

# Rheological and AFM Characteristic of Hydrolysates Produced by Limited Enzymatic Hydrolysis of Soy Flour Extrudates\*

M. WITCZAK\*\*, A. PTASZEK, D. ŻMUDZIŃSKI, K. SURÓWKA, and M. GRZESIK

*Faculty of Food Technology, University of Agriculture, PL-30 149 Kraków  
e-mail: rrwitcza@cyf-kr.edu.pl*

Received 1 April 2005

Effect of limited proteolysis of extruded soy flour on rheological properties and microstructure of the obtained hydrolysates was investigated. Flow curves with a controlled shear rate and time-dependent curves with a constant shear rate were determined. The surface of investigated material was studied with atomic force microscope Quesant “Nomad” model. Rheological characteristics have been studied using a Cheng and Evans structural theory. Multiple exponential flow law was used to calculate non-Newtonian equilibrium viscosity. The shear stress was modified using structural parameter accounting for time-dependent effect. These studies have shown a significant influence of the enzyme used upon the rheological properties and structure of suspensions of hydrolysis products.

Hydrolysis and extrusion are important methods used to modify the physicochemical properties of protein preparations. Implementation of these methods allows obtaining products with significantly changed characteristics in comparison to the original matter. Thus, it is frequently employed in food technology.

The unique properties of extruded soy protein preparations are a result of high temperature, pressure, and shear forces existing in the extruder barrel. In such conditions protein molecules undergo unfolding or disaggregation. High shear may well force molecules to favour relatively extended chain conformations, which then become oriented in the shear field. At high temperatures and low moisture contents met on extrusion processes, forces usually essential for protein—protein interactions, *e.g.* hydrogen bonds and hydrophobic interactions, usually do not operate. Electrostatic forces, still very strong, become the dominant forces in controlling the mode of interaction between the macromolecules in extruder. Disulphide and non-covalent bonds are subsequently formed on cooling [1]. As a result texturized products with fibrous structure are obtained. Such thermally modified products can serve as raw materials for limited enzymatic proteolysis. To this last method, applied to non-extruded proteins, particular attention has been devoted during the last decade. Hydrolysates, besides their usually enhanced biological value, are charac-

terized by many properties superior to those ones of starting protein [2, 3]. Properly controlled proteolytic enzymes activity can contribute to increase solubility, foaming and emulsifying properties, while maintaining relatively high water-holding capacity and viscosity. The results of hydrolysis depend not only on the enzyme used and conditions of the process, but also on the kind of raw material and the preparation procedure. Such relationships were indicated by *Henn and Netto* [4] who showed that various commercially available soy protein isolates hydrolysed with pancreatin required various time to reach the same degree of hydrolysis. Moreover, the profiles of molecular masses of peptides in the final products were differentiated with a direct impact on their functional properties. Recently, it was found by SDS-PAGE electrophoresis that enzymatic proteolysis of extrudates, despite of reaching similar degree of hydrolysis, proceeds to higher extent than hydrolysis of non-texturized proteins [5, 6]. Hence, the functional properties of appropriate hydrolysates will be different.

The aim of the present work was to examine the effect of limited proteolysis of extruded soy flour (ExSF) on rheological properties and microstructure of the obtained hydrolysates. It was assumed that limited proteolysis would allow obtaining product, which would combine features typical for extrudates and hydrolysates.

\*Presented at the 32nd International Conference of the Slovak Society of Chemical Engineering, Tatranské Matliare, 23—27 May 2005.

\*\*The author to whom the correspondence should be addressed.

## EXPERIMENTAL

In order to obtain ExSF defatted soy flour Nutrisoy 7B (ADM, Decatur, IL, USA), with a total protein content of 50.6 % ( $N \times 6.25$ ), was subjected to extrusion in an industry-standard twin-screw extruder Llesse BV (Arnhem, Netherlands) equipped with two screws in a high shear configuration. Moisturised raw material was introduced into the extruder at a rate of  $3 \text{ kg min}^{-1}$ . The obtained ExSF was dried in the tray cabinet dryer at room temperature, ground in an impact mill and sieved to collect the fraction of  $200 \mu\text{m}$ .

