Starch Conversion During Extrusion as Affected by Added Gelatin

R. Wulansari, J.R. Mitchell, and J.M.V. Blanshard

ABSTRACT

Waxy maize starch was modified by twin-screw extrusion cooking in the presence and absence of gelatin. X-ray diffraction, microscopy, rheology, and differential scanning calorimetry studies showed that, at constant specific mechanical energy and extruder heater temperatures, the degree of starch conversion decreased with increasing levels of gelatin. It was hypothesized that during extrusion cooking, the gelatin acts as a lubricant, protecting the starch from being converted since more mechanical energy would be dissipated in the gelatin phase than the starch phase.

Key Words: extrusion cooking, starch conversion, gelatin, waxy maize starch, DSC

INTRODUCTION

THE INFLUENCE OF EXTRUSION ON STARCH CONVERSION has been extensively studied. The loss of order, which can be seen microscopically or from the disappearance of the differential scanning calorimetry (DSC) gelatinization endotherm, is followed or accompanied by other changes, including depolymerization of polysaccharides and disruption of granular structure (Della Valle et al., 1995; Fan et al., 1996; Willett et al., 1997). One characteristic of extrusion cooking that distinguishes it from conventional cooking is the application of intense mechanical forces. This mechanical energy input clearly has a major effect on the degree of starch conversion during extrusion (Diosady et al., 1985; Colonna et al., 1989; Wang et al., 1989). Attempts have been made to deconvolute the influence of thermal and mechanical energies on starch conversion, using an energy equivalent principle (Wang et al., 1992). This enabled the relative contributions of mechanical and thermal energy to be quantified. Using capillary rheometry, Zheng and Wang (1994) attempted to measure the minimum shear stress required to convert starch and showed that this was strongly dependent on temperature.

There have been few attempts to investigate the extent to which starch conversion is influenced by composition of the product at constant mechanical and thermal energy input. This can be of practical importance since the degree of starch conversion influences textural and other properties of the product. Our objective was to investigate the hypothesis that starch conversion is governed by the specific mechanical energy input and temperature and is largely independent of product composition.

The product composition was varied by adding different amounts of gelatin to waxy maize starch. Gelatin and starch mixtures are widely used in confectionery production. Waxy maize starch was used to avoid possible complications from the formation of amylose lipid complexes.

MATERIALS & METHODS

Materials

A limed hide, 225 Bloom gelatin (Rousselot) was used without

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further purification. Waxy maize starch was obtained as Amioca powder from National Starch and Chemical Co. (Manchester, U.K.). The moisture content of materials was determined by oven drying at 105 °C to be around 13.8% dwb for gelatin and 13.3% dwb for the starch. Bacterial collagenase (derived from *Clostridium hystolyticum*) type IA was obtained from Sigma Chemical Co., (St. Louis, Mo., U.S.A.).

Extrusion

Gelatin and waxy maize starch were mixed with ratios of gelatin:starch (8:2), (6:4), (4:6), and (2:8), according to dry weights in a Kenwood Peerless planetary mixer for 1 h prior to extrusion.

Unexpanded ribbons were prepared by a co-rotating, intermeshing, Clextral BC-21 twin-screw extruder (Firminy, France). The 400-mm extruder barrel had a 16:1 length to diameter ratio, and consisted of 4 modules, each 100-mm long, equipped with a water cooling system. The screw configuration consisted of double thread elements with 33 mm pitch in the first zone, 25 mm pitch in the second zone, and 16.7 mm pitch in the third and fourth zones. A 25-mm length of reverse pitch element (RPE) was located 25 mm behind the die. The extrusion was performed using a slit die of 1 mm × 30 mm × 47 mm dimension. The screw speed was 200 rpm, feed rate 5 kg·h⁻¹, and temperature profile of 40, 90, 110, and 80 °C from zone 1 to 4, respectively. During extrusion, distilled water (0.35 to 1.2 L·h⁻¹) was pumped into the first zone of the barrel to varymoisture contents and, hence, the specific mechanical energy (SME). In these experiments, the magnitude of the SME ranged from 340 to 830 kJ·kg⁻¹, which was in the range of much of the experimental studies that have been reported on the extrusion process (Meuser and van Lengerich, 1984; Colonna et al., 1989). The temperature of the product at the die was monitored continuously with a combined temperature and pressure transducer.

