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PERTETA

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Ucapan Terima Kasih

Technical Paper

Flow Behavior of Isolate Protein from Soybeans var. Grobogan and Whey Protein Isolate at Acidic Condition under Various Heating Times

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Abstract

Flow behavior of Soy Protein Isolate (SPI) suspension and Whey Protein Isolate (WPI) solution at pH 2.0 under various heating times were studied using steady shear viscosity measurements. Shear rate sweeps with increasing shear rates (up ramp) was performed to investigate the structural breakdown of the proteins during shearing. Down ramp shear rates were performed to check structural recovery of the proteins. The results showed that unheated SPI suspension has Newtonian flow; meanwhile, unheated WPI solution was slightly shear thickening. Heating the proteins at 80ºC for 4, 8, 12, and 16 h changed flow behavior of the proteins. Flow curve of SPI suspension heated for 12 h and 16, fitted Ostwald model with flow behavior index (n) of 0.625 and 0.264, respectively. This index indicates pseudoplastic (shear thinning) behavior, which also observed in heated WPI solution. The changes in flow behavior was attributed by the changes in protein structures, i.e., globular structures into fibrillar structures under prolonged heating at acidic condition. This conversion also increased the apparent viscosities of the proteins. SPI fibrils have higher apparent viscosities than WPI fibrils. This difference might be attributed to the detail fibril structures. SPI fibrils have branched and curvy structures; meanwhile, WPI fibrils are long and straight.

Keywords: flow behavior, protein fibrils, soy protein isolate viscosity, whey protein isolate.

Abstrak

Perilaku aliran suspensi *Soy Protein Isolate* (SPI) dan larutan *Whey Protein Isolate* (WPI) pada pH 2.0 pada berbagai lama pemanasan diinvestigasi. *Shear rate* yang meningkat diaplikasikan untuk mengetahui kerusakan struktur protein selama geseran. *Shear rate* dengan pola menurun dilakukan untuk mengetahui apakah struktur protein kembali ke struktur awal setelah mengalami kerusakan. Hasil penelitian menunjukkan bahwa suspensi SPI yang tidak dipanaskan memiliki perilaku aliran *Newtonian*; sementara larutan WPI yang tidak dipanaskan bersifat sedikit *shear thickening*. Pemanasan protein pada 80ºC selama 4, 8, 12, dan 16 jam mengubah perilaku aliran suspensi SPI dan larutan WPI. Kurva aliran suspense SPI yang dipanaskan selama 12 jam dan 16 jam sesuai dengan model Ostwald dengan indeks perilaku aliran (*n*) masing-masing 0.625 dan 0.264. Indeks ini mengindikasikan perilaku aliran bersifat *pseudoplastic* (*shear thinning*), yang juga teramati pada larutan WPI yang dipanaskan. Perubahan perilaku aliran disebabkan oleh perubahan struktur protein dimana SPI dan WPI awalnya memiliki struktur *globular* lalu menjadi struktur f*ibrillar* akibat pemanasan yang lama pada kondisi asam. Perubahan struktur juga meningkatkan nilai *apparent* viskositas, dimana viskositas fibril SPI lebih tinggi daripada fibril WPI. Perbedaan ini diakibatkan oleh perbedaan struktur fibril protein dimana SPI berbentuk fibril yang bercabang dan melengkung sedangkan WPI berbentuk fibril yang lurus dan panjang.

Kata kunci: fibril protein, sifat aliran, *soy protein isolate*, viskositas, *whey protein isolate*.

Introduction

Soybean-based products are well-known for their high protein content. The famous soybean-based products from Indonesia are tofu, tempeh, and sweet soy sauce. These products are affordable and consumed daily. One of superior local cultivar is var. Grobogan (*Glycine max* (L.) Merill) that is originally cultivated in Grobogan Regency, Central of Java, Indonesia. It has white yellowish pod of seed with the weight of 16 to 20 g per 100 seeds and contains high protein, 42.3% (Nurrahman 2015). High quality of local soybean cultivars, as represented by their protein content, opens possibilities to utilize them for much higher added-value product than tofu, tempeh, or sweet sauce.