Prepared extrudates were suspended in water ( $w = 0.138$ ) and a desired pH of the suspension was adjusted using either  $3 \text{ mol dm}^{-3}$  HCl or  $3 \text{ mol dm}^{-3}$  NaOH solutions. Each suspension was heated to reach an appropriate temperature, pH value was adjusted, and then enzyme was added. During proteolysis conducted in a water bath with agitation, pH was checked every 10 min and readjusted, if required, to reach the optimal value. After proteolysis, suspension was heated to  $95^\circ\text{C}$  for 10 min to inactivate the enzyme, and then quickly cooled, neutralised, and freeze-dried. Dry product was crushed and sieved to collect the fraction of  $200 \mu\text{m}$ .

For protein extrudates hydrolysis Neutrase of *Bacillus* origin ( $\rho = 1.5 \text{ AU g}^{-1}$ ) and Protamex of *Bacillus* origin ( $\rho = 1.5 \text{ AU g}^{-1}$ ), both produced by Novo Nordisk A/S (Bagsvaerd, Denmark), were used. Hydrolysates were obtained at optimum conditions:  $50^\circ\text{C}$ , pH = 6.8, 60 min, 13 g of water added per g of protein. The amount of enzymes used for hydrolysis was  $18 \text{ AU g}^{-1}$  and  $12 \text{ AU g}^{-1}$  for Neutrase and Protamex, respectively [6].

Measurement of rheological properties of hydrolysates was performed using a rotational Rheometer RheoStress RS 150 (Haake, Germany) in the cone-plate system (cone diameter 35 mm, angle  $2^\circ$ , gap size 0.105 mm). Samples of powdered hydrolysates were mixed with water in a mass ratio 1 : 3.5, equilibrated at  $4^\circ\text{C}$  overnight, de-aired, put into a measuring system, allowed to relax, and heated to the measuring temperature for 10 min.

Flow curves with a controlled shear rate (CR mode) at a temperature of  $(25 \pm 0.2)^\circ\text{C}$  were determined. Shear rate varied within a range 1–1000  $\text{s}^{-1}$ . Time-dependent curves with a constant shear rate (1–1000  $\text{s}^{-1}$ ) at a temperature of  $(25 \pm 0.2)^\circ\text{C}$  were also registered.

Surface of investigated material was studied with atomic force microscope Quesant “Nomad” model. Hydrolysate samples were deposited on a glass microscopic plate and allowed to dry at ambient temperature. Measurements were performed at room temperature, in the high resolution to 1024 in non-contact mode. Image size was about  $10 \times 10 \mu\text{m}$  and a scan rate was fixed to 10 Hz.

## THEORETICAL

The yield stress was determined by plotting a strain-stress curve with the stress being logarithmically increased from 1 Pa to 100 Pa during 10 min [7]. This analytic method is used to calculate the yield stress by making linear and polynomial regression of the 3rd grade in the two regressed curves marking the yield stress. This is a material property indicating the transition between “solid-like” and “liquid-like” behaviour, and it is related to the minimum shear stress corresponding to the first evidence of flow [8]. Although, the elastic behaviour below the yield stress is always accompanied by some modest flow [9, 10], however in most cases, this creep is so small that it can be ignored in comparison with material elasticity [11]. It can also be noted that material is nearly elastic below its yield stress and flows as non-linear viscous fluid due to the structure break-down above this limit [11].

Non-Newtonian viscosity of the fluids as a function of shear rate could be expressed using the exponential model [12]

$$\eta(\dot{\gamma}) = \tau_0 \dot{\gamma}^{-1} + \sum_{p=1}^{\infty} \eta_p \exp(-t_p \dot{\gamma}) \quad (1)$$

where  $\dot{\gamma}$  is the shear rate,  $\tau_0$  is the yield stress, and  $\eta_p$  and  $t_p$  are constants.

This model is known to behave very satisfactorily in its prediction for several foodstuff data [13, 14]. However, for time-dependent behaviour, the state equation is modified to include a structural parameter  $\lambda$ . According to *Cheng* and *Evans* structural theory [15] and the work of *Tiu* and *Boger* [16] the structure could refer to a distribution of particles. Then, the shear stress is modified and the state equation could be rewritten as

$$\eta(\dot{\gamma}) = \lambda \left( \tau_0 \dot{\gamma}^{-1} + \sum_{p=1}^{\infty} \eta_p \exp(-t_p \dot{\gamma}) \right) \quad (2)$$

According to *De Kee et al.* [14] changes of structural parameter with time follow an  $n$ th-order kinetic equation

$$\frac{d\lambda}{dt} = -k(\lambda - \lambda_{\text{eq}})^n \quad (3)$$

where  $t$  is time,  $\lambda_{\text{eq}}$  is the value of structural parameter at equilibrium, and the rate constant  $k$  is a function of shear rate.

