Effect of Shear Stress Extrusion Intensity on Plasticized Corn Flour Structure: Proteins Role and Distribution

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ABSTRACT: Plasticized corn flour-based materials were prepared by extrusion and injection molding. Extrusion of corn flour blends (75% wet basis (wb)—glycerol (5 or 10% wb)—water) was performed in a twin-screw extruder with either one or three shearing zones. Native corn flour is mainly composed of corn starch granules surrounded by proteins layers. Therefore, the destructuration of corn flour by thermomechanical treatments was analyzed (i) by techniques essentially allowing to monitor corn starch amorphization (differential scanning calorimetry, X-ray diffractometry, determination of water sorption isotherms, susceptibility to hydrolysis by amylolytic enzymes) (ii) and via proteins layers role and distribution observed by confocal scanning laser microscopy and comparing the susceptibility of corn starch to hydrolysis by amylolytic enzymes in the presence or not of a protease. Both corn starch granules amorphization and proteins dispersion and aggregation were more pronounced for materials extruded in a screw profile with three shearing zones. For materials extruded in a screw profile with one shearing zone, the amorphization of starch was higher in materials made with 5% wb glycerol, whereas the proteins dispersion and aggregation was more pronounced in materials made with 10% wb glycerol. A barrier role of proteins to hydrolysis of corn starch by amylolytic enzymes was demonstrated and discussed. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 123: 2177–2186, 2012

Key words: extrusion-injection molding; plasticized corn flour-based materials; corn starch granules structure; proteins; enzymatic amylolysis

INTRODUCTION

The growing interest in easing the environmental burden of petrochemically derived polymers has stimulated the development and production of biodegradable polymers-based materials. For instance, starch can be processed into thermoplastic materials in the presence of plasticizers using heat and shear, as recently reviewed by Liu et al.[1] Unfortunately, starch-based materials often do not match the requirements for the applications, namely because thermoplastic starch is highly hydrophilic and hygroscopic and swells or even dissolves in water and has poorer mechanical properties than most of synthetic polymers. To overcome this limitation, plasticized starch has been blended with more hydrophobic biodegradable polymers such as synthetic aliphatic polyesters (polycaprolactone or polylactic acid) or other biopolymers (proteins).[2,3] Habeych et al.[4] blended zein with wheat starch to reduce its sensitivity to water. Zeins, the storage proteins of corn, represent 60% of this protein fraction and are located in the protein bodies.[5] Their hydrophobicity is due to their high glutamic acid (21–26%), leucine (20%), alanine (10%), and proline (10%) contents.[6]

Recently, corn starch-zein blends were also considered as model systems to provide a better understanding of the impact of extrusion on corn flour-based extruded materials.[7–9] A clear advantage of using raw materials like corn flour is that they are far cheaper than starch, since extraction of starch generates a high energetic cost, whatever its botanical origin. Leblanc et al.[10] performed thermogravimetric, calorimetric, X-ray diffraction, mechanic, and

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TABLE I
Different Plasticizer Combinations Used and Number of Shearing Zones Applied During Extrusion Before Injection Molding of Corn Flour-Based Materials

Formulation: native corn flour : glycerol : water (%)	Number of shearing zones	Nomenclature
100 : 0 : 0	–	corn flour
75 : 5 : 20	1	G5S1
75 : 5 : 20	3	G5S3
75 : 10 : 15	1	G10S1
75 : 10 : 15	3	G10S3

morphological experiments on extruded wheat flour-based materials and materials prepared from starch extracted from the same wheat flour. They concluded that except a reduction of 30% of strain to break values for wheat flour-based films no drastic difference was observed. More recently, Dias et al.[11] reported that edible films prepared from rice flour by casting had similar mechanical properties but higher water vapor permeabilities than films made with rice starch. Taken together, these observations support the idea that flour-based materials are possible alternatives to starch-based compositions and other costly biopolymers and deserve further study.

Corn flour is composed of the endosperm, which generally contains between 75 and 87% starch and 6–8% proteins.[6] Extrusion process induces structural modifications of starch that have been well described, such as starch gelatinization and molecular weight decrease.[12] This latter depends on shear stress intensity.[13,14] This molecular weight decrease leads to an intrinsic viscosity reduction.[15] In addition to thermomechanical stress, some additives commonly used as plasticizers can modulate these changes, like glycerol that has been shown to facilitate the formation of smaller polymer fragments during extrusion.[16] The common point between all the studies cited above is that they all focused on studying the changes that can undergo the carbohydrate starch matrix.

When studying corn flour-based instead of corn starch-based extruded materials, the role of protein layers surrounding the starch granules in native corn flour should also be considered. Because of their film forming properties, zein molecules could participate to set up the physicochemical properties of corn flour-based materials. Hermansson[17] reported that after extrusion, the starch matrix forms a continuous amorphous phase, while proteins become a discontinuous phase. Confocal scanning laser microscopy (CSLM) observations of proteins in extruded or molded corn flour-based materials allowed distinguishing two types of morphology randomly dispersed in the matrix: (i) damaged remnant native organization (likely resulting from the mechanical effect tending to disrupt the native organization) and (ii) protein aggregates dispersed in a starch matrix (likely resulting from the effect of the temperature on the constitutive proteins following the mechanical disruption of their native organization).[8]

In the present study, nonedible corn flour-based materials were prepared by extrusion followed by injection molding after blending with glycerol. The total plasticizer (water and glycerol) concentration was 30% (w/w) on a wet basis (wb) and two different glycerol concentrations in the blend (5 or 10% wb) were considered. To control corn flour destructuration, two different screw profiles in the feeding zone of a corotative twin screw extruder were used (one or three shearing zones profiles). Taken together, this experimental design allowed to assess the respective effects of formulation and thermomechanical extrusion treatment. The structure and morphology of native corn flour starch and of the four different extruded-molded corn flour-based materials were investigated by differential scanning calorimetry (DSC), X-ray analysis (RX), and determination of the water sorption isotherms at 25°C. Tensile testing allowed to characterize the mechanical properties of the four corn flour-based materials.