High protein content of soybean potentially can be extracted and formed as soy protein isolate (SPI), i.e. containing at least 90% of protein (Warji *et al.* 2017). SPI functions as an emulsifier (Keerati-u-rai and Corredig 2009), a gelling agent (Cramp *et al.* 2008, Liu *et al.* 2015), a biomedical application (Liu *et al.* 2017, Wongkanya *et al*. 2017), and forms amyloid fibrils at acidic conditions under long heating (Akkermans *et. al.* 2007, Hefnawy and Ramadan 2011, Lassé *et al.* 2016, Tang and Wang 2010, Tang *et al.* 2012, Xiao *et al.* 2016, Xia *et al.* 2017, Yildiz *et al.* 2017). SPI fibrils can be used as building blocks of multilayer microcapsules (Gomez-Mascaraque and Lopez-Rubio 2016, Lee 2016, Warji *et al.* 2017, Purwanti *et al.* 2017). Other products based on SPI fibrils might be formed but their characteristics need to be elucidated; therefore, appropriate processes can be applied.

An important characterization that could be used as basis to design certain unit operations or to control the quality of food manufacturing process and final products is flow behavior (Bazinet *et al.* 2004). An example, flow behavior obtained from the action of mixing could be related to the power input during dough processing (Steffe 1996). Dough mixing is also a measure for flour quality and dough behavior during bakery such as dough development time, stability and degree of softening. Flow behavior of food materials could be drastically altered due to various processes such as heating, cooling, compounding, homogenization, etc. Specifically, other parameters such as concentrations and pH value affect flow behavior of proteins (Bazinet *et al.* 2004, Dissanayake *et al*. 2013). Combination of those two parameters and temperature would greatly affect proteins structures, thus, its flow behavior (Kamada *et al*. 2017). This research specifically studied flow behavior of SPI suspension and solution of SPI fibrils at acidic condition under various heating times in which the protein was isolated from soybean var. Grobogan. As references, flow behavior of WPI and WPI fibril solutions was also determined.

Materials and Methods

Materials

Soybeans var. Grobogan (non-GMO) was supplied by Grobogan Soybean House, Grobogan Regency, Central of Java, Indonesia. WPI (BiPro® JE 193-3-420) was kindly provided by Davisco Food International Inc. (Le Sueur, Minnesota, USA) with a reported protein content of 97.9% on dry basis. The chemicals used were n-hexane (AR, Cat. No. A-1045, Smart-Lab, Indonesia), 2-Mercaptoethanol for synthesis (Cat. No. 805740, Merck, Germany), Tris (hydroxymethyl) aminomethane (Cat. No. 108382, Merck, Germany), and HCl 37% (Cat.No. 1000317, Merck, Germany). Double distilled water was used to dissolve protein.

Preparation of protein isolate from soybean var. Grobogan

The protein was isolated according to Warji *et al.* (2017) which was modified methods from Kuipers *et al.* (2005) and Akkermans *et al.* (2007). In brief, soybean meal (SBM) was prepared from dehulled soybeans and it was defatted five times using hexane with SBM/hexane ratio of 1:3 at room temperature. The defatted SBM was then suspended in 30 mM Tris-HCl buffer pH 8 that contained 10 mM of 2-mercaptoethanol with SBM/buffer ratio of around 1:10. After 1.5 h stirring, the suspension was centrifuged at 19,000 ×*g*, 20°C for 30 min (Beckman, Model J2-21 Ultracentrifuge, USA) and filtered (Whatman, Schleicher & Schuell, type 595, Cat. No.1001125). The supernatant was collected and its pH was set to pH 4.8 to induce protein precipitation. The precipitate was harvested using centrifugation at 19,000 ×*g*, 20°C for 30 min and it was washed twice using double distilled water. After washing, the precipitate was re-suspended in double distilled water, set the pH to pH 8, stirred overnight and then, freeze-dried for 24 h. The result was soy protein isolated from soybean var. Grobogan.

Preparation of protein suspension/solution

SPI powder was dispersed in double distilled water (2% w/w) and formed SPI suspension. WPI solution was also prepared at the same concentration. SPI suspension and WPI solution were stirred overnight at room temperature to enhance solubilization. Afterwards, pH of the suspension/solution was set to 2.0 using 6 N HCl solution. The protein suspension/solution was then heated in a water bath at 80°C (internal temperature of the suspension/solution) while stirring for 4, 8, 12 and 16 h.

