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The Viscoelastic Properties of the Protein-rich Materials from the Fermented Hard Wheat, Soft Wheat and Barley Flours

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ABSTRACT

To study the viscoelastic properties of the remain residues after fermentation of crops, the linear and non-linear rheological properties of the suspensions of remain residues for the Hard Red Spring wheat (HRS) flour, soft wheat (Pastry) flour, barley flour, as well as the of HRS flour, pastry flour and barley flour after fermentation were investigated. The linear and non-linear rheological properties of the suspensions for vital wheat gluten and barley protein were also investigated and compared with the results for suspensions of residues from fermented HRS (FHRS), Fermented Pastry Flour (FP) and Fermented Barely flour (FB), respectively. The linear viscoelastic properties for all the materials in this study exhibited viscoelastic solid behaviors. While the non-linear rheological properties of the suspensions for all measured samples displayed shear-thinning behaviors which can be well described by a power law constitutive model. Both linear and non-linear rheological properties for the FHRS suspension were similar to those for the vital wheat gluten suspension and much stronger than those for the HRS suspension. The linear and non-linear viscoelastic properties for FP were stronger than those for Pastry flour suspension but weaker than those for the wheat gluten suspension. For barley, both linear and non-linear rheological properties for FB and barley protein suspensions were close to each other and stronger than those for un-fermented barley flour suspension. According to this study, it is highly expected that the FHRS and FB will be utilized as alternative materials for vital wheat gluten and barley protein, respectively.

Key words: Barley flour, barley protein, fermentation, rheology, viscoelastic properties, wheat flour, wheat protein

INTRODUCTION

Biofules are attractive to the scientists not only because they can replace petroleum products gasoline and diesel but also they can prevent further increase of emission of carbon dioxide (Keshwani and Cheng, 2009; Hill, 2009). Now ethanol has been widely used by blending with gasoline at various percentages. However, butanol has some advantages over ethanol. Because butanol's longer hydrocarbon chain makes it more similar to gasoline than ethanol and its longer hydrocarbon chain causes it to be fairly non-polar (Balat, 2011; Ni and Sun, 2009). Other advantages are higher energy content, better blending ability, lower volatility, being used in conventional engines without modifying engines and lower water absorption (Schwarz and Gapes, 2006). But the research for bio-butanol is much less than for bio-ethanol (Zverlov *et al.*, 2006). It is expected that more and more research and utilizations for bio-butanol will be launched.

Fermentation of cereal grains to make butanol and ethanol produces a protein-rich material after the alcohol is removed (Ruane *et al.*, 2010). It is very important for the commercial success of the overall butanol or ethanol process when we optimally utilize the remains from the butanol and ethanol productions. Most varieties of wheat and barley contain more proteins than the corn used in alcohol industry. The fermentation of wheat and barley will create more protein-rich materials than the corn (Dale *et al.*, 2009). So the wisely usage of the protein-rich residues from the fermented wheat and barley becomes even more crucial for the commercial success of the entire alcohol process. The objective of this work was to determine the effect of the butanol production fermentation on the rheological properties of the remaining residue after fermentation of wheat and barley flours. Information presented here will be useful for utilizing the by-product of wheat and barley flours fermentation for butanol and any other fermentation products.

MATERIALS AND METHODS

Materials: Hard Red Spring (HRS) wheat flour (74% starch content), soft wheat flour (Pastry flour, 78% starch) and barley flour (75% starch) were obtained from North Dakota Mill (Grand Forks, ND, USA), Flaktex (Schaumburg, IL, USA) and Honeyville Grain (Brigham City, UT), respectively.

Culture maintenance and inoculum development for fermentation: *Clostridium beijerinckii* P260 that produce acetone butanol ethanol (ABE) was obtained from Professor David Jones (Otago University, Dunedin, New Zealand). Spores of *C. beijerinckii* P260 were maintained in sterile distilled water at 4°C. Medium for stage I inoculum was prepared as follows: 2.5 g cooked meat medium (CCM: Difco Laboratories, Detroit, MI, USA) and 0.25 g glucose (Sigma Chemicals, St. Louis, MO, USA) were added to 25 mL distilled water contained in a 50 mL screw capped bottle (Pyrex obtained from Fisher Scientific, USA) and soaked for 15 min prior to autoclaving at 121°C for 15 min. After autoclaving the bottle was cooled to 35°C.