In addition, since the morphology of proteins in corn flour-based materials was far less studied than that of starch in corn starch-based materials, confocal scanning laser microscopy observations of proteins were achieved. To complete these observations, the susceptibility to hydrolysis by amylolytic enzymes of native corn flour and materials obtained from it by thermomechanical processing was assessed. More originally and to get a better insight in the proteins role, the acceleration of enzymatic amylolysis by proteolytic enzymes allowed to assess the barrier role of proteins against enzymatic hydrolysis of corn flour starch as a function of its thermomechanical destructuration.

MATERIALS AND METHODS

Preparation of corn flour-based materials

Corn flour-based materials were prepared by twin-screw extrusion followed by injection molding of corn flour-glycerol-water mixtures (Table I). Extrusion of corn flour blends [corn flour (75% wet basis (wb))—water—glycerol (5% or 10% wb)] was performed in a twin-screw extruder with either one or three shearing zones. Glycerol with a 99.5% purity and corn flour were provided by Grosseron (Saint-Herblain, France) and Cérégrain (Bourg en Bresse, France), respectively. The elementary composition of corn flour in carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) was 44.8% ± 0.3%, 6.68% ± 0.06%, 1.51% ± 0.11%, and 0.20% ± 0.02%, respectively. It was determined with a FlashEA series 1112 apparatus (Thermoelectron Corp., Waltham, MA). The protein content of corn flour (9.46% ± 0.73%) was calculated from nitrogen content (N × 6.25). Lipids content from corn flour (about 4.5%) was determined following extraction of lipids by continuous hot cyclohexane percolation under reflux in a hot vapor continuous extractor. Considering the presence of about 2% of cellulosic fibers in corn flour in addition to 4.5% lipids and about 10% protein, the amount of starch in flour was thus ~ 83%. Prior to extrusion, corn flour was stored for at least 1 week at 23°C in a 50% relative humidity (RH) atmosphere until equilibrium: a moisture content of 10% (w/w) was thus reached. The extrusion was performed in a corotative twin-screw extruder, Evolum HT 32 from Clextral (Firminy, France). The screw had a diameter of 32 mm, with an L/D ratio of 44. The screw profile used was made of shearing zones, separated by conveying zones. Two different screw profiles were used to obtain various shearing conditions (Fig. 1).

The temperature profile ranged from 85 to 100°C, while the total flow rate (corn flour, glycerol, and water) was set to about 18 kg h^{-1}. The destructured flour was then pelletized and stored at 23°C and 50% RH during 1 week prior to injection. The granules were injected with a 140 tons injection molding machine from Billion (Oyonnax, France). A temperature profile ranging from 70 to 120°C was used along the screw barrel. Dumbbell specimens (NF EN ISO 1A standard) were prepared, with the following dimensions: useful length of 85 mm, useful width of 10 mm and thickness of 4 mm. These pieces were crushed with a Polymixis PX-MFC 90D apparatus (Kinematica.CH, Switzerland) set at a speed of 5000 revolutions per min for 30 s. The crushed material was then sieved onto 0.2-mm cut-off filters. The corn flour and corn flour-based material powder obtained following crushing were then submitted to further analysis.

Figure 1 Screw profiles used for corn flour–glycerol–water blends extrusion.

Methods

Differential scanning calorimetry (DSC)

Differential scanning calorimetry was used to determine the thermal properties of native corn flour and extruded-injection molded materials prepared from it. DSC analyses were performed with a DSC 2920 apparatus (TA Instruments, Guyancourt, France). Samples (20 mg) and deionized water (100 mg) were weighed directly in appropriate pans and sealed hermetically. Pans were heated from 20 to 140°C with a heating rate of 5°C min^{-1}. Native and residual gelatinization enthalpies, ΔH (J g^{-1}), were calculated by integration of the obtained endothermic peaks. The level of residual crystalline starch in the flour corresponds to the ratio $\Delta H_{residualCF}/\Delta H_{nativeCF}.X_{starch}$, where $\Delta H_{residualCF}$ is the measured residual gelatinization enthalpy of the transformed flour, $\Delta H_{nativeCF}$ is the gelatinization enthalpy of the native flour, and X_{starch} is the concentration of starch in the flour (83% (w/w).).

X-ray analysis (RX)

Diffraction diagrams of 1 g of each sample were recorded using a Bruker D8 Advance diffractometer (Champs-sur-Marne, France) with the *para*-focusing Bragg-Brentano geometry under the following conditions: Cu Kα radiation, 40 kV, 40 mA, scanning velocity 0.015° s^{-1}. Dynamic scintillation counter detector was used to monitor the diffracted intensities. All diffractograms were normalized at the same total area under scattering curve over the Bragg angle range 3° and 30° (2θ).