Measurement of flow behavior

Flow behavior of unheated and heated proteins as described above was measured using a controlled-stress rheometer (Rheometer Anton Paar MCR301, Anton Paar GmbH, Austria). The protein suspension/solution was placed in a concentric cylinder measuring system (CC17) attached to the Peltier temperature device for concentric cylinder system (C-PTD 200,

Anton Paar GmbH, Austria). Shear rate sweeps with up ramp from 1 s^{-1} to 500 s^{-1} and down ramp from 500 s^{-1} to 1 s^{-1} were performed at 30°C. Thirty data points for each ramp were recorded in which each data point was measured for 5 s. The results were presented as viscosity (mPa·s) and shear stress (Pa) as a function of shear rate (s^{-1}). The data were fit in Ostwald model as follows:

$$\eta = k\dot{\gamma}^{n-1} \tag{1}$$

or in terms of shear stress:

$$\sigma = k\dot{\gamma}^{n} \tag{2}$$

where η is the viscosity (mPa·s), σ is shear stress (mPa), $\dot{\gamma}$ is the shear rate (s^{-1}), k (Pa·s^{n}) is the consistency and n is the power-law index, respectively (Barnes 2000). The power law index indicates flow behavior of the solution. The solution that behaves as Newtonian fluid is shown by n=1. If n<1, then the solution behaves as pseudoplastic (shear thinning) and if n>1, then the solution behaves as dilatant (shear thickening).

When different flow curves during up ramp and down ramp shear rates exist (hysteresis), it means that protein structures might be thixotropic materials. The thixotropic properties of a material can be evaluated using its hysteresis curves. Area under the hysteresis loop is used to determine the thixotropic strength of the material structures under shearing. Hysteresis loop area is calculated from the data of shear stress as a function of shear rate using Eq. (3) (Liu *et al.* 2017). The area was obtained by the difference between integrated shear stress during up ramp shear rates and down ramp shear rates.

$$Hysteresis\ loop\ area = \int_{\dot{\gamma}_1}^{\dot{\gamma}_2} k\dot{\gamma}^{n} - \int_{\dot{\gamma}_2}^{\dot{\gamma}_1} k'\dot{\gamma}^{n'} \tag{3}$$

where k is the consistency coefficient during up ramp shear rates, k' is the consistency coefficient during down rump shear rates, n is flow behavior index for up ramp shear rates, and n' is flow behavior index for down ramp measurements.

The thixotropic magnitude can also be evaluated using a breakdown coefficient, K_d, which is defined as the ratio of hysteresis areas under up ramp and down ramp flow curves as shown in the following equation:

$$K_d = \frac{A_{up} - A_{Down}}{A_{up}} \tag{4}$$

Where, A_{up} and A_{down} are the areas under up ramp and down ramp flow curves, respectively (Dokic *et al.* 2010). In addition, K_d is an index of the energy required to break down the thixotropic structures.

Results and Discussion

Soybeans var. Grobogan and the protein isolates

Figure 1 showed soybeans var. Grobogan (a), the isolated protein from the soybeans (b) and whey protein isolate (c). The soybeans have yellowish color; meanwhile the isolated protein is white. The color of SPI powder is similar to that of WPI powder. The color difference between the SPI powder and the soybeans is mainly attributed by the fat contained in the beans. The fat was removed during protein isolation and as the result, white protein powder was obtained.

Flow behavior and viscosity of SPI and WPI

Figure 2 shows the flow curves of unheated 2% w/w SPI suspension at pH 2.0 and the heated suspension. The up-ramp shear rates of unheated SPI suspension and the heated suspension for 4 h and 8 h show that the protein suspension/solution has Newtonian behavior, i.e., the viscosity does not change by increasing the shear rates. The flow curves fit Ostwald model with flow behavior index (n) of one, which confirm Newtonian flow (Fig. 2(a)). This flow behavior did not change when down-ramp shear rate was applied as shown in Fig. 2(b).