Integrating eqn (3) for constant  $\dot{\gamma}$  yields

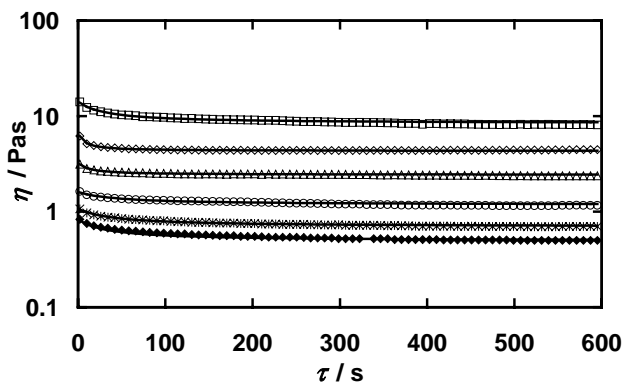
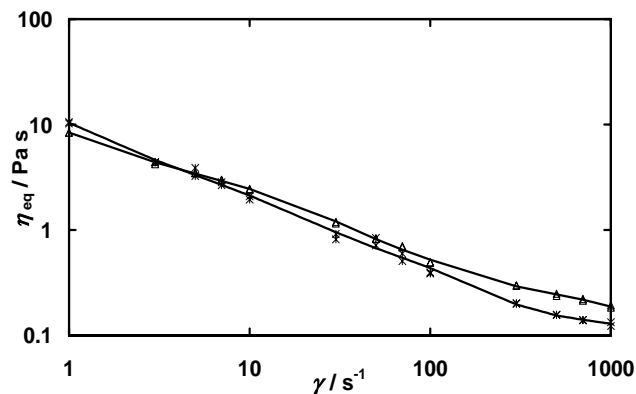
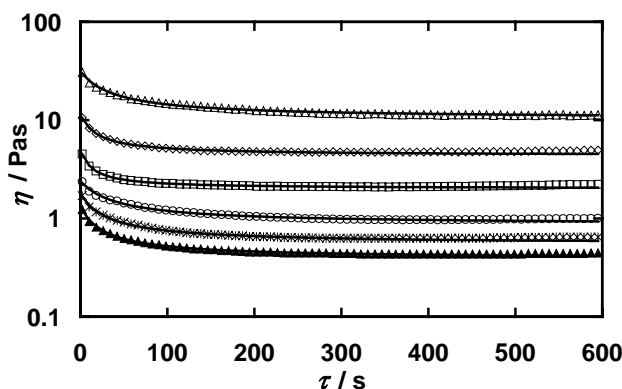
$$\eta(\dot{\gamma}) = ((\eta_{t \rightarrow 0} - \eta_{\text{eq}})^{1-n} - kt(1-n)\eta_{\text{eq}}^{1-n})^{\frac{1}{1-n}} + \eta_{\text{eq}} \quad (4)$$

where equilibrium viscosity ( $\eta_{\text{eq}}$ ) is given by the relation

$$\eta_{\text{eq}}(\dot{\gamma}) = \tau_0 \dot{\gamma}^{-1} + \sum_{p=1}^{\infty} \eta_p \exp(-t_p \dot{\gamma}) \quad (5)$$

**Table 1.** Values of Estimated Parameters of Eqn (5)

Enzyme	$\tau_0/\text{Pa}$	$\eta_1/(\text{Pa s})$	$t_1/\text{s}$	$\eta_2/(\text{Pa s})$	$t_2/\text{s}$	$\eta_3/(\text{Pa s})$	$t_3/\text{s}$	$R^2$
Neutrased	5.65	0.296	0.000485	0.580	0.0117	1.97	0.0609	0.999
Protamex	8.29	0.148	0.000204	0.546	0.0097	1.50	0.0842	0.997