To germinate, 0.1 mL of spores were transferred to 1.5 mL presterilized Appendorf™ plastic tube followed by heat shocking at 75°C for 2 min. Transferring the spores to the CCM medium followed this. Then the inoculated bottle was incubated at 35°C in an anaerobic jar for 12-16 h. The culture grown in cooked meat medium was called stage I inoculum.

Medium for stage II inoculum was prepared as follows: 3 g of glucose and 0.1 g of yeast extract (Difco) were dissolved in 93 mL distilled water contained in 125 mL screw capped bottle. The bottle was autoclaved at 121°C for 15 min followed by cooling to 35°C. To the bottle 1 mL each of P2 medium stock solutions (Vitamin, buffer and mineral) were added (Qureshi and Blaschek, 1999). Then the bottle was inoculated with 7 mL of stage I inoculum and placed in an anaerobic jar at 35°C for cell growth for 12-16 h. This was called stage II inoculum.

Fermentation: In order to prepare grain flour based medium, 7.2 g flour was dissolved in 100 mL distilled water. To solublize starch, the medium was heated at 90°C for approximately 10 min in a 200 mL beaker. To the medium 0.1 g yeast extract was added and mixed thoroughly. Then the medium was transferred to a 125 mL screw capped bottle and autoclaved at 121°C for 15 min. After autoclaving the bottle was cooled to 35°C and 1 mL each of P2 medium stock solutions were added and mixed. Prior to autoclaving pH of the medium was adjusted to 6.5-6.7 with dilute H_2SO_4/NaOH.

Then the bottle was inoculated with 5-7 mL of stage II inoculum and placed in an anaerobic jar for cell growth and fermentation to butanol. At the end of fermentation which took approximately 72-96 h, 1 mL sample was taken followed by centrifugation at 12,000 rpm in a microfuge centrifuge to remove cells and sediments. The clear liquid was analyzed for ABE production by gas chromatography. During fermentation CO_2 and H_2 gases were produced which stopped when fermentation was complete. These procedures were performed for each of Hard Red Spring (HRS) wheat flour, soft wheat flour (Pastry flour) and barley flour. After the fermentation, the remains were freeze-dried and named as Fermented HRS (FHRS), Fermented Pastry (FP) and Fermented Barley (FB), respectively.

The control was prepared by dissolving 7.2 g flour and 0.1 g yeast extract in 100 mL distilled water. After dissolving the flour, 1 mL each of P2 medium stock solutions were added and mixed thoroughly but it was not let to ferment. The control was needed to show if there is any factor that affects the fermentation process.

Acetone butanol ethanol (ABE) analyses: Fermentation products (ABE) were analyzed by gas chromatography (6890N; Agilent Technologies, Wilmington, DE, USA) using a packed column as described previously (Qureshi *et al*., 2007; Qureshi *et al*., 2008a, b). Before injection into the GC, the samples were diluted 4 fold with distilled water. In these studies approximately 22±0.5 g L^{-1} total ABE was produced of which butanol was 13.5±0.4 g L^{-1}.

Size-exclusion high-performance liquid chromatography (HPLC): Samples (10 mg) were dispersed in 1 mL of 0.1 M phosphate buffer (pH 6.9) with 0.5% SDS. Each sample was then extracted for 1 h at 40°C, then centrifuged at 3000 g for 15 min and filtered through a 0.45 μm syringe filter. The instrument used for the size-exclusion HPLC was an HP Series 1100 system (Agilent Technologies, Santa Clara, CA). Fifty microliters of sample was injected for each run and the detector was set at 214 nm wavelength. SE-HPLC was conducted using a BioSep SEC-S4000 column (Phenomenex, Torrance, CA, USA). The sample was run using isocratic 50/50 acetonitrile/water containing 0.1% TFA at a flow rate of 0.5 mL min^{-1} for 60 min.

Rheological measurements: The control flours, fermented residue flours and proteins for the rheological experiments were prepared by suspended in a 0.05M sodium phosphate buffer (pH7.0 at 25°C) at a concentration of 20% by weight (Xu *et al*., 2001). The samples were well dispersed and stored at 4°C and used within 2 days after preparation to avoid sample degradation. At least two suspension samples were made for each sample.