The apparent viscosity of the heated SPI suspension for 4 h is slightly lower than the unheated one. Visually, dispersion of 2% w/w SPI in water formed a suspension. The suspension turned into solution-like system after heating for 4 h and even longer time. It seems that heating helped to hydrolyze the protein better, therefore, a protein solution was eventually obtained and its viscosity was slightly lower than a protein suspension. Further heating the protein solution for 8 h increased the apparent viscosity. The

(a)   (b)   (c)

Figure 1. (a) soybeans var. Grobogan, (b) the isolated protein powder of (a), and (c) whey protein isolate.

increase in viscosity might indicate changes in protein structures. This is confirmed by flow behavior of the protein solution that was pseudoplastic/shear thinning after heating for 12 and 16 h (n = 0.625 and 0.264 for SPI solution heated for 12 and 16 h, respectively). Shear thinning might occur if protein structures re-arrange themselves in the flow direction or undergo structural breakdown upon shearing that reduces their resistance to flow. These phenomena might not take place in dilute globular structures. This shear thinning behavior does not change when down-ramp shear rates was applied. However, the flow behavior index of down-ramp shear rates is higher than that of up-ramp shear rates (Table 1), which indicates hysteresis.

Figure 3 shows flow curves of unheated and heated WPI solutions at the same conditions as SPI suspension/solutions. Unheated WPI solution was slightly shear thickening (n=1.106) during up-ramp shear rates (Fig. 3(a)). The down-ramp shear rates also show similar flow behavior index (n = 1.066)

which indicates that the flow behavior of unheated WPI solution remained the same during backward measurement (Fig. 3(b)). Heating WPI solution seems to change globular structures of the protein, even at shortest heating time applied in this research (4 h). The heated WPI solution was shear thinning with similar flow index behavior at all heating times (n = 0.585, 0.581, 0.558, and 0.640 for WPI solution heated for 4, 8, 12 and 16 h, respectively). These indices might suggest that fully conversion of globular structures of WPI was completed after 4 h of heating.

The viscosity changes between unheated – heated SPI suspension and unheated – heated WPI solution are significantly different. The increase in apparent viscosity of heated SPI suspension is much higher than that of heated WPI solution. For an example, the apparent viscosity of heated WPI solution for 16 h, at shear rate of 1 s^{-1} is 72.65 mPa·s, which is approximately 10 times lower than that of heated SPI suspension for the same time. The difference might

Figure 2. Flow curves of unheated SPI suspension and heated SPI solutions at pH 2.0, measured at 30 °C: (a) up-ramp and (b) down-ramp shear rate measurements. (–⊖–) denote unheated SPI, (–⊟–) heated SPI heated at 80°C for 4 h, (–◇–) 8 h, (–⊟–) 12 h, and (–◆–) 16 h.