Determination of sorption isotherms

Sorption isotherms were obtained by gravimetric method using an Autosorp 3.0 apparatus (Biosystemes, Couternon, France) at 25°C. The apparatus comprised a closed chamber where temperature and relative humidity were regulated and controlled electronically (± 0.2°C and ± 0.02%, respectively). A rotating disk supported and weighted the products automatically using an electronic balance (±0.0001 g). A ventilator provided a homogenous gas phase. For absorption process, 0.1g of each dried sample was placed in the chamber maintained at 25°C. The relative humidity of the chamber was controlled by an atomizing humidification system at values ranging from 0 to 90%.

Mechanical properties

Tensile testing of dumbbell specimens of corn flour-based materials was done according to ISO 527-2 standard by using an Adamel Lhomargy (Roissy en Brie, France) testing machine. Samples for tensile measurements were conditioned at 50% relative humidity (RH) and 23°C for 2 weeks before testing. The tests were performed at 23°C using a load cell of 1 kN at a crosshead speed of 5 mm min^{-1}. An average value of three repeated tests was taken for each type of samples. Determination of Young modulus is provided to tangent at origin of the curve stress-elongation.

Amylolytic hydrolysis

Since corn flour is mainly composed of starch, an enzymatic preparation containing α-amylase and amyloglucosidase from *Aspergillus niger* was used. This enzymatic preparation (Hazyme® DCL) was a gift from DSM Food Specialties (Seclin, France). A unit of α-amylase or a unit of amyloglucosidase (U) is defined as the amount of enzyme that liberates 1 mg of maltose or 1 mg of glucose from starch in 3 min, respectively, (at 20°C and pH 6.9). Thirty grams per liter crushed material suspensions were prepared in a reaction buffer containing sodium acetate 0.05 mol L^{-1} (pH 5.5), sodium azide (0.02% w/w) to prevent bacterial growth and calcium chloride (50 mg L^{-1}) to stabilize the fungal amylolytic enzymes. Enzymes were then added to the desired final concentration (50 U mL^{-1}) (except in control tubes). The tubes containing the reaction mixtures were incubated in a chamber thermostated at 50°C (optimal temperature for enzymes activity)[18] under rotary shaking (6 rpm). The enzymatic hydrolysis of starch from corn flour and materials prepared from it were monitored by following the release of reducing sugars in the reaction mixture. The reducing sugars were measured by Miller method[19]: briefly, 400 μL of reaction mixture were sampled out at regular intervals for 30 min and 40 μL of HCl (0.01 mol L^{-1}) were added immediately to stop the enzyme action. After centrifugation for 10 min at 10,000 × g and 4°C, 600 μL of 3,5-dinitrosalycilic acid (DNS, 10 g L^{-1}) solution were added to the supernatant before heating at 100°C for 15 min. After cooling, the absorbance was measured at 640 nm using a spectrophotometer (Jenway 6300, Essex, United Kingdom). The linearity of absorbance versus incubation time for the 15 first minutes allowed determining the initial velocity of enzymatic amylolysis. All enzymatic hydrolysis experiments were performed in triplicate.

Simultaneous proteolytic and amylolytic hydrolysis

To assess the potential barrier role of protein layers surrounding starch granules in native corn flour and the impact of thermomechanical treatments destructuring native corn flour on this barrier role, corn starch hydrolysis by amylolytic enzymes was also followed in the presence of protease B from *Bacillus amyloliquefaciens* (Brewers® Protase B MG preparation, DSM Food Specialties, Seclin, France). This enzymatic preparation was added in the reaction medium at an E/S (enzyme/substrate) ratio of 100μg g^{-1} with amylolytic enzymes (Hazyme® DCL, 50 U mL^{-1}). The hydrolysis by amylolytic enzymes of powders from corn flour and injected-molded materials derived from it was performed and monitored under the same conditions as described above.

Confocal scanning laser microscopy (CSLM)

CSLM was used to observe the protein organization around starch granules in the native and extruded materials. For each sample, 12.5-μm-thick slices of the sample were prepared using a microtome at room temperature. The sections were placed on a flat glass slide. Protein staining was performed with 0.01% (w/v) acid fuchsin dissolved in 1% (v/v) acetic acid for 5 min, as described by Chanvrier et al.[8] The slices were then extensively washed with distilled water, placed on a microscope slide, covered with a cover slide and sealed with nail varnish to avoid drying out. The samples were analyzed using an inverted Zeiss CSLM 510 META microscope (Zeiss, Oberkochen, Germany). The CSLM consisted in an Aviovert 200*M* BP inverted microscope, using a He/Ne laser with 543-nm wavelength. The images were analyzed on a computer using Zeiss LSM Image Browser.

Statistical analysis

All experiments were performed using at least three samples (replicates). The results presented are the averages and standard deviations that were calculated from these replicate measurements. Statistical differences between samples were calculated using Fisher test with a significance level of $P > 0.05$ (Statgraphics centurion XV_ software, version 15.0.10, Sigmaplus, Levallois-Perret, France).