The extrudates were ground and passed through a 422-μm laboratory sieve. Ground samples were used directly, while the remainders were stored at –20 °C for other measurements.

DSC measurements

A DSC-7 (Perkin Elmer Corp, Norwalk, Conn., U.S.A.) was used for calorimetric measurements. Data were collected and analyzed using Pyris Software for Windows™ version 2.0. Around 2.5 to 3 mg of sample was weighed into an aluminum-volatile sample pan, and water was added with a syringe. The amount of water added was adjusted to a water to sample ratio of 4:1 (w/w), and the pan was hermetically sealed and re-weighed. The sample was equilibrated overnight before measurement to ensure the diffusion of the water into the granules, and 3 to 5 replicates were analyzed. An empty pan was used as a reference. Heat flow and temperature were calibrated, using cyclohexane (onset temperature, T_o, of 6.7 °C) and indium (T_o of 156.78 °C, and Heat of fusion of 28.45 J·g⁻¹).

Samples were scanned from 0 to 100 °C at 10 °C/min. T_o, T_p (peak temperature), and T_e (endset temperature) were recorded.

Degree of conversion (DC) of starch was calculated as follows (Wang et al., 1989):

$$DC(\%) = [\Delta H_o) - (\Delta H_i)]/(\Delta H_o) \times 100\% \qquad (1)$$

where (ΔH_0) is the specific heat of gelatinization of the native

starch material (11.7 $J \cdot g^{-1}$), and (ΔH_i) is the specific heat of gelatinization of the starch in extrudate.

Reported results are means of 3 to 5 determinations.

X-Ray Scattering

Wide Angle X-ray Scattering (WAXS) was performed with an X-ray diffractometer Phillips APD-15 system (The Netherlands), operating at 40 kV and 50 mA, producing a CuKα radiation at a wavelength of about 1.54Å. The fresh extrudate was placed on an aluminum holder, and the X-ray pattern was recorded over the range of 4 to 38° (2θ diffraction angle).

Enzymatic extraction of gelatin from extrudates

To enable examination of starch in the gelatin containing extrudates without interference, the gelatin was degraded by collagenase and washed out from the ground extrudate. The ground extrudate was incubated in tris-buffer (0.1 M, pH 7.4), containing $CaCl_2$ (50 mM), overnight to ensure the complete swelling of gelatin. Collagenase was added in excess, and the mixture was incubated at 37 °C with occasional stirring for 5 h. These conditions were the minimum necessary for effective enzymatic digestion of gelatin (Bernal and Stanley, 1986). After centrifugation at (4000 × *g*) for 10 min and washing with 95% ethanol and water, the starch was recovered by drying in a 70 °C oven with moisture content around 5% to 10%. The yield of residual starch was calculated assuming that all gelatin had been washed out.

Rheological measurement

Viscoelastic measurements were carried out using a Bohlin CS-10 rheometer, equipped with parallel plate geometry (40-mm dia, 1-mm gap between plates). Oscillation was performed on aqueous starch dispersions of 20% w/w (dry basis), with frequency of 0.1 Hz, strain 0.02, and temperature gradient increasing from 25 to 90 °C at 3 °C/min.

Optical microscopy analysis

Thin films of extrudate were obtained by cryo-microtoming (Bright Instrument Company Ltd., Huntingdon, U.K.) the extrudate to a thickness of about 6 to 8 μm. The extrudate films were then mounted on a microscope slide and stained with iodine vapor by placing the specimen in a jar above a wet filter paper that had been sprinkled with iodine crystals. The sample was then examined under a Leitz Diaplan compound light microscope (Ernst Leitz Wetzlar GmbH, Germany), fitted with a polarizing filter.

Statistical evaluation

Data reported were mean values from minimum of 2 replications. Standard of deviation and analysis of variance were performed with confidence interval of 95% ($p < 0.05$).