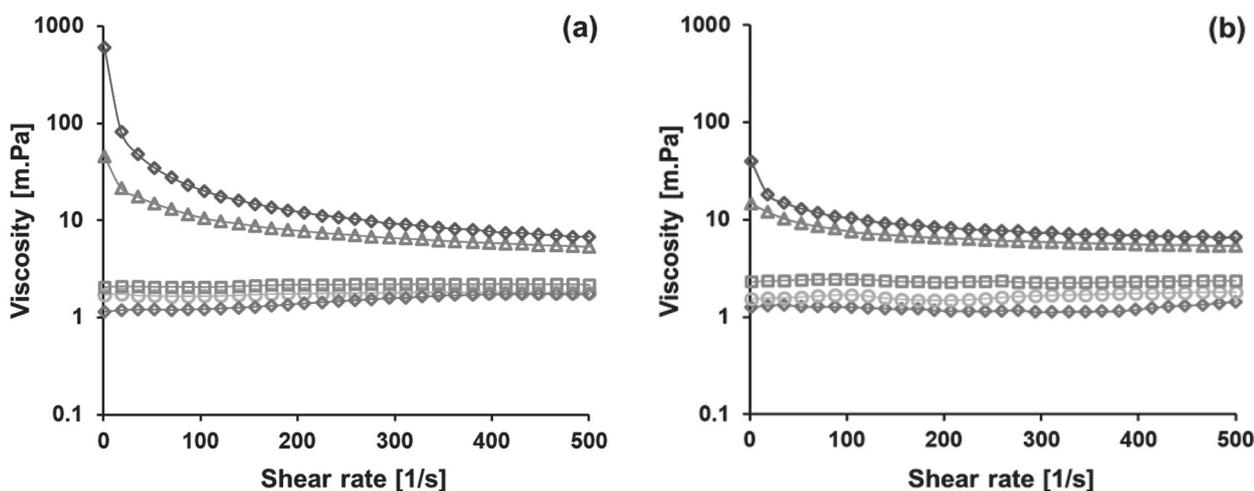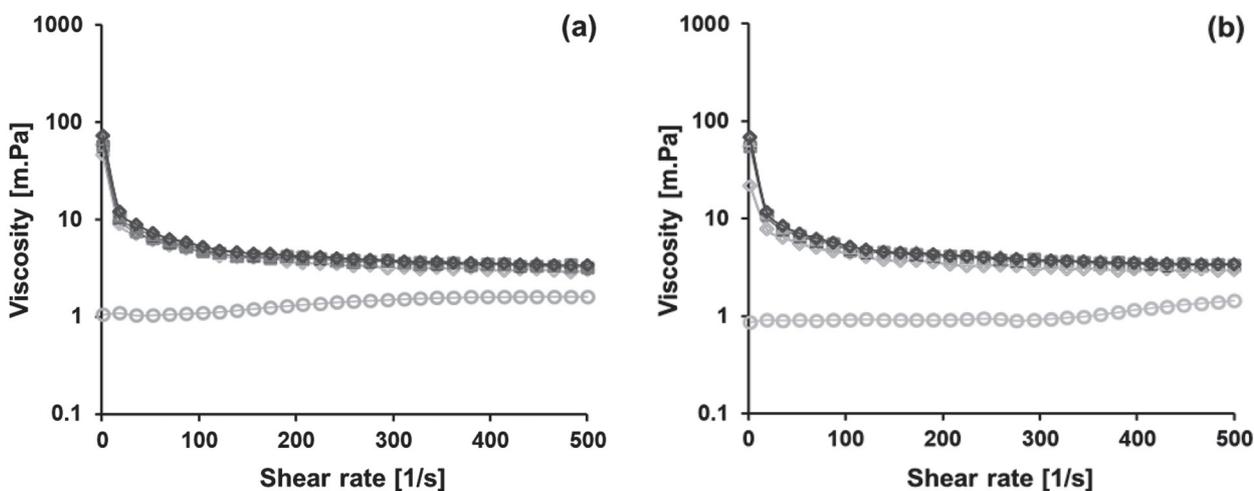Figure 3 Flow curves of unheated and heated WPI solutions measured at 30 °C and pH 2.0: (a) up-ramp and (b) down-ramp shear rate measurements.(–⊖–) denote unheated SPI, (–⊟–) heated SPI heated at 80°C for 4 h, (–◇–) 8 h, (–⊟–) 12 h, and (–◆–) 16 h.

Table 1. Parameters from data fitting to Ostwald model and thixotropic quantities.

Heating time (hours)	Up ramp shear rates			Down ramp shear rates			Kd	Loop area
	k	n	R^2	k'	n'	R'^2		
SPI								
0 (unheated)	0.003	0.936	0.995	0.002	1.006	0.996	0.046	9.934
4	0.001	1.108	0.979	0.001	1.155	0.984	0.064	10.341
8	0.002	1.040	0.997	0.002	0.995	0.996	0.004	0.943
12	0.057	0.625	0.992	0.018	0.808	0.997	0.123	105.222
16	0.625	0.263	0.991	0.042	0.699	0.999	0.257	327.096
WPI								
0 (unheated)	0.001	1.087	0.974	0.001	1.060	0.955	0.024	3.438
4	0.020	0.679	0.997	0.035	0.585	0.985	0.029	12.186
8	0.040	0.581	0.979	0.039	0.581	0.972	0.016	7.659
12	0.045	0.558	0.969	0.045	0.559	0.976	0.006	2.579
16	0.051	0.540	0.973	0.048	0.551	0.974	0.005	2.486

be attributed by different protein structures. Warji et al. (2017) showed that heating SPI suspension at pH 2.0 for 16 h converted globular structures of proteins into fibrillar structures that were branched and curvy; meanwhile, heating WPI solution at the same conditions resulted in straight and long protein fibrils.

