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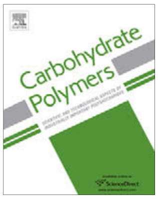

Enhanced mechanical properties of partially beta-amylase trimmed starch for material applications

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ABSTRACT

The influence of partial beta-amylase dextrinisation on the recrystallisation properties of wheat starch was monitored by DSC. The melting peak endotherm in the 100–140 °C range decreased regularly at increasing degradation levels, and disappeared with the limit dextrin. Mechanical properties of the obtained partial dextrins displayed unusual stress/strain couple value. Dextrinisation leads to an increase of strain at break with retention of strength. Dextrinisation appears to enhance the orientation ability during processing.

1. Introduction

Starch, the most abundant α glucopyranose plant reserve polysaccharide, is commonly described as a natural complex of two polymeric families: 1/5th of linear amylose and 4/5th of highly branched amylopectin (Buléon, Colonna, Planchot, & Ball, 1998). Starch macromolecules have been identified in the last decades as potential candidates for the substitution of some oil based technical polymers. Most research efforts in this field have been dedicated to modifying the glass transition by destructurization/formulation of starch material, using plasticisers (Arvanitoyannis & Biliaderis, 1999; Follain, Joly, Dole, & Bliard 2005a, 2005b, reference therein). The conversion of glassy structured native starch into rubbery plasticised destructurized starch has been hailed as the solution for optimising the material's mechanical properties. Unfortunately none of the attempts in this direction seems to have succeeded in increasing starch's mechanical properties to reach the ones of reference synthetic polymers for common applications. The modulation of the molecular mobility by gradual introduction of plasticisers only changes the properties from brittle to ductile behaviours, but the precocious rupture character of starch materials remains the same for all types of stress strain relation. This precocious rupture could be attributed to the presence of defects in the structure as well as low ability for establishing crystalline or physical entanglements at the macromolecular level.

In the case of classical semi-crystalline polymers, crystallisation enhances mechanical behaviour by creating physical crosslinking between polymer chains. The opposite effect observed with the crystallisation of starch material could be attributed to intramolecular recrystallisation of the highly branched amylopectin, instead of intermolecular crystallisation. Native granular starches, possess a level of natural crystallinity as seen by early X-ray diffraction experiments (Katz & Van Itallie, 1930; Zobel, 1964). The two main A and B crystallinity types found in starch have been described as two types of packing polymaltosidic rod-like double-helices (Imberty, Buleon, Vinh, & Perez, 1991). When hydrothermal treatment in excess water is applied between 50 and 90 °C, the granules swell and are progressively destructurized. During this process the genuine crystallinity is lost; but spontaneous recoiling of the polymaltosidic double-helices happens upon cooling, leading to recrystallisation and starch's ubiquitously used stiffening properties. Similarly in plasticised starch the transition glass temperature (T_g) decreases rapidly with increasing water or plasticiser content and when T_g is below 20 °C, the material is at the rubbery state at room temperature. In the rubbery state the macromolecules possess a high level of local mobility and, providing they have a sufficient mobility, linear α (1–4) glucosidic chains present in amylose or amylopectin side chains will spontaneously recoil as double-helix rods, inducing recrystallisation (Van Soest, Hulleman,

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De Wit, & Vliegenthart, 1996; Van Soest & Knooren, 1997). Amylose would crystallize rapidly whereas amylopectin would develop crystallinity over a much longer period of time. Unfortunately retrogradation is not the only drawback for starch mechanical behaviour since totally amorphised materials also present very low properties.

The average shape factor (molecular length to width ratio) of starch constituents seems to be unsuitable for creating effective entanglements, mainly due to the (highly branched) amylopectin contribution (Robin, Mercier, Charbonniere, & Guilbot, 1974). This remark on the shape factor effect of starch components can be generalised to other natural or renewable polymers which are not naturally well designed for optimal entanglement. On a strain at break vs stress at break diagram presented in Fig. 1, different structural effects can be depicted:

(i) Extracted hemicelluloses display low mechanical behaviour, mainly attributable to low molecular weight despite their short branching.
(ii) Destructurized unfolded proteins display much better properties, close to the ones present in the family of plasticised polylactic acid. This can be linked to the high shape factor of proteins.
(iii) Plasticised starch shows intermediate properties, between hemicellulose and proteins.