RESULTS & DISCUSSION

Extrudates

Non-expanded extruded ribbons were obtained in all cases. The measured product temperature immediately before the die was 80 ± 2 °C and did not show any systematic dependence on gelatin-starch ratio. Thermograms were compared (Fig. 1) for native starch, extruded starch, extruded gelatin, and extruded gelatin-starch mixtures, containing differentratios. The SME of all extrudates was in the range of 600 ± 50 $kJ \cdot kg^{-1}$. Thermograms showed that the peaks corresponding to the transitions of gelatin and starch were well separated, enabling them to be analyzed individually. Apparently a starch gelatinization endotherm was present in the extrudates containing gelatin but not in the starch extruded alone, showing that the degree of starch conversion in the extrusion was reduced by the presence of gelatin. This was confirmed by microscopic examination of the sample, which revealed the existence of "Maltese cross" and slightly swollen starch granules in the samples containing gelatin, while no

such crosses were seen in samples of starch extruded alone. This suggested that in the gelatin containing extrudates a substantial starch molecular order remained.

Residual starch

To provide further information on the properties of starch in the sample, the gelatin was removed by enzymatic digestion. The procedure also resulted in a significant loss of starch (Table 1), particularly at higher starch-gelatin ratios. Possibly water-soluble starch was created and was washed out with the gelatin hydrolysate. When there was no gelatin present, the yield was much higher, which might be explained by the presence of gelatin preventing the formation of a network of converted starch and, hence, making the material easier to wash out. It seems reasonable to assume that the unconverted starch remained within the pellet, and a fraction of the highly converted water-soluble starch was washed out with the digested gelatin.

Wide angle X-ray scattering (WAXS)

X-ray diffraction patterns (Fig. 2) were examined for the residual starch, following gelatin extraction. In all cases, the A-type pattern corresponding to native starch was seen, except in the case of material extruded without gelatin. This confirmed the DSC and microscopy observations that showed that the inclusion of gelatin reduced starch conversion.

Differential scanning calorimetry

The residual starch was analyzed by DSC by adjusting to a ratio of water:starch (4:1), and the sample that was treated and run in the DSC in the same way that the extrudates were compared (Table 2) for SMEs about 450 and about 600 $kJ \cdot kg^{-1}$. By comparison with the heat of gelatinization of the native starch, it was seen that this residual starch contained between 32% and 68% unconverted material. The onset temperature of the gelatinization endotherm was 3
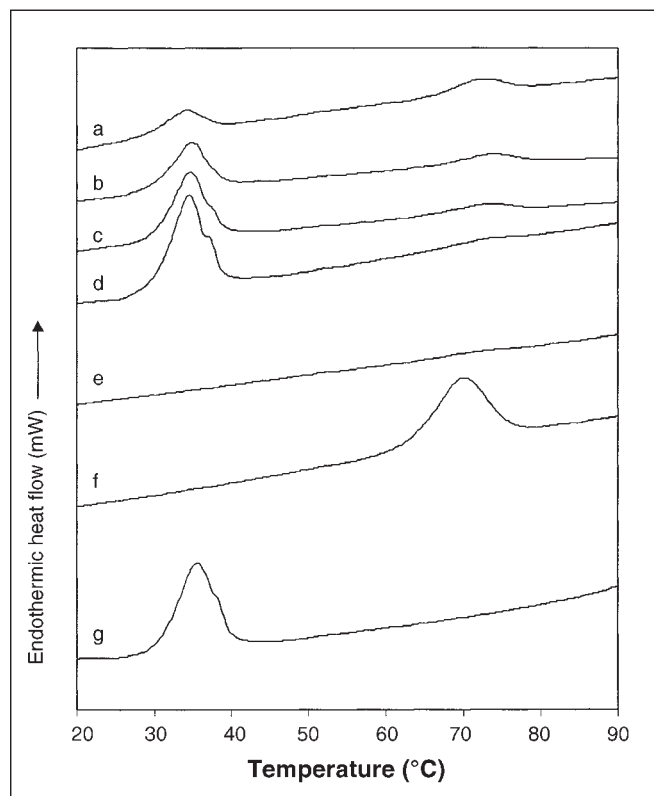

Fig. 1—DSC thermograms of extrudate with SME about 600 $kJ \cdot kg^{-1}$ at various gelatin:starch ratios: (a) 2:8; (b) 4:6; (c) 6:4; (d) 8:2; (e) extruded starch; (f) native waxy maize starch; (g) extruded gelatin.