**Fig. 1.** Time-dependent curves of ExSF hydrolysates produced with Neutrased measured at different shear rates:  $1 \text{ s}^{-1}$  ( $\square$ ),  $3 \text{ s}^{-1}$  ( $\diamond$ ),  $10 \text{ s}^{-1}$  ( $\Delta$ ),  $30 \text{ s}^{-1}$  ( $\circ$ ),  $70 \text{ s}^{-1}$  ( $*$ ),  $100 \text{ s}^{-1}$  ( $\blacklozenge$ ); model (—).**Fig. 3.** Equilibrium viscosity curves of ExSF hydrolysates produced with: Neutrased ( $\Delta$ ), Protamex ( $*$ ); model (—).**Fig. 2.** Time-dependent curves of ExSF hydrolysates produced with Protamex measured at different shear rates:  $1 \text{ s}^{-1}$  ( $\square$ ),  $3 \text{ s}^{-1}$  ( $\diamond$ ),  $10 \text{ s}^{-1}$  ( $\Delta$ ),  $30 \text{ s}^{-1}$  ( $\circ$ ),  $70 \text{ s}^{-1}$  ( $*$ ),  $100 \text{ s}^{-1}$  ( $\blacktriangle$ ); model (—).

Evaluation of the model could be found elsewhere [12, 17]. Estimation of parameter of eqn (5) was based on minimisation of a suitable mean square deviation function

$$S = \sum_{i=1}^N (\eta_{\text{eq},i}^{\text{exp}} - \eta_{\text{eq},i}^{\text{cal}})^2 \quad (6)$$

where exp refers to the value calculated for experiments at  $\dot{\gamma} = \text{const}$ , cal refers to the computation value and  $N$  is a number of the experiments at  $\dot{\gamma} = \text{const}$ .

Model parameters as well as the results of statistical analysis were obtained using the Marquardt—Lavenberg method.

## RESULTS AND DISCUSSION

Figs. 1 and 2 present variation of apparent viscosity with time for ExSF suspension hydrolysed with Neutrased, and using Protamex, respectively. Only selected data are plotted in these drawings. Proposed rheological model (solid lines) fitted the experimental data well.

Apparent viscosity of both samples decreased with increasing time. It is also notable that the time to reach equilibrium state decreases with increasing shear rate. It is seen that dispersions of the hydrolysate produced using Neutrased exhibit a greater rheological stability in time. The analysis of viscosity decay *vs.* time indicates that in both cases the equilibrium between rate of break-down and rebuilding of structure is achieved in a short time. Narrow range of viscosity changes obtained for hydrolysate produced using Neutrased is related to a high stability of its structure.

Fig. 3 shows the curve of equilibrium viscosity ( $\eta_{\text{eq}}$ ) as a function of shear rate. The equilibrium viscosity variation was represented very well using a three-parameter ( $p = 3$  in eqn (5)) exponential model. This model was successfully applied to fit all results from analysed range of shear rates. The results of fitting are presented in Table 1.

At equilibrium state, higher values of apparent viscosity were recorded for hydrolysate produced using Neutrased. On the contrary, at  $\tau \rightarrow 0$ , higher values of apparent viscosity were observed for the suspension of hydrolysate produced using Protamex.

Fig. 4 shows the equilibrium flow curves and selected experimental flow curves ( $\tau = 450 \text{ s}$ ). It is seen that experimental flow curves reach an equilibrium

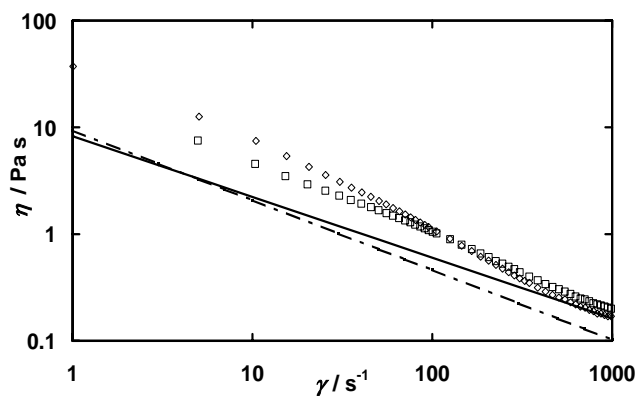


Fig. 4. Comparison of equilibrium flow curves (ExSF hydrolysed with: Neutrase (—), Protamex (---)) with experimental flow curves for  $\tau = 450$  s (ExSF hydrolysed with: Neutrase ( $\square$ ), Protamex ( $\diamond$ )).