Classical approaches to limit recrystallisation in starch have been limited to performing low levels of chemical grafting. Low amounts of functional modification considerably decrease the level of chain reorganization. Yet, no efficient strategies can be found to enhance the shape factor of amylopectin. In previous studies we presented various strategies destined to counteract the detrimental effect of the retrogradation by using a photo-crosslinking process (Delville, Joly, Dole, & Bliard, 2002, 2003), or compatible hydrophilic polymer addition and combined processes (Follain et al., 2005a, 2005b). In the present study we explore the beneficial effects of a controlled enzymatic trimming on the retrogradation of the constituting macromolecules and the consequences on the resulting material's mechanical properties. Beta-amylase has been widely used for starch structural investigation studies (Bourne, Haworth, Macey, & Peat, 1948; French, 1960; Watanabe & French, 1980). It is classically used in cocktails with other enzymes in the industrial production of high dextrose equivalent (DE) syrups from starch (Alexander, 1992).

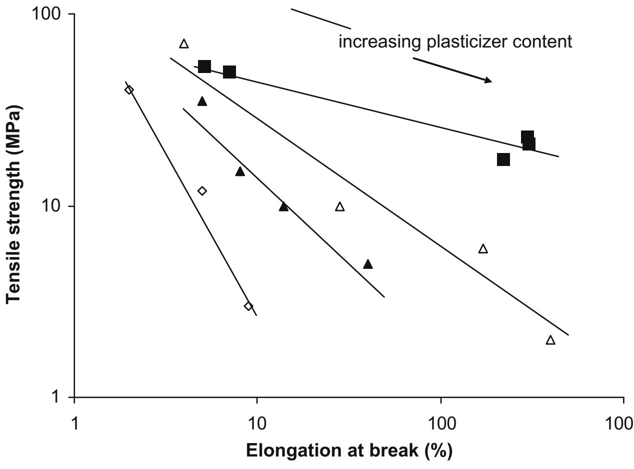

Fig. 1. Comparison of the plasticisation effect on mechanical properties in different natural or renewable polymers: ■ acetyl tri-*n*-butyl citrate plasticised PLLA, (from Baiardo et al., 2003), △ glycerol & lactic acid plasticised wheat gluten, (from Pommet, Redl, Guilbert, and Morel (2005)), ▲ sorbitol plasticised starch, (from (Gaudin, Lourdin, Le Botlan, Ilari, and Colonna (1999)), ◇ sorbitol plasticised hemicelluloses, (from (Groendahl, Eriksson, and Gatenholm (2004)).

In amylopectin partial dextrinisation is expected to result in limiting intramolecular reorganization and reducing the overall width, slenderising the shape of the macromolecules without markedly reducing their original molecular length. In this paper, we investigate the result of partial β-enzymolysis of starch on the retrogradation properties of the obtained dextrins, and the effect on the resulting plasticised materials' mechanical properties.

2. Materials and methods

2.1. Starch, enzyme, glycerol

Native wheat starch (12% humidity) from Chamtor (Bazancourt, France) was defatted by ethanol soxlet extraction for 24 h and deproteinated as described previously (Bertoft, 1989).

Beta-amylase (EC 3.2.1.2, type II.B Crude, from Barley A 7130) from Sigma was used as received, after checking that the maximum degradation level of starch was corresponding to a 50% mass loss, as reported in literature. The produced maltose was analysed by HPLC to be the sole product of the reaction using a Rezex RSO oligosaccharide Ag+ column from Phenomenex at 85 °C in a thermosphere TS130 temperature control system using 0.2 μm filtered pure water eluent.

Glycerol was purchased from Sigma–Aldrich (L'Isle d'Abeau Chesnes, France) and was used without further purification.