The methods of rheological measurements were similar to those described by Xu *et al*. (2001, 2007). A strain-controlled TA ARES rheometer (TA Instruments, New Castle, DE, USA) was used to perform the rheology studies. 50 mm diameter cone-plate geometry was used. The temperature was controlled at 25±0.1°C by a water circulation system. To ensure that all the measurements were made within the linear viscoelastic range, a strain-sweep experiment was conducted initially. An applied shear strain in the linear range was adopted for the other viscoelastic property measurements for the same material; fresh samples were used for each experiment. Linear viscoelastic behavior indicates that the measured parameters are independent of applied shear strain. The linear range for all measured suspensions was less than 0.8% of strain. Small-amplitude oscillatory shear experiments were conducted over a frequency (ω) range of 0.1-500 rad sec^{-1}, yielding the oscillatory shear storage or elastic (G') and loss (G") moduli. At low frequencies (0.1-1 rad sec^{-1}), the measurement time was between 2 to 10 min while at relative high frequencies (1-500 rad sec^{-1}), the measurement time was between few seconds to 2 min. The storage or elastic modulus (G') represents the material's non-dissipative component of mechanical properties and is characteristic of elasticity. The "rubber-like" behavior is suggested if the G' spectrum is independent of frequency and greater than the loss modulus (G") over a certain range of frequency. The loss modulus (G") represents the material's dissipative component of the mechanical properties and is characteristic of viscous flow. The phase shift or phase angle (δ) is defined by $\delta = \tan^{-1}$ (G"/G') and indicates whether a material is solid with perfect elasticity ($\delta = 0$) or liquid with pure viscosity ($\delta = 90°$), or something in between. Non-linear rheological studies were conducted at the same ARES instrument with the same geometry described above. The steady shear measurements were conducted with the shear rate increased step-wise in the range of shear rate of 0.1-500 sec^{-1}. The delay time was 10 sec. Shear-thinning rheological behavior can be characterized by a power law constitutive equation (Bird *et al.*, 1977).

RESULTS AND DISCUSSION

The SE-HPLC profile of the all fermented samples had an increased quantity of high-molecular weight and low-molecular weight proteins (data not shown) compared to the un-fermented flours. This result indicated and confirmed that the protein concentration increased after the fermentation of wheat and barley flours (Kindred *et al.*, 2008; Lacerenza *et al.*, 2008). In addition, proteins can be easily extracted due to the absence of the starch.

The linear rheological studies of dynamic frequency sweep results for the 20% (wt.%) HRS flour suspension, FHRS suspension and vital wheat gluten suspension are shown in Fig. 1. Both FHRS suspension and HRS flour suspension displayed viscoelastic solid behaviors - the shapes of the G' and G" curves were parallel; the G' were much greater than G" and the values of G' and G" were almost independent on the frequency. However, the FHRS suspension exhibited much stronger viscoelastic properties than the HRS flour suspension. The frequency-independence storage or elastic modulus (G') for the HRS flour suspension was 78 Pa at 10 rad sec^{-1}. While the frequency-independence storage modulus (G') for the FHRS suspension was 1677 Pa at 10 rad sec^{-1} which was 20 times greater than that for the HRS flour suspension (Fig. 1). The phase shifts for both FHRS and HRS flour suspensions were in the same range of 14-29°. Because fermentation made the starch and sugar become ABE and removed. The FHRS possessed mostly proteins and fibers. Apparently, the results above indicated that wheat proteins mainly contributed to the viscoelastic properties of the wheat flour. Comparing FHRS suspension and vital wheat gluten suspension, we found that they showed very similar properties. The storage modulus (G') for the gluten suspension was 1626 Pa at 10 rad sec^{-1} which was identical to that for the FHRS suspension (Fig. 1). There were some slight differences between FHRS properties and gluten properties. The shapes of the G' and G" curves for gluten suspension showed slightly more frequency dependent than those for the FHRS suspension. And the phase shifts for the gluten suspension were in the range of 20-37° which was a little bit larger than that for the FHRS suspension. The linear dynamic rheological property difference between FHRS and vital wheat gluten suggested that the two materials should have slightly structure difference. The first reason for the difference was that the procedures for producing vital wheat gluten and FHRS were totally different (Liao *et al.*, 2010). Second, vital wheat gluten was produced from several different wheat flours that made the protein content different. Overall, the linear rheological properties of FHRS and vital wheat gluten were very close to each other. Soft wheat Pastry flour suspension showed much weaker viscoelastic behaviors comparing to HRS flour suspension (Fig. 1, 2). The G' for the 20% (wt.%) Pastry