Table 1—Starch in residue following extraction of gelatin by enzymatic digestion.

Samples	Yield (%)[a]
Extruded starch	80.4 ± 1.9[b]
SME about 450 kJ/kg	
Gelatin-starch	
2:8	48.6 ± 4.5
4:6	42.0 ± 5.1
6:4	84.5 ± 1.9
8:2	85.4 ± 1.0
SME about 600 kJ/kg	
Gelatin-starch	
2:8	39.3 ± 5.2
4:6	45.9 ± 2.1
6:4	76.8 ± 5.4
8:2	80.0 ± 1.5

[a]Yield (%) = [(wt of residual starch)/(wt of original starch)] × 100.
[b]Values from duplicates and range of yield values.

Table 2—Phase transition temperatures and heats of gelatinization (ΔH) of starch from DSC

	Onset, T_o (°C)	Peak, T_p (°C)	Endset, T_c (°C)	ΔT (°C)	ΔH^a J·g⁻¹ Starch	ΔH^b J·g⁻¹ Starch
Native starch	63.1 ± 0.5[c]	70.2 ± 0.1	76.3 ± 0.5	13.2		11.7 ± 0.5
SME about 450 kJ/kg						
Gelatin:starch						
2:8	67.7 ± 0.5	73.7 ± 0.3	79.5 ± 0.5	11.8	5.0 ± 0.3	2.4 ± 0.1
4:6	66.7 ± 0.3	73.5 ± 0.2	79.5 ± 0.4	12.8	6.5 ± 0.2	2.7 ± 0.1
6:4	65.3 ± 1.3	73.0 ± 0.1	78.8 ± 0.3	13.5	5.9 ± 0.1	4.9 ± 0.1
8:2	63.5 ± 0.3	72.3 ± 0.1	78.7 ± 0.2	15.2	7.0 ± 0.9	6.0 ± 0.3
SME about 600 kJ/kg						
Gelatin:starch						
2:8	66.8 ± 0.3	73.0 ± 0.3	79.0 ± 0.5	12.2	5.6 ± 0.2	2.2 ± 0.1
4:6	66.6 ± 0.3	73.5 ± 0.1	79.4 ± 0.4	12.8	5.0 ± 0.3	2.3 ± 0.1
6:4	64.4 ± 0.3	72.2 ± 0.4	77.7 ± 0.6	13.3	3.8 ± 0.2	2.9 ± 0.1
8:2	62.3 ± 0.3	72.3 ± 0.3	79.1 ± 0.5	16.8	6.4 ± 0.4	5.0 ± 0.3

[a]Expressed per weight of material in residual starch.
[b]Expressed per weight of total starch in extrudate.
[c]Means and standard deviations from 3 to 5 measurements.

to 4 °C higher than the native starch for extrudates where the original gelatin level was less than half the total dry weight in the formulation. A possible reason for the increase in onset temperature is that the extrusion preferentially converted granules that had a lower onset temperature. That is, the unconverted material may have contained the more thermally resistant granules. At the higher gelatin levels where more protection to conversion occurred, the onset temperature was reduced, suggesting that the less thermally resistant granules were then protected. Published studies using microscopy showed that starch granules did not lose their birefringence at the same temperature upon heating (Whistler and BeMiller, 1997). The higher endset temperature might be explained by the relatively low contribution of resistant granules to DSC responses when native starch was compared with the residual material. There is no evidence, however, that, at the lowest amount of starch investigated, the endset temperature had decreased as might be expected

from this explanation, since more of the less-resistant granules would be included in the residual starch sample. Another possibility would, therefore, be that the extrusion process had an annealing effect on the starch that was converted, resulting in an overall increase in peak and endset temperatures.

The heat of gelatinization decreased with increasing SME and decreasing amount of gelatin in the original mixture. This suggested that, although protection was provided to starch gelatinization in extrusion cooking, the effect could be reduced by changing the ratio and/or applying more mechanical energy.