2.2. Beta-Amylolysis kinetics parameter measurement

Twenty gram of purified starch was destructurized in 400 mL of water for 20 min in an non-magnetic stainless steel commercial autoclave from SEB France, at 120 °C under gentle stirring on a magnetic stirrer while heating, as described previously (Delville et al., 2002). The hot mixture was diluted with a (50 mMol) pH 4.8 acetate buffer to obtain 1 L of solution. When the resulting solution had cooled to 50 °C, 1 mL of 1.7 mg L^{-1} beta-amylase solution (50 units) was added. The resulting mixture was gently stirred at 50 °C for a total of 400 min. After enzyme addition, every 20 min 5 mL aliquot were sampled. Each aliquot was immediately boiled for 5 min prior to reducing power analysis using the Luff–Schoorl method, calibrated on known maltose standards (Schoorl, 1929). The results of the hydrolysis kinetics (Fig. 2) gives the evolution of the equivalent maltose concentration reducing power (r) as a function of hydrolysis time (t) with the following parameters $r = 0.0162t + 0.7241$ with a correlation coefficient R^2 value of 0.9948.

2.3. Production of the partial dextrins

The degradation times corresponding to the 5%, 10%, 20% and 30% partial dextrins D5, D10, D20, D30 were determined from

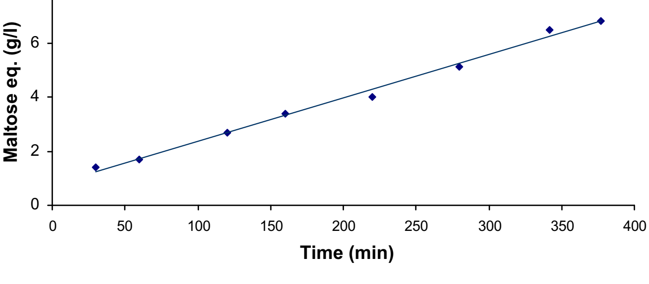

Fig. 2. Beta-amylase hydrolysis kinetics.

the previous kinetics parameters and four starch samples (20 g) were reacted at 50 °C as described above, during 17, 79, 202 and 326 min respectively. The hydrolysis reaction was then stopped by addition of 50 mL ethanol and boiling for 5 min. The partial dextrins were then purified by diafiltration in a 2.5 L stirred frontal ultrafiltration cell (MILLIPORE, Saint Quentin en Yvelines, France) on 10 K MWCO regenerated cellulose membranes (13661AM MILLIPORE). The ultrafiltrate maltose concentrations were controlled in the initial ultrafiltrates as described above, before diluting and diafiltering the dextrin concentrates (typically 4 L) until the ultrafiltrates showed no indication of reducing power. The cell contents were then reduced to 300 mL and freeze-dried on a HETO Powerdry L 3000 (JOUAN, Saint Herblain, France). Both the obtained dextrin yield and maltose concentration were coherent with the calculated degradation level.

2.4. Plasticisation and material processing

The partial dextrin samples (generally from 10 to 15 g) were shaken for at least 30 min in 100 mL absolute ethanol solution with 20.5% glycerol (w/w dry sample). After diffusion of the plasticiser to the sample, ethanol was evaporated under reduced pressure. The new crumbly material was kept at 80 °C for 1 h to remove ethanol. Then the resulting mixture was kept for 4 days at 80% RH in order to ease the extrusion. After this period all the different plasticised samples were extruded in a micro-compounder ThermoHaake Minilab Rheomex CTW5. The screw rotation speed and the temperature of the sheath were set at 70 rpm and 110 °C. The obtained 3–4 mm strands were granulated prior to extrusion in the SCAMIA Rheoscam single screw extruder equipped with a specific 500 μm diameter multi-thread extruding die. The screw rotation was set to 50 rpm and the temperature of the three zones were set to 110, 115 and 120 °C. The resulting threads were kept at 50% RH for one week before mechanical testing.