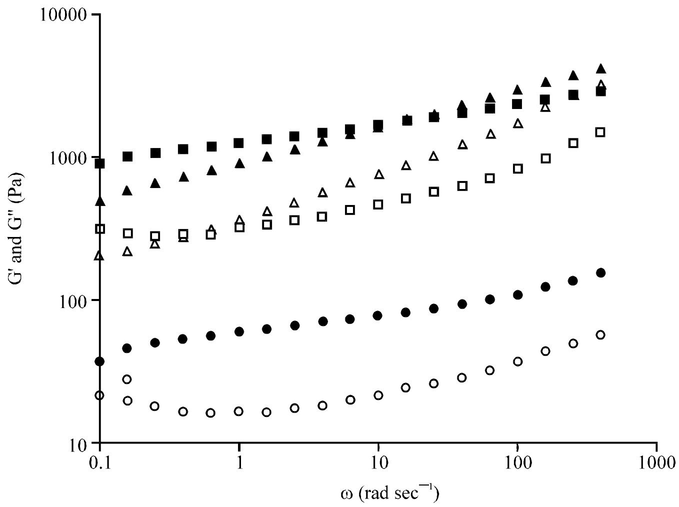

Fig. 1: The linear viscoelastic properties of dynamic frequency sweep measurements for the 20% (wt.%) suspensions of HRS flour, FHRS and vital wheat gluten at the strain of 0.8% and at the temperature of 25°C. Filled symbols: G'; opened symbols: G". (●, ○): G' and G" for the HRS flour suspension. (■, □): G' and G" for the FHRS suspension. (▲, Δ): G' and G" for the vital wheat gluten suspension

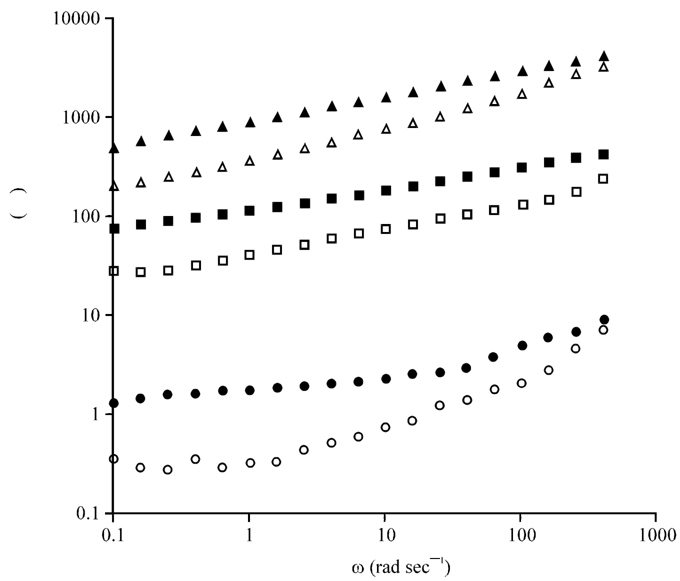

Fig. 2: The linear viscoelastic properties of dynamic frequency sweep measurements for the 20% (wt.%) suspensions of soft wheat pastry flour, FP and vital wheat gluten at the strain of 0.8% and at the temperature of 25°C. Filled symbols: G'; opened symbols: G". (●, ○): G' and G" for the Pastry flour suspension. (■, □) G' and G" for the FP suspension. (▲, Δ): G' and G" for the vital wheat gluten suspension