The degree of conversion was calculated (Fig. 3) as a function of SME and gelatin-starch ratio. Calculations were carried out on the assumption that all unconverted starch was retained in the residue following collagenase extraction. The results were consistent with the more limited data from analysis of thermograms of the whole extrudate. At any SME the degree of conversion increased with ratio of starch to gelatin, approaching that of the extruded waxy maize starch alone. Conversion of starch in the gelatin containing samples generally increased with SME, suggesting a dominant effect of mechanical energy. The exception was the gelatin starch (2:8) product where the conversion approached that of waxy maize starch alone. At this high starch level a starch continuous phase would be expected, and, hence, gelatin would have little influence on the transfer of mechanical stress to the starch phase.

Dispersion rheology

The storage modulus effect as a function of temperature was determined (Fig. 4) on heating 20% w/w dispersions (SME about 600 kJ·kg⁻¹) for residual starch following gelatin extraction. G′ started to increase when the temperature reached 55 to 68 °C, while that of the starch extruded alone did not show any increase with increasing temperature. The dispersion rheology would be dominated by the volume fraction occupied by the swollen granules; this is a further indication that the starch that was extruded in the absence of gelatin was fully converted, as there would be no intact granules left that would swell when the temperature was increased. The increase in temperature for the rise in G′ with increasing starch content in the original formulation partly reflected the increase in DSC onset temperature. However, for formulations containing higher gelatin levels, there was an increase in storage modulus below the DSC onset temperature and also below the temperature found for the increase in G′ for unprocessed starch. This must indicate a contribution from the swelling of intact granules that had reduced molecular order.

The water content in the extruder varied from 18% to 32% wwb. In a quiescent environment, the heating time at 100 °C, necessary for complete thermal conversion (DC = 0%, Eq. 1) of a
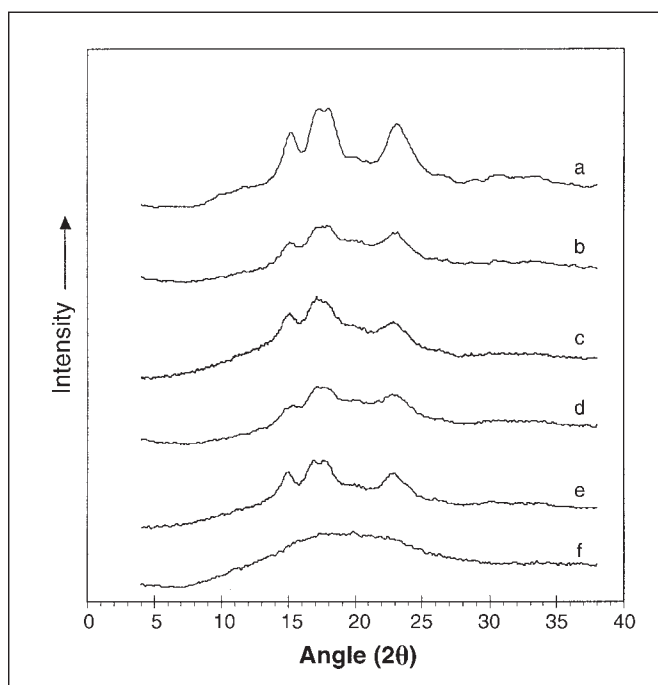

Fig. 2—X-ray scattering of residual starch obtained by collagenase digestion of gelatin from extruded gelatin:starch: (a) native waxy maize starch; (b) 2:8; (c) 4:6; (d) 6:4; (e) 8:2; (f) extruded starch. SME involved during extrusion about 600 kJ·kg⁻¹.