2.5. Accelerated ageing and DSC measurement

Partial dextrin samples (D10–30) were grinded, equilibrated at 50% RH; 30% water was then added and samples were sealed in highly hermetic pans. Sealed pans were aged after a previously performed thermal treatment at 170 °C for 5 min intended to eliminate their own thermo-mechanical history. The absence of leak was controlled by weighing the pans after this first temperature scan. The sample weights were typically 15–50 mg according to the used pans. Accelerated ageing was performed at 60 °C for 65 h. After this time DSC measurement was performed at a 5 °C/min heating rate from −40 to 220 °C. Thermal analysis were performed on a TA Instruments 2920 DSC with DSC TM option and equipped with a refrigerated cooling system (RCS). Temperature and enthalpy were calibrated using indium. The same pan type and thermal program (heating rate), as in the corresponding experiments, were used for the calibrations.

2.6. Tensile testing of threads

Tensile tests were performed on a tensile testing machine at 50% RH (Test 108 from GT-Test, France), with a crosshead speed of 10 mm/min and a pressure transducer of 2 kN piloted by the Testwinner 220 software. A minimum of 30 samples for each Dextrin was tested. Young's modulus, ultimate tensile strength, and elongations at beak (EB) were obtained from the measurement of the force–strain curve.

3. Results and discussion

Beta-amylase is an exo-enzyme that specifically binds to a double (1–4-alpha-D-glucopyranosyl-1–4-alpha-D-glucopyranosyl-) unit from the non-reducing ends of polymaltosidic polysaccharides, and sequentially cleaves glucose disaccharidic units until it encounters any structural variation, producing maltose as sole hydrolysis product. In starch, amylopectins are known to leave up to 50% enzyme resistant material, named beta-limit-dextrins, bearing 1–6-alpha-D-glucose branched non-reducing ends (Whelan, 1964), whereas in the amylose fraction, non-branched amylose molecules are degraded completely to maltose or maltotriose for uneven DPs. Slightly branched amylose molecules are degraded from the non-reducing end down to the first branched point. Solutions of defatted, deproteinated destructurized wheat starch were hydrolyzed with beta-amylase at 50 °C. The maltose liberation kinetics was followed over 326 min (Fig. 2). From the kinetics parameters the four hydrolysis times, leading to 5%, 10%, 20% and 30% degradation levels, were determined. After hydrolysis, diafiltration ensured the removal of small molecules from the produced dextrins.

3.1. Retrogradation properties

As storage, temperature and humidity directly influence the development of crystallinity of starch material (Delville et al., 2002), the resulting dextrins were subjected to an accelerated ageing process prior to DSC analysis by storing material to be tested at 88% RH at 60 °C. The measurement of the enthalpy variation (Fig. 3) in the 100–140 °C range shows a rapid collapse of the melting endotherm value reported on (Fig. 4) with increasing dextrinisation.

3.2. Mechanical properties

In the previously produced partially degraded samples the 10%, 20% and 30% degraded materials were selected for mechanical evaluation. For technical applications, limit dextrin has limited interest because of treatment length. Moreover the produced maltose can act as an *in situ* generated co-plasticiser up to a level of 30% (Godbillot, Dole, Joly, Roge, & Mathlouthy, 2006) in a large scale process, therefore avoiding the costly and time consuming maltose separation step. In this study the mechanical properties were measured on maltose-free dextrins, in standardized formulation plasticised at 20.5% glycerol in order to monitor the effect of the degradation alone. As described in Follain's review Follain et al. (2005a,b), at this plasticiser level short distance interactions and plasticiser microdomains poorly influence mechanical properties, whereas long distance interactions (including entanglements) are supposed to be the key factor.

In order to amplify the observed effect of this degradation on the mechanical properties, the samples were processed in the forms of millimetre-size threads. At this size linear macromolecules can be easily orientated. This characteristic is commonly used for improving the mechanical properties of classical synthetic polymers. During the thread processing two macromolecule orientation events can occur: first during high shear step happening at the entrance of the narrow die and then by post die stretching of the thread during the rolling step.