RESULTS AND DISCUSSION

Differential scanning calorimetry and X-ray analysis of corn flour and materials prepared from it

Figure 2 gives the thermograms obtained by DSC analysis of the native corn flour and extruded and injection molded corn flour-based materials. The gelatinization endothermic enthalpy (ΔH) values of corn flour, extruded and injection molded corn flour-based materials can be classified as follows: $\Delta H_{\text{native corn flour}}$ (3.6 J g^{-1}) > ΔH_{G10S1} (0.35 J g^{-1}) > ΔH_{G5S1} (0.07 J g^{-1}) ≥ ΔH_{G10S3} and ΔH_{G5S3} (0 J g^{-1}) (G5S1, G5S3, G10S1, and G10S3 codification is given in Table I). Whatever the glycerol content (5 or 10% wb) in the formulation and the number of shearing zones in the twin-screw extruder (one or three), the thermomechanical treatment of native corn flour led to a marked decrease of the gelatinization endothermic enthalpy: their residual values following extrusion-injection molding treatment were always less

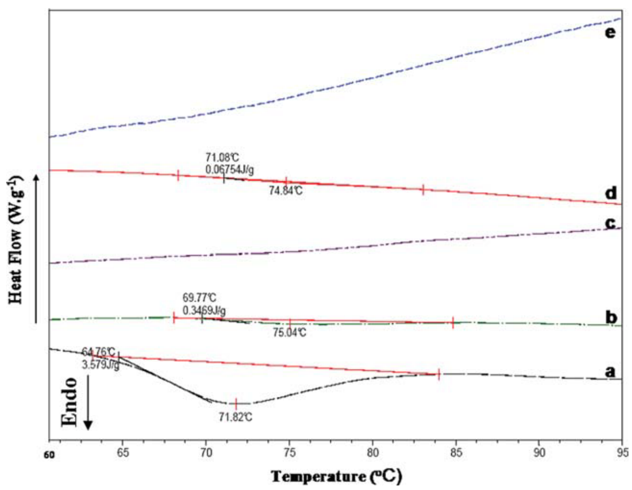

Figure 2 DSC thermograms of native corn flour (a), injection molded materials made from corn flour with 5 or 10% wb glycerol extruded with a twin-screw extruder with a screw profile with one or three shearing zones [G5S1 (d), G5S3 (e), G10S1 (b), and G10S3 (c)]. Thermograms were shifted along the *Y* axis for clarity. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

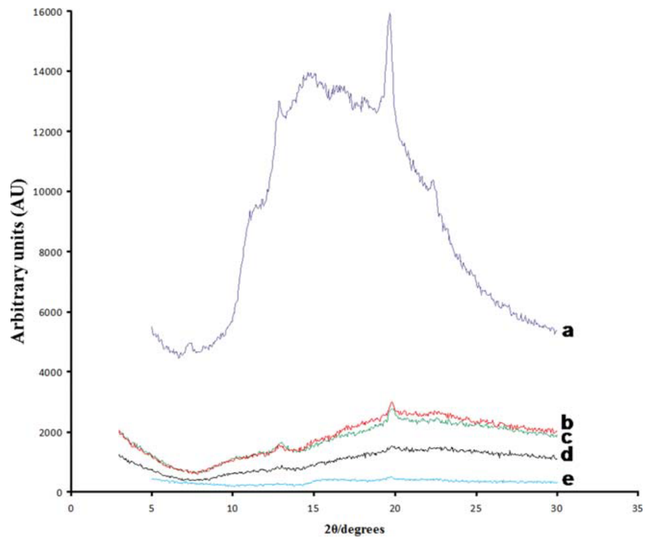

Figure 3 *X*-ray diffraction patterns of native corn flour (a), and injection molded materials made from corn flour with 5 or 10% wb glycerol extruded with a twin-screw extruder with a screw profile with one or three shearing zones [G5S1 (b), G10S1 (c), G5S3(d), G10S3(e)]. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

than 8% of the value measured for native corn flour. The residual gelatinization enthalpy value of the 10% wb glycerol formulation extruded in a twin-screw extruder containing one shearing zone (G10S1) at a temperature not exceeding 100°C before injection molding at less than 120°C (8% of that of native corn flour) is consistent with those reported by Chanvrier et al. for corn flour processed in a Rheoplast® at 100 and 128°C to simulate extrusion (13.5 and 2.9% of that of native corn flour, respectively).

Figure 3 shows X-ray diffraction diagrams of the native and extruded corn flour-based materials. Native corn flour presented an intense peak at $2\theta = 19.6°$ which considerably decreased after extrusion and injection molding. X-ray analysis confirmed that only the materials processed in the twin-screw extruder with one shearing zone (G5S1, G10S1) contained residual native starch, whereas starch was completely amorphous in the materials processed in the extruder with three shearing zones whatever their glycerol content (G5S3, G10S3).

Taken together, these results confirm that increasing shearing stress leads to decrease starch crystallinity. It can also be observed that the residual gelatinization enthalpy of corn flour-based materials processed with 5% wb glycerol (0.07 J g^{-1}) was close to zero and significantly lower than that of materials processed with 10% wb glycerol (0.35 J g^{-1}). This result highlights that glycerol content notably affects the residual crystallinity degree of corn starch. Our results are in agreement with those of Van Soest et al.[20] who showed that the residual gelatinization enthalpy increased significantly when the glycerol percentage increased in starch materials.