waxy corn starch containing 30% water, has been estimated at about 20 min (Zheng and Wang, 1994). Mean residence times in the small extruder were less than a minute. The lack of puffing of the material at the die and the measured product temperature prior to the di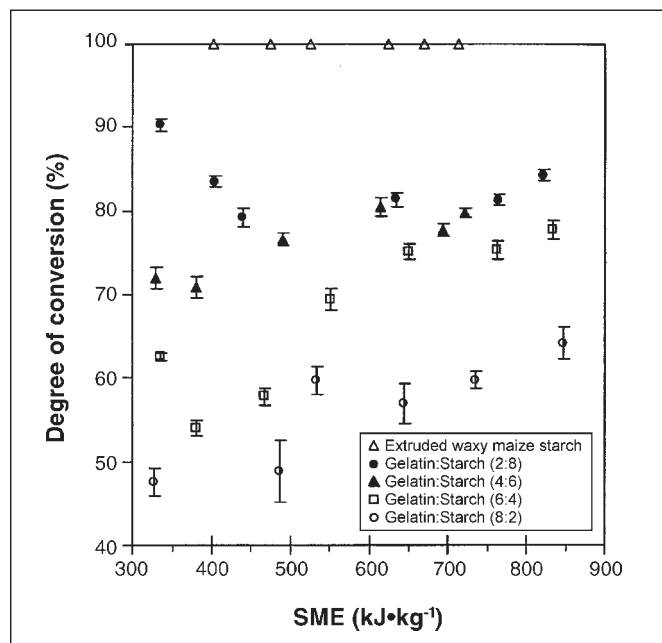e make it improbable that a temperature as high as 100 °C would be reached at any time during the process. The conversion of starch should, therefore, be dominated by the mechanical rather than thermal energy.

In general, mixtures of biopolymers at low water contents would be expected to phase separate. Studies with infrared microscopy and dynamic mechanical thermal analysis have shown that many products, although appearing visually homogeneous, contain different starch-rich and gelatin-rich phases (Mousia et al., 1999). A possible explanation for the starch conversion results obtained is that the shear stress is lower in the starch phase of such mixed systems than it would be for starch alone. For a viscoelastic material this shear stress would depend on viscosity and shear rate as well as strain and modulus of elasticity (Wang et al, 1992). It seems probable that the gelatin would melt first within the extruder before the starch was converted. This would result in a relatively low-viscosity gelatin phase that would be subjected to a high shear rate. Thus, gelatin would act as a lubricant to the systems and more mechanical energy would be dissipated in gelatin phase than the starch phase. Such gelatin is more charged and hydrophobic than the starch; it could also interfere with ingress of water to the starch during gelatinization in the extruder. Also the interaction at the interface between the starch and gelatin domains may be important. A weak interaction at this interface would result in slip or fracture, also reducing the magnitude of mechanical energy that would be dissipated within the starch domain.

Fig. 3—Degree of conversion (%) of starch as a function of the SME of extrusion.

CONCLUSIONS

THE DEGREE OF STARCH CONVERSION DEPENDED ON THE FORmulation, as well as the heating conditions and the SME. Incorporation of gelatin produced a reduction in conversion. This reduction increased with the increased level of gelatin in the formulation. The native starch remaining after processing in the presence of gelatin showed a higher DSC gelatinization temperature than unprocessed native starch. This may be due to annealing effects or conversion of the less-resistant granules. The work is relevant to confectionery products containing starch and gelatin and may have general applicability, since it suggests potential means of controlling starch conversion during extrusion.