Moreover the chosen process allowed us to produce numerous samples from limited amount of purified dextrins. However a non-stabilized extrusion process introduces variations of sample geometry. This point was used to our advantage to study the relation between the mechanical behaviour and sample geometry.

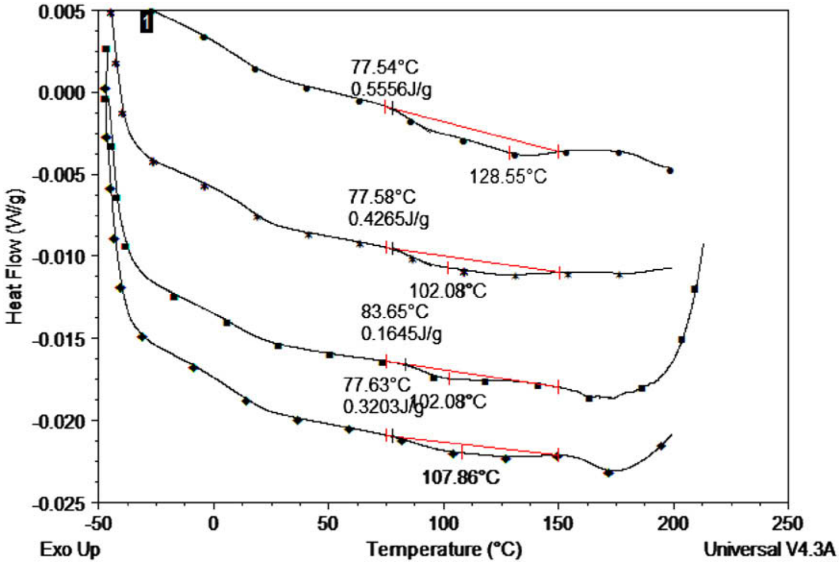

Fig. 3. DSC analysis of the retrogradation of starch and partial dextrins after accelerated ageing: ● starch reference material, * dextrin D10, ■ dextrin D20, ◆ dextrin D30.

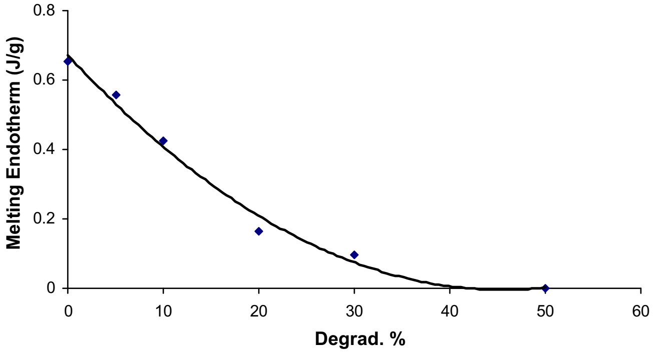

Fig. 4. Variation of the partial dextrin's melting endotherm with the dextrinisation rate.

As seen on Fig. 5, large variations of the Young's modulus can be observed as a function of the cross-section area and sample type. A progressive decrease of the force at break is observed with increasing dextrinisation. Non-degraded starch samples were produced with a narrower cross-section area range probably due to a larger melt viscosity. But in this obtained range no relation can be seen between cross-section area and modulus, while a clear relationship can be seen with all studied partial dextrins. Youngs modulus increases with decreasing cross-section area suggesting that orientation occurs. This phenomenon is seen at the smallest cross-section area. Fig. 6 displays the variation of the force at break with the cross-section area. A general relation between force and cross-section area can be seen allowing the calculation of strength for all samples. The values are almost identical but the standard errors decrease with the degradation yield. As seen on Fig. 7, unlike strength, strain at break shows a clear dependence on the level of dextrinisation. Strain at break increases with the dextrinisation level, from 2.6% for non-degraded starch to 31% for 30% – dextrinised samples. Though these compared values are obtained with samples having very different cross-section areas, a clear difference can be seen between non-degraded, 10% and 20% – dextrinised samples in the 1–1.5 mm^2 range, and between 20% and 30% dextrinised samples in the 0.5–1 mm^2 range.