Water sorption isotherms of corn flour and materials prepared from it

Sorption isotherms of native corn flour and extruded-injection molded materials prepared from it are shown in Figure 4. All the studied samples showed a sigmoidal water sorption profile, which is the generally obtained form for food products.[21]

The main differences between isotherms were located in the 0.6–0.9 water activity range. A classification based

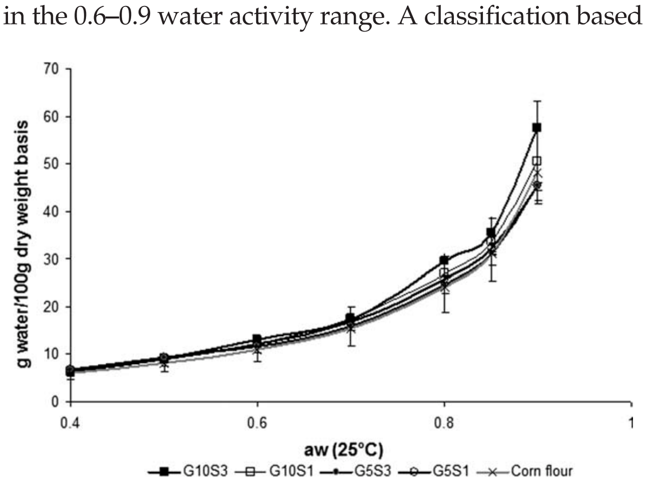

Figure 4 Corn flour and injection molded materials made from corn flour with 5 or 10% wb glycerol extruded with a twin-screw extruder with a screw profile with one or three shearing zones corn flour–water–glycerol blends extrudates (G5S1, G10S1, G5S3, G10S3) adsorption isotherms at 25°C.

TABLE II
Mechanical Properties of Corn Flour-Based Materials

Samples	Tensile breaking strength (MPa)	Elongation at break (%)	Young modulus (MPa)
G5S1	2.6 ± 0.2[a]	17.4 ± 5.7[a]	88.3 ± 9.2[a]
G5S3	7.1 ± 0.8[b]	6.4 ± 3.5[b]	355.1 ± 104.2[b]
G10S1	2.3 ± 0.1[c]	19.2 ± 7.6[a]	74.4 ± 24.9[a]
G10S3	7.0 ± 0.3[b]	14.6 ± 3.3[a]	198.8 ± 34.5[c]

Each value is the mean of three determinations. Error bars correspond to standard deviation. Means with the same letter are not significantly different at $P < 0.05$ as determined by Fisher's least significant difference procedure.

on the quantity of sorbed water on a dry weight basis in this water activity range can be established: G10S3 > G10S1 > G5S3 ≥ G5S1 ≥ native corn flour. Water sorption was thus mainly correlated with glycerol content in extruded-injection molded materials but also to a lesser extent with the applied shearing stress level during extrusion. Lourdin et al.[22] also observed that increasing the glycerol content in starch-based materials significantly increases their capacity to adsorb water. In the same context, other studies demonstrated that glycerol-plasticized films had higher moisture content than nonplasticized ones at high RH and compared to several other plasticizers, glycerol showed higher affinity to water.[23,24]

Concerning the thermomechanical treatment, the shearing stress increase (from one to three shearings) led to an increase in moisture content from 50 to 57 wt % at 25°C and 90% relative humidity for materials prepared with 10% wb glycerol (G10S1 and G10S3, respectively). However for materials made from a 5% wb glycerol formulation, moisture content under the same conditions remained constant (45 g adsorbed water per 100 g material) regardless of the number of shearing zones in the twin screw extruder.

Mechanical properties of corn flour-based materials

Tensile breaking strength, percentage of elongation at break, and Young modulus values of dumbbell specimens of corn flour-based materials are given in Table II. Increasing the number of shearing zones in the twin-screw extruder from one to three resulted in a significant increase of tensile breaking strength and Young modulus of corresponding materials. Conversely, a significant ($P > 0.05$) and not significant ($P < 0.05$) decrease of the percentage of elongation at break were observed for materials containing 5 and 10% glycerol, respectively.

Mechanical properties were more affected by the increase of the number of shearing zones from one to three than by the increase of glycerol content from 5 to 10%: materials prepared by extrusion with three shearing zones (G5S3, G10S3) in the twin-screw extruder were more resistant and less elastic than materials prepared by extrusion with one shearing zone. The lower strain at break values of these latter materials (G5S1, G10S1) might be linked to the presence of residual starch granules, while starch was completely amorphous in G5S3 and G5S10 materials: Chanvrier et al.[9] proposed that residual starch granules in the amorphous starchy matrix constitute heterogeneity and are potentially responsible for this fragile behavior. On one hand, increasing glycerol content from 5 to 10% did not significantly ($P < 0.05$) modify the mechanical properties of materials containing residual starch granules (G5S1, G10S1). On the other hand, G5S3 and G10S3 materials had a similar tensile breaking strength, while G10S3 materials had a significantly ($P > 0.05$) higher percentage of elongation at break and a significantly ($P > 0.05$) lower Young modulus than G5S3 materials. This is coherent with the observation that plasticizers such as glycerol weaken intermolecular forces between the adjacent polymer chains,[25] thereby increasing elasticity and decreasing resistance of starch-based materials, although this trend would be more marked for higher levels of glycerol addition. It is also important to note that since dumbbell specimens were conditioned at 50% RH and 23°C before the tensile tests, the materials with glycerol are also plasticized with water (the equilibrium moisture under these conditions is about 0.1 g water per g dry solids).