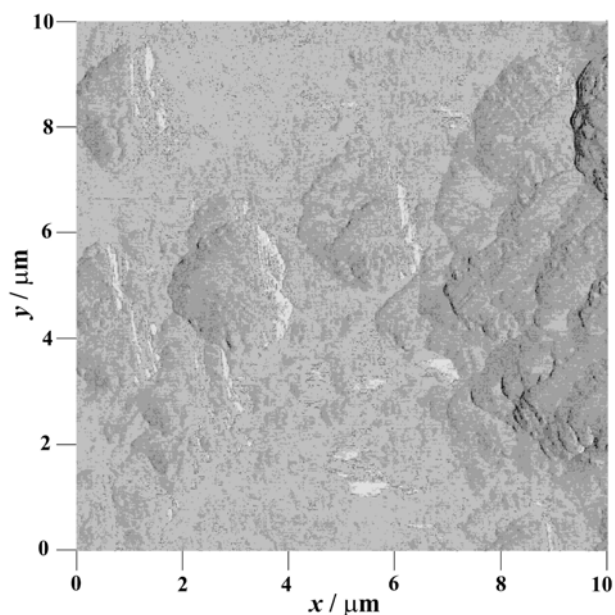


Fig. 5. The image of suspension of ExSF hydrolysed with Neutrase in phase mode.

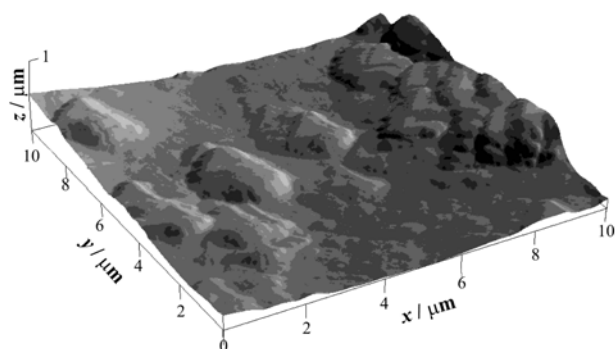


Fig. 6. The image of suspension of ExSF hydrolysed with Neutrase in wave mode.

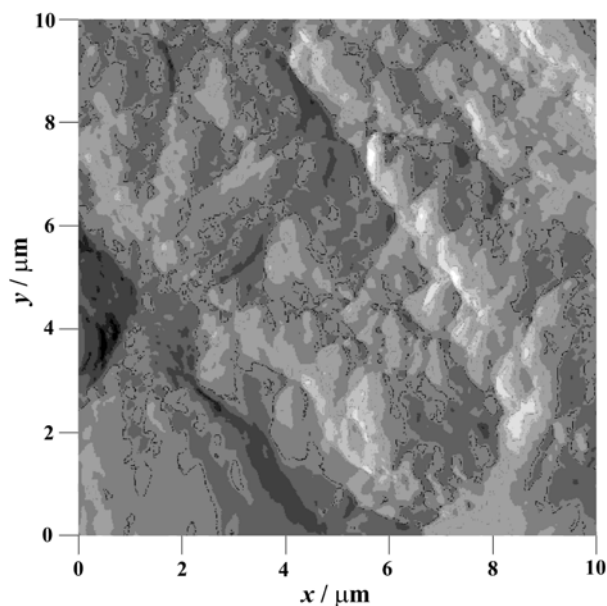


Fig. 7. The image of suspension of ExSF hydrolysed with Protamex in phase mode.

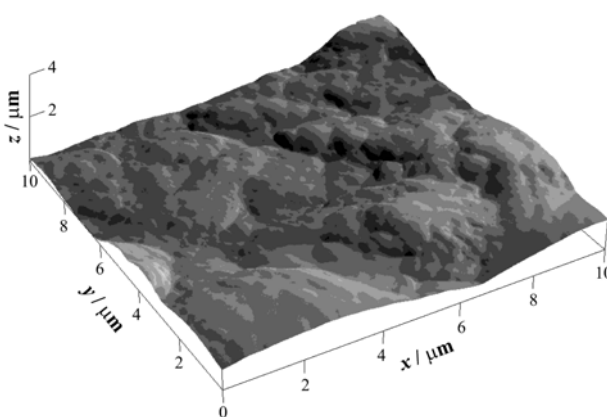


Fig. 8. The image of suspension of ExSF hydrolysed with Protamex in wave mode.

state at high shear rate, and shear rate required to reach equilibrium state is dependent on shearing time.