Fig. 8 summarizes the results of the present work on a strain at break/stress scheme as described before in a short review dedicated to starch material's mechanical properties (Follain et al., 2005a,b). In Follain's review all starch material display systematic decrease of strength with the increase of strain at break.

In the present work the increase of strain at break observed with increasing degradation is obtained with total retention of strength.

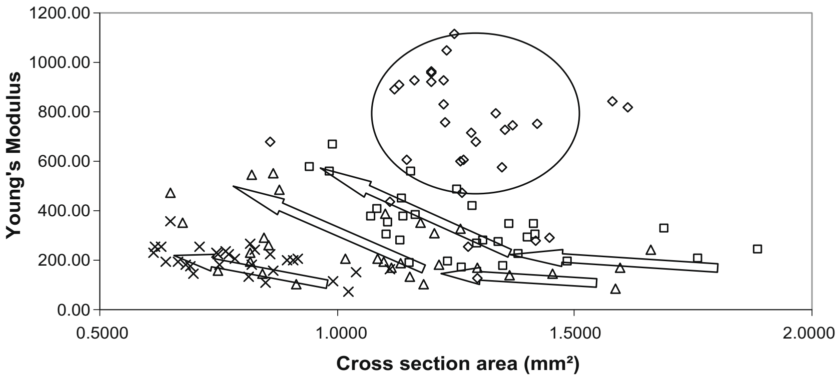

Fig. 5. Young's modulus evolution with the cross-section area. ◇ Starch reference material, □ dextrin D10, △ dextrin D20, × dextrin D30. The arrows represent the general tendencies.

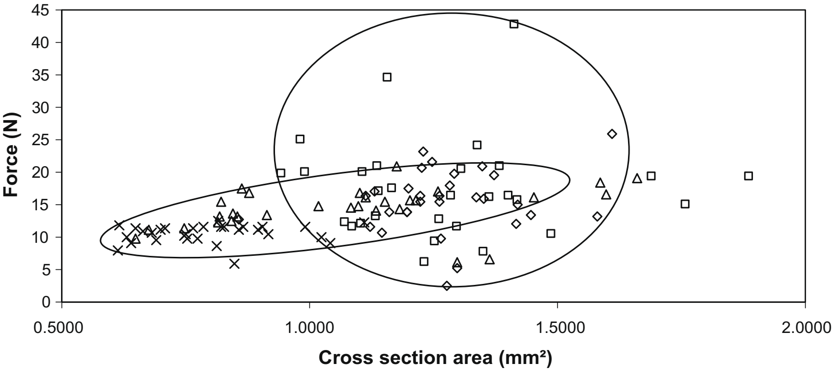

Fig. 6. Relationship between limit force and cross-section area. ◇ Starch reference material, □ dextrin D10, △ dextrin D20, × dextrin D30.

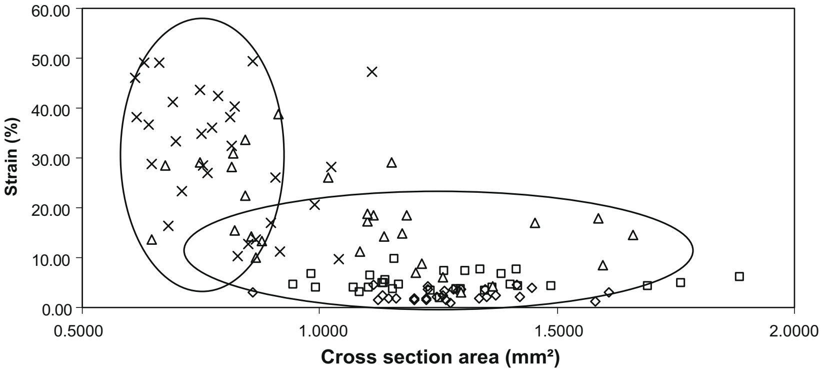

Fig. 7. Relationship between limit strain and cross-section area. ◇ Starch reference material, □ dextrin D10, △ dextrin D20, × dextrin D30.