Hysteresis loop

Flow curves as shown in Figure 2 and 3 can also be depicted in terms of shear stress as function of shear rates as shown in Figure 4. Shear stress profiles of unheated and heated SPI for 4 and 8 h are similar. Prolonged heating to 12 and 16 h resulted in much higher shear stresses and increased the area of hysteresis loop.
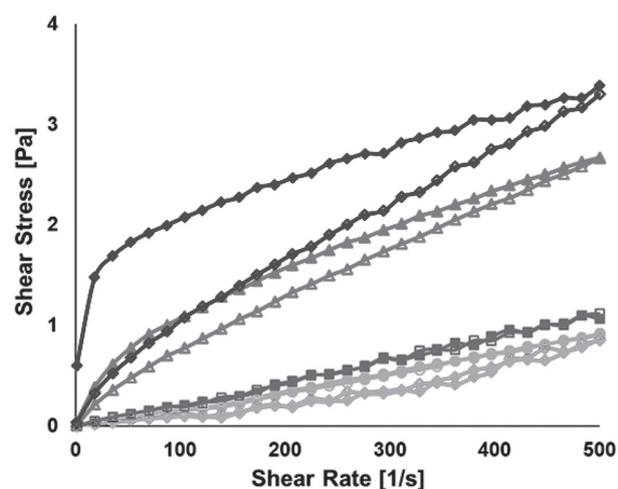
Shear stress profiles for unheated and heated WPI solutions at the same conditions are depicted in Figure 5. All heated WPI solutions have similar shear stress profiles, which are completely different from shear stress profile of unheated solution. The shear stresses developed during heating were also much lower that those developed by SPI suspension. The shear stress profiles of WPI solutions indicate that hysteresis did not occur.

Hysteresis loop is only observed in heated SPI which is an indication that heated SPI was thixotropic material. The loop is clockwise, which means that shear stresses in increasing shear rates are higher than those in decreasing shear rates (Liu *et al.* 2017). The clockwise hysteresis loop is considered as

Figure 4. Shear stresses of unheated and heated SPI as function of shear rates. (–●–) denotes up ramp unheated SPI and (–○–) the down ramp; (–◆–) up ramp heated SPI for 4 h and (–◇–) the down ramp); (–■–) up ramp heated SPI for 8 h and (–□–) the down ramp; (–▲–) up ramp heated SPI for 12 h and (–△–) the down ramp; (–◆–) up ramp heated SPI for 16 h and (–◇–) the down ramp.
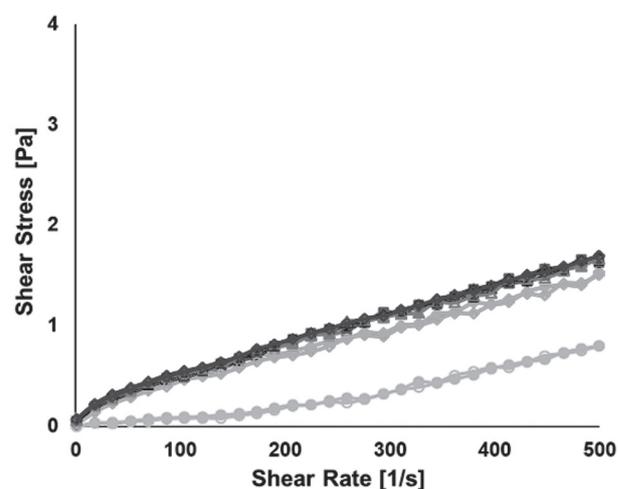
Figure 5. Shear stress curves of unheated and heated WPI solutions. (–●–) denotes up ramp unheated SPI and (–○–) the down ramp; (–◆–) up ramp heated SPI for 4 h and (–◇–) the down ramp); (–■–) up ramp heated SPI for 8 h and (–□–) the down ramp; (–▲–) up ramp heated SPI for 12 h and (–△–) the down ramp; (–◆–) up ramp heated SPI for 16 h and (–◇–) the down ramp.

structural breakage by shearing or new rearrangement of structures (Liang *et al.* 2014). Analysis results of hysteresis loops to describe thixotropic properties of the proteins are listed in Table 1.