Figs. 5–8 present AFM images of the suspensions of ExSF hydrolysed with Neutrase (Figs. 5 and 6) and Protamex (Figs. 7 and 8). Analysis of hydrolysate suspensions shows their complex internal structure. Structure of the hydrolysate produced using Neutrase is not homogeneous comprising suspended objects (Fig. 5). Rheological behaviour of analysed system depends only on the dissipative phase. It is related to small viscosity changes (Fig. 1).

Fig. 7 presents image of the hydrolysate produced using Protamex. This structure is homogeneous and compact. For that reason, the system needs more time to achieve equilibrium (Fig. 2).

Differences in structure and rheological properties between the products obtained with the use of different enzymes should be regarded as vitally important, *e.g.* enzymes used for proteins hydrolysis could be chosen depending on the hydrolysate target application.

From the rheological point of view, it can be deduced that extruded soy protein concentrates undergoing limited enzymatic proteolysis could be used as ingredients in the formulation of such products as salad dressing, concentrated soups and drinks, seasoning mixtures as well as in confectionery, bread-making, and meat-processing industries.

*Acknowledgements.* This research was carried out within the project financed by the Polish State Committee for Scientific Research (Project No. PBZ-KBN/021/P06/99/01).

### SYMBOLS

$k$	rate constant	$s^{-1}$
$n$	order of reaction	
$\dot{\gamma}$	shear rate	$s^{-1}$
$\eta$	viscosity	Pa s
$\eta_{eq}$	equilibrium viscosity	Pa s
$\eta_p$	constant in the exponential model	Pa s
$\lambda$	structural parameter	
$\lambda_{eq}$	value of the structural parameter at equilibrium	
$t$	time	s
$\tau_0$	yield stress	Pa
$t_p$	constant in the exponential model	s

### REFERENCES

- Ledward, D. A. and Mitchell, J. R., in (Blandshard, J. M. W and Mitchell, J. R., Editors) *Food Structure – its Creation and Evaluation*, p. 219. Butterworths, London, 1988.
- Arai, S. and Fujimaki, M., in (Fox, P. F., Editor) *Food Enzymology*, Vol. 2, p. 83. Elsevier, London, 1991.
- Darewicz, M., Dziuba, J., and Caessens, P. W. J. R., *Pol. J. Food Nutr. Sci.* 9/50, 3 (2000).
- Henn, R. L. and Netto, F. M., *J. Agric. Food Chem.* 46, 3009 (1998).
- Surówka, K., Zmudziński, D., and Surówka, J., *Trends Food Sci. Technol.* 15, 153 (2004).
- Surówka, K., Zmudziński, D., Fik, M., Macura, R., and Łasocha, W., *Innovative Food Sci. Emerging Technol.* 5, 225 (2004).
- Steffe, J. F., in (Singh, R. P. and Wirakartakusumah, A., Editors) *Advances in Food Engineering*, p. 363. CRC Press, Boca Raton, 1992.
- Doraiswamy, D., Mujumdar, A. N., Tsao, I., Beris, A. N., Danforth, S. C., and Metzner, A. B., *J. Rheol.* 35 (4), 647 (1991).
- Barnes, H. A. and Walters, K., *Rheol. Acta* 24 (4), 323 (1985).
- Barnes, H. A., *J. Non-Newtonian Fluid Mech.* 81, 133 (1999).
- Mujumdar, A. N., Beris, A. N., and Metzner, A. B., *J. Non-Newtonian Fluid Mech.* 102, 157 (2002).
- De Kee, D. and Turcotte, G., *Chem. Eng. Commun.* 6, 273 (1980).
- Chevalley, J., *J. Texture Stud.* 6, 177 (1975).
- De Kee, D., Code, R. K., and Turcotte, G., *J. Rheol.* 27, 581 (1983).
- Cheng, D. C. H. and Evans, F., *Br. J. Appl. Phys.* 16, 159 (1965).
- Tiu, C. and Boger, D. V., *J. Texture Stud.* 5, 329 (1974).
- Carreau, P. J., De Kee, D., and Daroux, M., *Can. J. Chem. Eng.* 57, 135 (1979).