flour suspension was in the range of 1.3-8.9 Pa and the phase shift was in the range of 10-38°. The Fermented Pastry (FP) suspensions exhibited much stronger dynamic viscoelastic properties than un-fermented Pastry flour suspension (Fig. 2). The storage modulus (G') for FP was in the range of 74-426 Pa; the phase shift was in the range of 18-25°. The FP contained mainly proteins and fibers due to the fermentation removing the starches and sugar. Therefore, the FP suspension exhibited much stronger linear dynamic viscoelastic properties than un-fermented Pastry flour suspension. However, the FP displayed weaker properties compared with vital wheat gluten (Fig. 2). The reason for this should be that the Pastry flour contains much less wheat proteins since it is soft wheat flour. The linear dynamic frequency sweep measurements results for the 20% (wt.%) barley flour suspension, FB suspension and barley protein suspension are illustrated in Fig. 3. Both barley flour suspension and FB suspension displayed viscoelastic solid behaviors. However, the FB suspension exhibited much stronger viscoelastic properties than the barley flour suspension. The frequency-independence storage or elastic modulus (G') for the barley flour suspension was 16 Pa at 10 rad sec^{-1}. While the frequency-independence storage or elastic modulus (G') for the FB suspension was 528 Pa at 10 rad sec^{-1} which was 35 times greater than that for the barley flour suspension (Fig. 3). The phase shifts for both FB and barley flour suspensions were in the same range of 13-26°. Because fermentation made the starch and sugar become ABE and removed. The FB possessed mostly proteins as well as fibers. Apparently, the results above indicated that barley protein mainly contributed to the viscoelastic properties of the barley flour. Comparing FB suspension and barley protein suspension, we found that they showed pretty similar properties. The storage modulus (G') for the barley protein suspension was 804 Pa at 10 rad sec^{-1} which was slightly higher than that for the FB suspension (Fig. 3). The shapes of the G' and G" curves for barley protein suspension showed more frequency dependent than those for the FB suspension. And the phase shifts for the barley protein suspension were in the range of 14-36° which was a little bit larger than that for the FB suspension. The linear dynamic rheological property difference

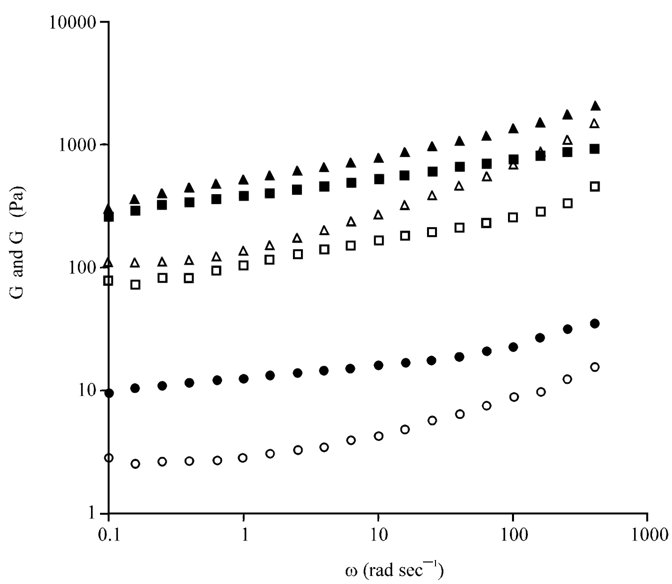

Fig. 3: The linear viscoelastic properties of dynamic frequency sweep measurements for the 20% (wt.%) suspensions of barley flour, FB and barley protein at the strain of 0.8% and at the temperature of 25°C. Filled symbols: G'; opened symbols: G". (●, ○): G' and G" for the barley flour suspension. (■, □): G' and G" for the FB suspension. (▲, Δ): G' and G" for the barley protein suspension

between FB and barley protein suggested that the two materials should have slightly structure difference. The reason for the difference was that the procedures for producing barley protein and FB were totally different (Mohamed *et al.*, 2007). Overall, the linear rheological properties of FB were slightly weaker but pretty close to those of barley protein.

To better understand the processing behavior, the non-linear steady shear viscoelastic properties for the suspensions of HRS flour, pastry flour, barley flour and FHRS, FP, FB, as well as vital wheat gluten and barley protein were studied. All of the studied suspensions exhibited shear-thinning behavior over the entire measured shear rates (Fig. 4-6). Shear-thinning rheological behavior can be characterized by a power law constitutive equation.