Kinetics of enzymatic amylolysis of corn flour and materials prepared from it

To assess both the effects of thermomechanical treatment (extrusion and shearing) and formulation on starch hydrolysis ability, the studied samples were subjected to amylolytic enzymes action and the produced reducing sugars were assayed (Fig. 5). The results showed that, whatever the number of shearing zones in the extruder, the thermomechanical process (extrusion-injection molding) had strongly increased the susceptibility of corn starch present in corn flour-based materials to hydrolysis by amylolytic enzymes (*A. niger* amyloglucosidase and α-amylase). Indeed, hydrolysis rates were respectively, 0.22 ± 0.02, 2.45 ± 0.09, 3.24 ± 0.02, 3.21 ± 0.03, and 4.03 ± 0.08 g glucose released L^{-1} min^{-1}, for native corn flour, G5S1, G5S3, G10S1, and G10S3. Avérous[2] also reported that thermomechanical treatments used to prepare glycerol-plasticized starch-based materials improve material biodegradation. Moreover, Mercier and Feillet[26] observed that a high temperature during extrusion of corn grits considerably

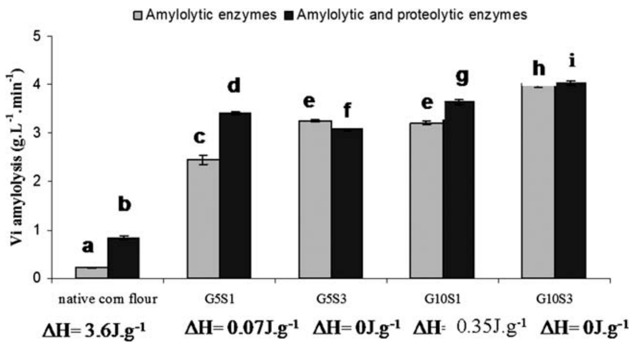

Figure 5 Initial rate of hydrolysis of corn starch by amylolytic enzymes alone or associated with proteolytic enzymes in native corn flour and injection molded materials made from corn flour with 5 or 10% wb glycerol extruded with a twin-screw extruder with a screw profile with one or three shearing zones (G5S1, G10S1, G5S3, G10S3). Enzymatic hydrolysis experiments were performed at 50°C and pH 5.5. Other experimental conditions are described in the Material and methods sections. [Data with different letters are significantly different (Fisher's test, $P < 0.05$)].

increases water-soluble carbohydrate amount and consequently the initial rate of starch hydrolysis by α-amylase. Extrusion process is known to induce structural modifications of starch as it has been described by Barron et al.[12] Indeed, native corn starch granules are a mixture of crystalline and amorphous states. Extrusion process induced granules destruction and formation of amorphous starch at the expense of crystalline one. Since crystalline starch is far less susceptible to the amylolytic enzymes action than amorphous one,[27] this might explain the observed correlation between amylolytic enzymes susceptibility and the destructuration level of starch granules. It is also noteworthy in Figure 5 that the higher the endothermic corn starch gelatinization enthalpy (ΔH), the lower the rate of corn starch hydrolysis by amylolytic enzymes. Since a higher ΔH is associated with a higher proportion of crystalline starch, this substantiates the hypothesis that starch aptitude to enzymatic attack mainly depends on their gelatinization degree.

The initial rates of starch hydrolysis by amylolytic enzymes alone or associated with a protease are also presented on Figure 5. Simultaneous hydrolysis by amylolytic and proteolytic enzymes induced a marked increase of starch hydrolysis for native corn flour and to a lesser extent for extruded-injection molded materials processed with the twin-screw extruder with one shearing zone (G5S1 and G10S1) but no significant changes for the materials processed with the twin-screw extruder with three shearing zones (Fig. 5). These results demonstrate that proteins are involved in mechanisms limiting corn starch hydrolysis by amylolytic enzymes for native corn flour and materials prepared from it by using a twin-screw extruder with only one shearing zone. The limiting effect of proteins was thus limited to materials for which the residual endothermic starch gelatinization enthalpy was not negligible, that is the starch granules present in corn flour were not completely gelatinized. This suggests thus that this limiting effect of proteins depended on starch granules destructuration level. Protein layers surrounding starch granules in native corn flour might constitute a structural barrier to the access of enzymes to starch. A previous study has shown that, to optimize corn flour thermal hydrolysis, it is necessary to destroy the protein layer surrounding starch granules in native corn flour by cooking at high temperature.[28] Therefore, to check the validity of this protein protective layer hypothesis, confocal scanning laser microscopy (CSLM) observations after protein staining with acid fuchsin of native corn flour and extruded-injection molded materials prepared from it were performed. The corresponding results are detailed in the next section.

