these three samples (G30:B70, G40:B60 and W10:G30:B60) were not significantly different and were still low, suggesting the absence of a continuous structure.

Comparing between the effect of plasticizer content on mechanical properties and viscosity, mechanical properties in term of Young's modulus presented the same trend as viscosity results. Extrudate that had a high viscosity presented high Young's modulus, except sample with 30 %water which presented low viscosity, but high Young's modulus due to moisture loss from sample during thermal molding as described earlier.

Conclusions

To develop biocomposite from whole DRB, properties of commercial DRB and extruded DRB were characterized. DRB is composed of a matrix of carbohydrate and protein reinforced with fibers. The thermal stability of DRB is 150–230 °C. Molecular size of extracted protein was in good agreement with literature [17], but the extracted protein yield was low due to thermal processing of commercial DRB. Moreover, plasticized DRB protein has viscoelastic properties.

The whole DRB with 30 % (G30:B70 and W30:B70) and 40 % plasticizers (G40:B60 and W10:G30:B60) was extruded into pellets. All extrudates presented pseudoplastic behavior as determined by capillary rheometer. Power-law index (n) and flow behavior consistency (K) of all DRB extrudates were respectively in a range of 0.30–0.32 and 1.2–3.8 × 10^4 which is closed to agropolymer. This may be explained by that extrusion process modified properties of DRB. In addition, extrusion process promoted protein aggregation. However, no significant effect of plasticizer on DRB protein aggregation obtained the same temperature processing. G40:B60 sample present a better flowablity properties. However, mechanical properties of sample were still low, suggesting the absence of a continuous structure. Therefore, DRB extrudate processing and properties should still be improved and will be further studied.

Acknowledgments This work was financially supported by The Thailand Research Fund (Grant No. TRG5580017).