Power law equation was used to fit shear-thinning viscosity for all measured suspensions. The experimental data were very well fitted by the power law constitutive equation (Fig. 4-6). The results of the fits are summarized in Table 1. The FHRS had higher viscosities than HRS flour suspension which is in the. The viscosities of same trend as their linear rheological property results FHRS suspension were very close to those of vital wheat gluten suspension; only slightly lower (Fig. 4). From Table 1, we can get the impression that FHRS suspensions and vital wheat gluten possess the same power law exponent of 0.01. Thus FHRS and gluten have the same shear-thinning extent. Combined with linear rheological behaviors shown above, we found that both linear and non-linear viscoelastic properties for FHRS and vital wheat gluten were very close to each other. Therefore, FHRS should possibly be used as an alternative material for gluten, or should be individually used as a viscoelastic material in both food and non-food utilizations. For instance, according to its viscoelastic behaviors, FHRS could be used as meat substitutes and as a gum material in food applications (Vinod *et al.*, 2008) and could also possibly be used as a plastic-like material by chemical modification (Lagrain *et al.*, 2010). The viscosities of FP were higher than those of Pastry flour suspension but lower than those of gluten suspension (Fig. 5). These results

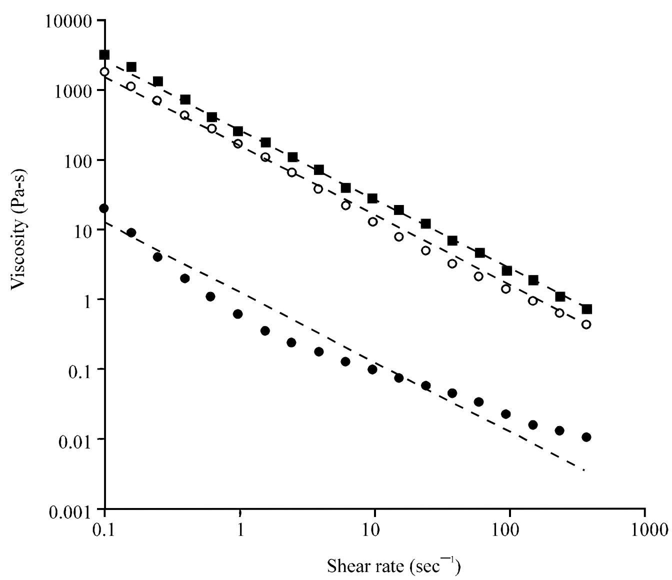

Fig. 4: The non-linear viscoelastic properties of the steady shear measurements for the 20% (wt.%) suspensions of HRS flour, FHRS and vital wheat gluten at the temperature of 25°C. Symbols are experiment results. Dashed lines are fitted with power law model. (●): viscosity for the HRS flour suspension. (○): viscosity for the FHRS suspension. (■): viscosity for the vital wheat gluten suspension

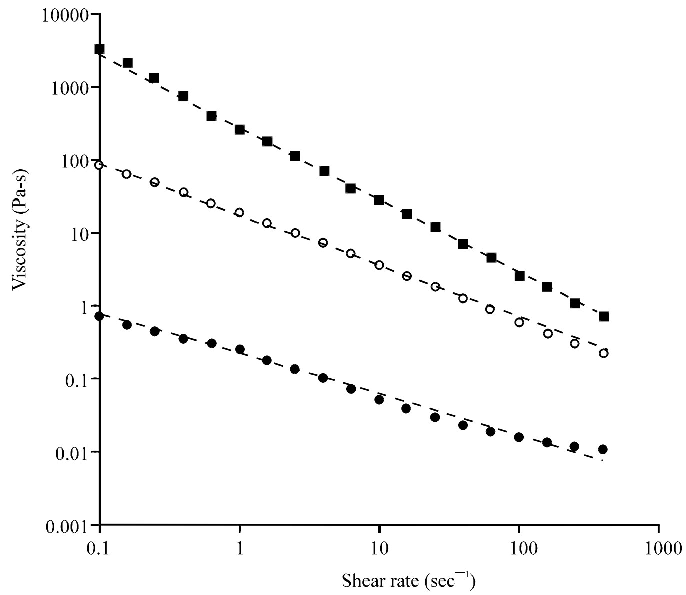

Fig. 5: The non-linear viscoelastic properties of the steady shear measurements for the 20% (wt.%) suspensions of soft wheat Pastry flour, FP and vital wheat gluten at the temperature of 25°C. Symbols are experiment results. Dashed lines are fitted with power law model. (●): viscosity for the Pastry flour suspension. (○): viscosity for the FP suspension. (■): viscosity for the vital wheat gluten suspension