Confocal scanning laser microscopy observations of corn flour and materials prepared from it

Microscopic observations of native corn flour [Fig. 6(a,b)] showed a continuous proteins matrix surrounding starch granules. For extruded-injection molded corn flour-based materials [Fig. 6(c–j)], the size of this external protein layer considerably decreased and proteins aggregates were observed. This is consistent with observations by Chanvrier et al.[8] CSLM images also revealed that increasing the number of shearing zones during extrusion process led to a significant increase in the proteins aggregation and to a destruction of the continuity aspect of the proteins layers observed in native corn flour starch granules: remnant protein layers were still observed on micrographs of materials processed with one shearing zone in the twin-screw extruder [Fig. 6(c,d,g,h)] but no more on micrographs of materials processed with three shearing zones in the twin-screw extruder [Fig. 6(e,f,i,j)]. Besides, a thorough comparison of CSLM photographs of materials made with 5% wb glycerol [Fig. 6(c–f)] and 10% wb glycerol [Fig. 6(g–j)] indicates that increasing the glycerol content favored the protein layer destructuration and dispersion. The initial rate of enzymatic amylolysis of materials made with 5% wb glycerol with the twin-screw extruder containing one shearing zone increased from 2.44 ± 0.07 g glucose released L^{-1} min^{-1} in the absence of proteolytic enzymes to 3.41 ± 0.03 g glucose released L^{-1} min^{-1} in their presence, while the initial rate of enzymatic amylolysis of 10% wb glycerol materials processed under the same conditions only increased from 3.21

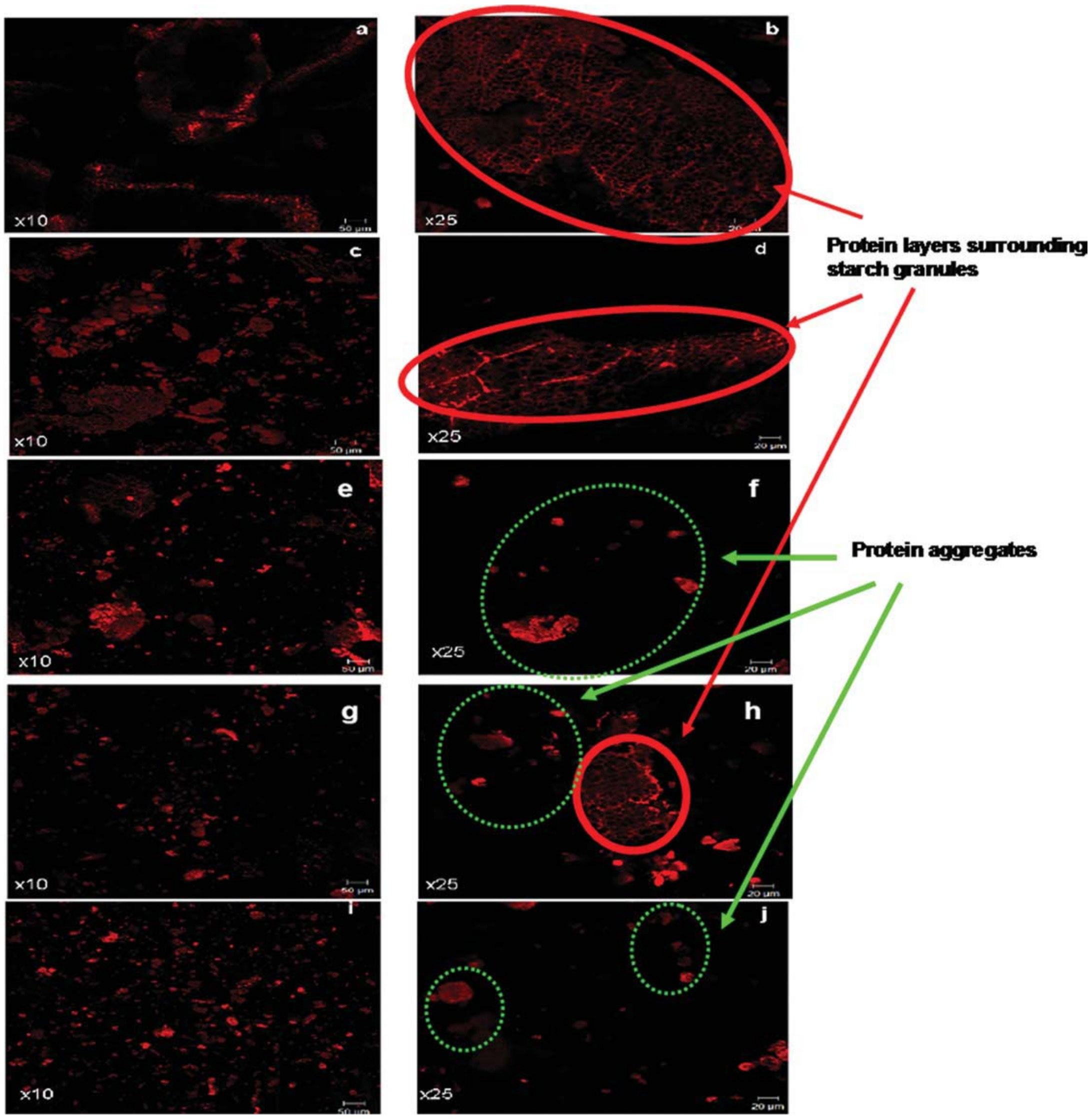

Figure 6 CSLM observations of native corn flour (a and b) and extruded-injection molded materials made from corn flour with 5 or 10% wb glycerol extruded with a twin-screw extruder with a screw profile with one or three shearing zones [G5S1 (c and d), G5S3 (e and f), G10S1 (g and h), and G10S3 (i and j)]. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