Heated SPI for 16 h has the highest breakdown coefficient (K_d), i.e., 0.257. This coefficient decreases with lower heating times. Since K_d is an energy index to break down the thixotropic structures, it means that the energy needed to break up SPI structures heated for 16 h is the highest among other heating times. The K_d for WPI solutions were also calculated and resulted in very low numbers, which is an indication that hysteresis barely exists.

Potential applications of SPI fibrils

Comparison of flow behaviors between SPI fibrils and other products is depicted in Figure 6. This comparison is a step to map potential application of SPI fibrils in food products. Based on slope of the curves shown in Figure 6, there is an indication that flow behavior of SPI fibrils is more pseudoplastic than that of strawberry puree but the pseudoplastic behavior of SPI fibrils is slightly less than that of yoghurt. The apparent viscosities of SPI fibrils are much lower than those of strawberry puree and yoghurt but they are much higher than fresh milk and WPI fibrils. Nevertheless, flow behavior and viscosities of SPI fibrils depicted in Figure 6 were based on 2% w/w of SPI powder. Strong pseudoplastic behavior or even different behavior and much higher viscosities might be obtained by increasing concentration of SPI fibrils. Based on this simple mapping, SPI fibrils might be applied as a food thickener or a new product ingredient, e.g., an ingredient for yoghurt.

Conclusions

Flow behavior of heated SPI and WPI pH 2.0 changed with increasing heating time with the strongest shear thinning behavior was observed in SPI heated for 16 h. This treatment also resulted in the highest apparent viscosities. The distinct flow behavior and apparent viscosities of heated SPI at acidic condition for l6 h were attributed by its structures, i.e., branched and curvy fibrils. Different protein structures as indicated in heated WPI resulted in less shear thinning solution and lower apparent viscosities. The flow behavior study indicated that SPI fibrils are thixotropic materials. It means that shear thinning behavior of the fibrils is time dependent.

Acknowledgment

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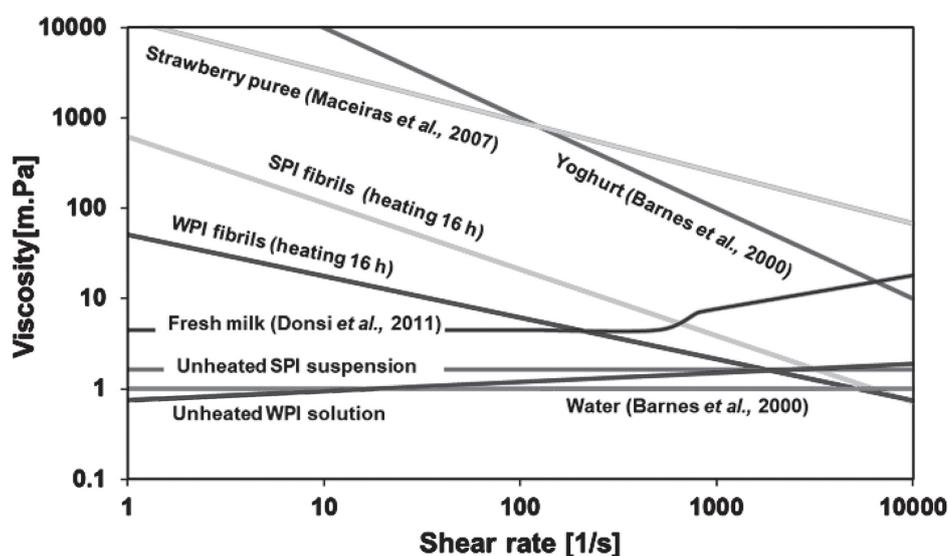Barnes, H.A. 2000. A Handbook of Elementary Rheology. Cambrian Printers: Wales, UK.

Figure 6. Flow behavior curves and viscosity profiles of WPI and SPI (unheated proteins and the fibrils) and other products (water, fresh milk (Donsi *et al.* 2011), yoghurt (Barnes *et al.* 2000), and strawberry puree (Maceiras *et al.* 2007) measured at room temperature (25° C – 30° C)).

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