Table 1: Power law model fitted parameters

Suspension of the sample	K (Pa-s^n)	n	R^2
HRS flour	1.25	0.01	0.88
Fermented HRS (FHRS)	161.88	0.01	0.98
Vital wheat gluten	270.70	0.01	0.97
Pastry flour	0.22	0.38	0.95
Fermented Pastry (FP)	17.72	0.30	0.97
Barley flour	0.55	0.17	0.90
Fermented Barley (FB)	55.96	0.01	0.97
Barley protein	87.50	0.01	0.95

K is the front factor and n is the power law exponent of the power law equation. R^2 is the coefficient of determination

had the same trend as the results for the linear viscoelastic properties of Pastry flour, FP and gluten suspensions. The results of both linear and non-linear rheological properties for pastry flour, FP and gluten suspensions suggested that FP display much stronger viscoelastic behaviors than Pastry flour but weaker than gluten. Thus, the FP may not replace the usage of gluten material. However, the FP can still be used as a viscoelastic material in many food and non-food applications, e.g. in cake and biscuits making and as cosmetic gels (Storz *et al.*, 2010; Perego *et al.*, 2007). The FB suspension had higher viscosities than barley flour suspension. The viscosities for FB and barley protein suspensions were very close to each other and they had the identical power law exponent of 0.01 (Fig. 6, Table 1). Overall, both linear and non-linear rheological properties of FB and barley protein were very similar to each other. Therefore, there should be a high potential to use FB as an alternative material for barley protein. Besides as an alternative material for barley

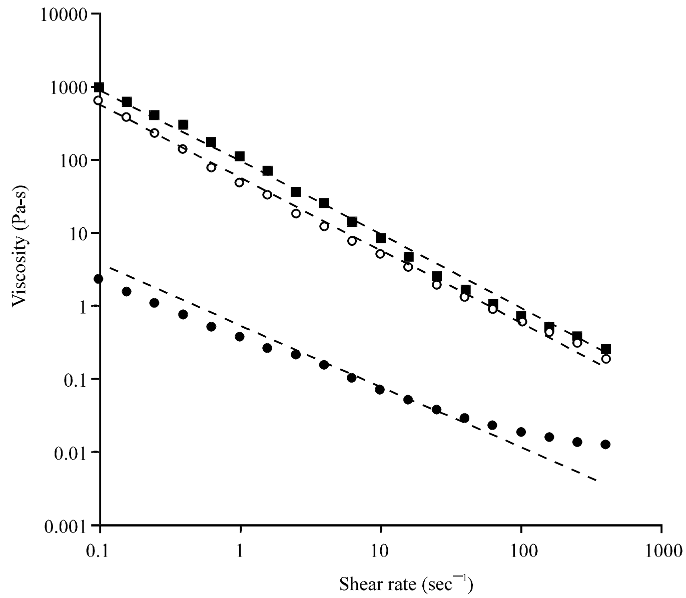

Fig. 6: The non-linear viscoelastic properties of the steady shear measurements for the 20% (wt.%) suspensions of barley flour, FB and barley protein at the temperature of 25°C. Symbols are experiment results. Dashed lines are fitted with power law model. (○): viscosity for the barley flour suspension. (●): viscosity for the FB suspension. (■): viscosity for the barley protein suspension

protein, FB can have many applications individually as a viscoelastic material in food and non-food industry. For example, the FB could be used as feed in poultry and as a binder and adhesive in polymer industry, etc. From Table 1, it is also noticed that FB suspension and barley protein suspension as well as FHRS and vital wheat gluten suspensions possessed the same power law exponent of 0.01. Thus FB and barley protein suspensions as well as FHRS and gluten suspensions had the same shear-thinning extent. These results suggested that FB, barley protein, FHRS and gluten should have similar behavior as they are in the processing.

CONCLUSION

In summary, the remains from the fermented hard red spring wheat flour (FHRS) exhibited similar linear and non-linear viscoelastic behaviors as vital wheat gluten. Similarly, both linear and non-linear viscoelastic behaviors of the remains from the Fermented Barley flour (FB) and barley protein were close to each other. Thus, the FHRS would be a possibly alternative material for vital wheat gluten and the FB would be a possibly alternative material for barley protein. In addition, as viscoelastic polymer materials, the FHRS and FB could individually have many applications in food and non-food industry. The residues from the fermented soft wheat Pastry Flour (FP) exhibited stronger rheological properties than the un-fermented Pastry flour but much weaker than vital wheat gluten. The FP could not replace the vital wheat gluten but still could be used as a viscoelastic material in many food and non-food applications.

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