± 0.03 g glucose released L^{-1} min^{-1} to 3.64 ± 0.03 g glucose released L^{-1} min^{-1} (Fig. 5). The observation by CSLM of the lower degree of destructuration of the protein layer surrounding starch granules in materials made with 5% wb glycerol than in materials made with 10% wb glycerol is thus consistent with the higher acceleration by proteases of enzymatic amylolysis of corn starch in materials made with 5% wb glycerol. Moreover, the absence of remnant protein layers surrounding starch granules on CSLM photographs and of acceleration by proteases of enzymatic amylolysis of corn starch for materials processed in a twin-screw extruder with three shearing zones further substantiates the hypothesis previously made concerning the protective role against enzymatic hydrolysis played by the protein layers still present around starch granules in materials with a lower degree of destructuration of starch granules.

GENERAL DISCUSSION

Corn flour starch granules destructuration–shear stress relationships

The experimental design of this study was constructed to assess the respective effects of thermomechanical extrusion-injection molding treatments and formulation on native corn flour destructuration. Native corn flour (as a control), and corn flour-based materials with 30% wb total plasticizer (water and glycerol) content either with 5 or 10% wb glycerol processed with a corotative twin-screw extruder containing one or three shearing zones in its profile were thus analyzed by differential scanning calorimetry, X-ray diffractometry, and their respective water sorption isotherms at 25°C were compared. Moreover, the susceptibility of corn starch to hydrolysis by amylolytic enzymes was investigated. A comparison of the results of all these analyses performed on native corn flour and on materials prepared from it indicated that the endothermic gelatinization enthalpy of corn starch granules and their crystallinity markedly decreased, whereas the water uptake (above 60% RH) and the rate of hydrolysis of corn starch by amylolytic enzymes significantly increased following the thermomechanical treatments. All these changes were more marked when materials were prepared with the twin screw extruder containing three shearing zones instead of one in its profile. Taken together, these results highlight the correlation of shear stress intensity with corn starch granules level of destructuration and formation of amorphous starch at the expense of crystalline one.

Corn flour starch granules destructuration–glycerol content relationships

The impact of glycerol content (5 or 10% wb) was not marked for materials obtained by extrusion with three shearing zones in the profile of the extruder, since their crystallinity strongly diminished and the residual endothermic gelatinization enthalpy of corn starch granules (ΔH) was negligible ($\sim$ 0 J g^{-1}). The only observed significant difference concerned the initial rate of hydrolysis of corn starch by amylolytic enzymes (3.24 $\pm$ 0.02 and 4.03 $\pm$ 0.08 g glucose released L^{-1} min^{-1} for materials made with a 5% wb or a 10% wb glycerol content, respectively). Far more significant differences were observed between materials made with a 5% wb or a 10% wb glycerol content by extrusion with only one shearing zone in the profile of the extruder and thus with a lower corn starch granules destructuration level. For instance, the residual ΔH values represented $\sim$ 1.5 and $\sim$ 8% of $\Delta H_{\text{native corn flour}}$ value for the materials made with 5% wb and 10% wb glycerol, respectively. The increase of glycerol content under these conditions thus led to a decrease of the corn starch granules destructuration level and amorphization. Consequently, since amorphous starch is far more susceptible to enzymatic amylolysis than crystalline one,[23] one would have expected that the rate of corn starch hydrolysis by amylolytic enzymes would have been higher for materials made with 5% wb glycerol. Indeed, the values of this rate were 2.45 $\pm$ 0.09 and 3.21 $\pm$ 0.03 g glucose released L^{-1} min^{-1} for materials made with 5 and 10% wb glycerol, respectively.

Plasticized corn flour structure: The proteins role

Since proteins surrounding corn starch granules may also prevent hydrolysis of corn starch by amylolytic enzymes, CSLM observations after staining proteins with acid fuchsin were performed and the kinetics of hydrolysis of corn starch by amylolytic enzymes in the presence or absence of proteases were determined. This allowed to observe that the destructuration of protein layers and their subsequent dispersion as proteins aggregates were less marked for materials made with 5% wb glycerol. Combined with the observation that in presence of proteases, the initial amylolysis rate increased from 2.45 $\pm$ 0.09 to 3.41 $\pm$ 0.03 g glucose released L^{-1} min^{-1} for materials made with 5% wb glycerol, while this rate only increased from 3.21 $\pm$ 0.03 to 3.64 $\pm$ 0.03 g glucose released L^{-1} min^{-1} for materials made with 10% wb glycerol, this substantiates the hypothesis that the residual protein layer still surrounding starch granules in these materials plays a barrier role limiting the action of amylolytic enzymes on corn starch.

CONCLUSIONS

In this study, the specificity of the hydrolytic activity of amylolytic and proteolytic enzymes was exploited to get a better insight in the structure of corn flour based materials made by thermomechanical processing. The results of this study highlighted that besides the contribution of the thermomechanical processing to the destructuration of corn starch granules and the formation of amorphous starch at the expense of crystalline one, the destructuration of the protein layer surrounding starch granules in native corn flour should also be considered in materials made from it.

Besides getting a better insight in the structure of corn flour-based materials, this study may also be a scientific basis to improve the understanding of the susceptibility of corn flour-based materials to biodegradation.

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