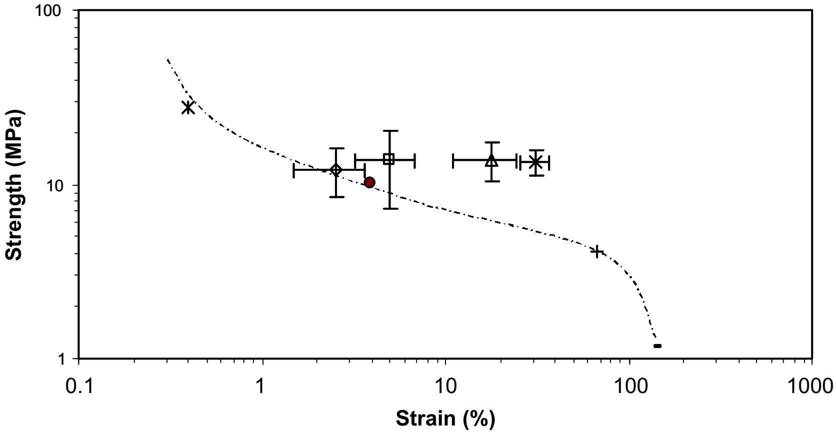

Fig. 8. Strength/strain at break of partial dextrins plasticised with 20.5% of glycerol. The dotted curve represents the evolution of reference starch plasticised with increasing glycerol amounts (* 5%, ● 17%, + 22% and – 30% wt). ◇ Starch reference material, □ dextrin D10, △ dextrin D20, × dextrin D30.

4. Conclusion

Starch beta-amylase partial degradation modifies the material's properties in various ways. The ability to recrystallize in accelerated ageing conditions decreased as a function of the degradation level. Mechanical properties were tested in conditions where retrogradation do not interfere i.e. at low relative humidity. In these conditions the modulus decreases and strain at break increases markedly with retention of stress.

These effects probably result from a complex combination of macromolecule orientation and molecule geometry. Orientation alone should result in increase of strength, modulus and strain. The direct effect of degradation is expected to result in alterations of all the properties, and particularly a decrease in strain at break. Such evolution of the mechanical properties associated with the orientation ability demonstrate that beta-amylase trimming leads not only to a mass reduction but also improves the ability for creating entanglements. These results bring the mechanical properties of starch's partial dextrins' close to the ones of protein material, as shown in Fig 1. It is worth noting that these results were obtained with a limited level of post extrusion orientation. This point should be further studied.

More complex is the question of the relative effect of dextrinisation on the two macromolecular families amylose and amylopectin. The continuous variations of retrogradation habilities as well as the strain at break increase suggest that a concomitant degradation of the two families occurs. This point is under current investigation.

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References

Alexander, R. J. (1992). Maltodextrins: Production, properties and applications. In F. W. Schenck & R. E. Hebeda (Eds.), *Starch hydrolysis products; worldwide technology, production, and applications* (pp. 233–276). New York: VCH Publishers.

Arvanitoyannis, I., & Biliaderis, C. G. (1999). Physical properties of polyol-plasticized edible blends made of methyl cellulose and soluble starch. *Carbohydrate Polymers, 38*, 47–58.

Baiardo, M., Frisoni, G., Scandola, M., Rimelen, M., Lips, D., Ruffieux, K., et al. (2003). Thermal and mechanical properties of plasticized poly(L-lactic acid). *Journal of Applied Polymer Science, 90*, 1731–1738.

Bertoft, E. (1989). Investigation of the fine structure of amylopectin using alpha- and beta-amylase. *Carbohydrate Research, 189*, 169–180.

Bourne, E. J., Haworth, N., Macey, A., & Peat, S. (1948). Amylolytic degradation of starch. A revision of the hypothesis of sensitization. *Journal of the Chemical Society*, 924–930.