REFERENCES

Bernal, V.M. and Stanley, D.W. 1986. Changes in the melting characteristics of bovine tendon collagen induced by a bacterial collagenase. J. Food Sci. 51(3): 834-835, 837.
Colonna, P., Tayeb, J., and Mercier, C. 1989. Extrusion cooking of starch and starchy products. In: *Extrusion Cooking*, C. Mercier, P. Linko, and J. M. Harper (Ed.), p. 247-320. Anerican Association of Cereal Chemists, Minneapolis, Minn., U.S.A..
Della Valle, G., Boche, Y., Colonna, P., and Vergnes, B. 1995. The extrusion behaviour of potato starch. Carbohydr. Polym. 28: 255-264.
Diosady, L.L., Paton, D., Rosen, N., Robin, L.J., and Athanassoulias, C. 1985. Degradation of wheat starch in a single screw extruder: Mechano-kinetic breakdown of cooked starch. J. Food Sci. 50: 1697-1699.
Fan, J., Mitchell, J.R., and Blanshard, J.M.V. (1996) The effect of sugars on the extrusion of maize grits, II. Starch conversion. Int. J. Food Sci. Technol. 31: 67-76.
Meuser, F. and van Lengerich, B. 1984. System analytical model for the extrusion of starches. In *Thermal Processing and Quality of Foods*, P. Zeuthen, J.C. Cheftel, C. Eriksson, M. Jul, H. Leniger, P. Linko, G. Varela, and G. Vos (Ed.), p. 175-179. Elsevier Applied Science, London.
Mousia, Z., Farhat, I.A., and Mitchell, J.R. 1999. Effect of water partitioning on the glass-transition behaviour of phase separated starch-gelatin mixtures. Polymer: In press.
Wang, S.S., Chiang, W-C., Yeh, A-I., Zhao, B., and Kim, I-H. 1989. Kinetics of phase transition of waxy corn starch at extrusion temperatures and moisture contents. J. Food Sci. 54(5): 1298-1301, 1326.
Wang, S.S., Chiang, W.C., Zheng, S., Zhao, B.L., and Cho, M.H. 1992. Application of an energy equivalent concept to the study of the kinetics of starch conversion. In *Food Extrusion Science and Technology*, J. L. Kokini, C-T. Ho, and M.V. Karwe (Ed.), p. 165-176. Marcel Dekker, Inc., New York.
Willett, J. L., Millard, M.M., and Jasberg, B. K. 1997. Extrusion of waxy maize starch: melt rheology and molecular weight degradation of amylopectin. Polymer 38: 5983-5989.
Whistler, R.L. and BeMiller, J.N. 1997. *Carbohydrate Chemistry for Food Scientists*, p. 116-152. American Association of Cereal Chemists, Minneapolis, Minn., U.S.A..
Zheng, X. and Wang, S.S. 1994. Shear induced starch conversion during extrusion. J. Food Sci. 59 (5): 1137-43
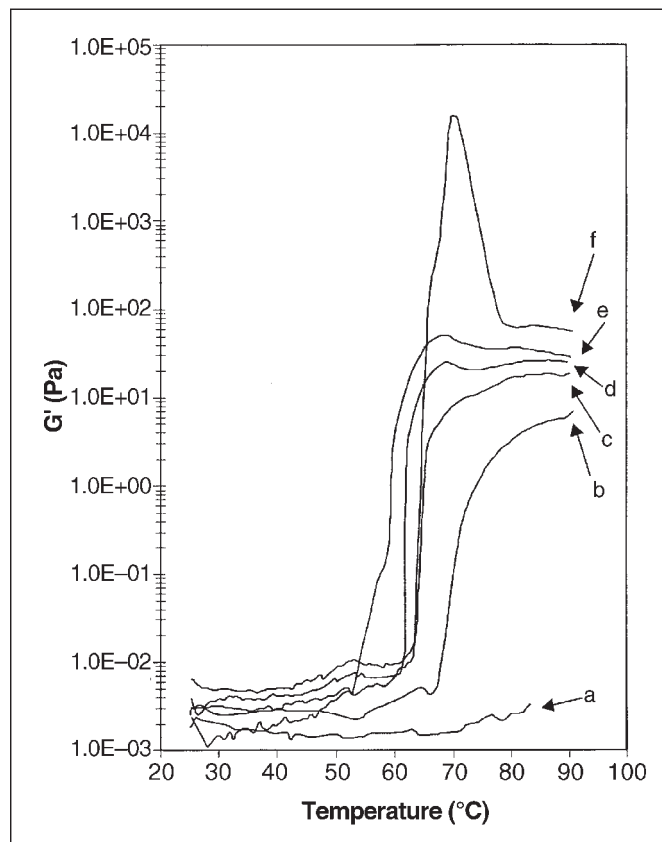Ms 0609 received 2/7/99; revised 6/22/99; accepted 7/10/99

Fig. 4—G′ as a function of temperature for 20% w/w (dwb) of residual starch from the extruded gelatin:starch: (a) starch alone; (b) 2:8; (c) 4:6; (d) 6:4; (e) 8:2; (f) native waxy maize starch. SME involved during extrusion about 600 kJ·kg⁻¹.