Buléon, A., Colonna, P., Planchot, V., & Ball, S. (1998). Starch granules: Structure and biosynthesis. *International Journal of Biological Macromolecules, 23*, 85–112.

Delville, J., Joly, C., Dole, P., & Bliard, C. (2002). Solid state photocrosslinked starch based films: A new family of homogeneous modified starches. *Carbohydrate Polymers, 49*, 71–81.

Delville, J., Joly, C., Dole, P., & Bliard, C. (2003). Influence of photocrosslinking on the retrogradation of hydrated solid wheat starch based films. *Carbohydrate Polymers, 53*, 373–381.

Follain, N., Joly, C., Dole, P., & Bliard, C. (2005a). Properties of starch based blends. Part 2. Influence of polyvinyl alcohol addition & crosslinking on starch based materials mechanical properties. *Carbohydrate Polymers, 60*, 185–192.

Follain, N., Joly, C., Dole, P., & Bliard, C. (2005b). Mechanical properties of starch-based materials. I. Short review and complementary experimental analysis. *Journal of Applied Polymer Science, 97*, 1783–1794.

French, D. (1960). Application of enzymic specificity to the determination of structures. Determination of starch structure by enzymes. *Bulletin de la Société de Chimie Biologique, 42*, 669–677.

Gaudin, S., Lourdin, D., Le Botlan, D., Ilari, J. L., & Colonna, P. (1999). Plasticization and mobility in starch-sorbitol films. *Journal of Cereal Science, 29*, 273–284.

Godbillot, L., Dole, P., Joly, C., Roge, B., & Mathlouthy, M. (2006). Analysis of water binding in starch plasticized films. *Food Chemistry, 96*, 380–386.

Groendahl, M., Eriksson, L., & Gatenholm, P. (2004). Material properties of plasticized hardwood xylans for potential application as oxygen barrier films. *Biomacromolecules, 5*, 1528–1535.

Imberty, A., Buleon, A., Vinh, T., & Perez, S. (1991). Recent advances in knowledge of starch structure. *Starch/Staerke, 43*, 375–384.

Katz, J. R., & Van Itallie, Th. B. (1930). The physical chemistry of starch and bread making. V. All varieties of starch have similar retrogradation spectra. *Zeitschrift Physikalische Chemie, 150*(Abt. A), 90–99.

Pommet, M., Redl, A., Guilbert, S., & Morel, M. H. (2005). Intrinsic influence of various plasticizers on functional properties and reactivity of wheat gluten thermoplastic materials. *Journal of Cereal Science, 42*, 81–91.

Robin, J. P., Mercier, C., Charbonniere, R., & Guilbot, A. (1974). Lintnerized starches. Gel filtration and enzymic studies of insoluble residues from prolonged acid treatment of potato starch. *Cereal Chemistry, 51*, 389–462.

Schoorl, N. (1929). Sugar titration. *Chemisch Weekblad, 26*, 130–134.

Van Soest, J. J. G., Hulleman, S. H. D., De Wit, D., & Vliegenthart, J. F. G. (1996). Changes in the mechanical properties of thermoplastic potato starch in relation with changes in B-type crystallinity. *Carbohydrate Polymers, 39*, 225–232.

Van Soest, J. J. G., & Knooren, N. (1997). Influence of glycerol and water content on the structure and properties of extruded starch plastic sheets during aging. *Journal of Applied Polymer Science, 64*, 1411–1422.

Watanabe, T., & French, D. (1980). Structural features of Naegeli amylodextrin as indicated by enzymic degradation. *Carbohydrate Research, 84*, 115–123.

Whelan, W. J. (1964). Hydrolysis with β-amylase and preparation of the β-amylase limit dextrin of amylopectin. *Methods in Carbohydrate Chemistry, 4*, 261–266.

Zobel, H. F. (1964). X-ray analysis of starch granules. *Methods in Carbohydrate Chemistry, 4*, 109–113.