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Nanostructural Morphology of Plasticized Wheat Gluten and Modified Potato Starch Composites: Relationship to Mechanical and Barrier Properties

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S *Supporting Information*

ABSTRACT: In the present study, we were able to produce composites of wheat gluten (WG) protein and a novel genetically modified potato starch (MPS) with attractive mechanical and gas barrier properties using extrusion. Characterization of the MPS revealed an altered chain length distribution of the amylopectin fraction and slightly increased amylose content compared to wild type potato starch. WG and MPS of different ratios plasticized with either glycerol or glycerol and water were extruded at 110 and 130 °C. The nanomorphology of the composites showed the MPS having semicrystalline structure of a characteristic lamellar arrangement with an approximately 100 Å period observed by small-angle X-ray scattering and a B-type crystal structure observed by wide-angle X-ray scattering analysis. WG has a structure resembling the hexagonal macromolecular arrangement as reported previously in WG films. A larger amount of β-sheets was observed in the samples 70/30 and 30/70 WG-MPS processed at 130 °C with 45% glycerol. Highly polymerized WG protein was found in the samples processed at 130 °C versus 110 °C. Also, greater amounts of WG protein in the blend resulted in greater extensibility (110 °C) and a decrease in both E-modulus and maximum stress at 110 and 130 °C, respectively. Under ambient conditions the WG-MPS composite (70/30) with 45% glycerol showed excellent gas barrier properties to be further explored in multilayer film packaging applications.

Wheat gluten-Modified potato Starch composites
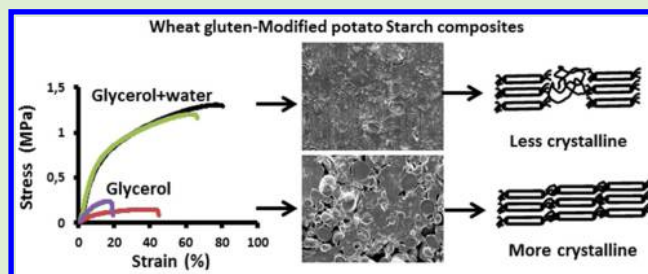
■ INTRODUCTION

During the last two decades, abundantly available and relatively inexpensive plant polymers such as wheat gluten (WG) protein and starch have become important alternatives in replacing petroleum based synthetic polymers for the production of bioplastics.[1] Wheat gluten, a byproduct of the wheat starch industry with attractive viscoelastic properties, has been successfully used for making gluten-hemp materials,[2−4] foams,[5] and nanoclay-composites.[6] Starches from various plants are widely used today as a raw material in the production of bioplastics, mainly for thermoplasticized packaging materials.[7] Several studies exist regarding the improved mechanical properties of thermoplastic material derived from chemically and genetically modified potato starch.[8,9] Both WG protein[2,10] and potato starch materials[8,11] show attractive mechanical and gas barrier properties. However, material properties are often negatively affected by processing parameters such as plasticization, and hence further development is needed to improve

these polymers use as packaging materials. A combination of WG and potato starch into a composite might be a promising and cost competitive alternative, which has not been previously explored.

Wheat gluten protein consists of polypeptides joined with disulfide and hydrogen bonds forming a three-dimensional polymer network,[12] and this network governs the functional properties of WG materials. In WG-based materials with glycerol (and urea with or without nanoclays), a hierarchical hexagonal structure of proteins was found to be interrelated with improved mechanical and barrier performance of the materials,[6,13] because protein structure and functional properties are closely related. This gives a background for further exploring WG proteins in various formulations, such as with

potato starch in order to explain the impact of these hexagonal structures on the functionality of those materials.

Potato starch is composed of amylose and amylopectin molecules having specific structures, mainly long linear chains for amylose while amylopectin is highly branched with shorter chains than amylose. Various ratios of amylose and amylopectin are known to be responsible for different physiochemical properties and end-use quality of starch-based materials.[14] In the present study, potato starch, modified to yield increased lengths of the glucan chains in amylopectin fraction and higher amylose content, was produced and combined with WG for making biocomposites using extrusion. It has been reported that long linear glucans, like amylose molecules, tend to form more entanglements compared to highly branched amylopectin molecules.[15] Therefore, the long-chained amylopectin starch used in the present study should act as a linear chain and have an influence on the chain entanglements development during extrusion. For biobased materials, from a technofunctional point of view it is of interest to explore a potato starch with amylopectin of longer chains length and decreased degree of branching as a good potential raw material for improved packaging materials.

From recent studies on protein-starch materials, egg albumen protein-corn/potato starch composites have shown higher strength (at low deformation) comparable to commodity plastics.[16] The functional properties of these albumen/starch-based materials were greatly influenced by the processing conditions used.[16] In addition, a study on the solution cast films, produced from separated wheat gluten and native wheat starch, investigated the water vapor sorption mechanisms and dynamics of both polymers.[17] To conclude, the need to control functional properties such as mechanical, gas/water barrier, water sorption, and so forth of protein and starch materials is currently under focus.

According to our knowledge, none of the previous studies have investigated the use of modified potato starch (MPS) in combination with WG proteins in order to produce a composite using extrusion processing. We aimed at studying the structural organization of WG proteins and MPS and the effects of WG protein polymerization, composition of the blend, and extrusion temperature on the mechanical and gas barrier properties of the composites.

■ EXPERIMENTAL SECTION

Materials. Wheat gluten was supplied by Lantmännen Reppe AB, Lidköping, Sweden. The gluten protein content was 77.7%, starch 5.8%, moisture 6.9%, and fat content 1.2%, according to the supplier. Starch from genetically modified potato Line 2012, further referred to as MPS, was developed at the Swedish University of Agricultural Sciences, Alnarp, Sweden as described in Methods. The MPS and its parental variety Dinamo were processed by Lyckeby Starch AB, Sweden. Glycerol (purity 99.5 wt %, 0.5 wt % water) was supplied by Karlshamns Tefac AB, Karlshamn, Sweden.

Methods. *Potato Starch Modification and Characterization.* Starch with an altered chain length distribution was developed by inhibition of the starch branching enzyme genes *SBE1* and *SBE2* in potato using RNA interference (RNAi). An RNAi fragment containing a small region of each *SBE* gene[18] was synthetically produced in tandem and cloned as an inverted repeat in the binary vector pGWIWgbss using the Gateway system (Invitrogen, Carlsbad, CA, U.S.A.). The binary vector pGWIWgbss is a modified version of the pK7GWIWG2(II) vector,[19] where the 35S promoter has been exchanged by a granular bound starch synthase promoter (GBSS) of *Solanum tuberosum* origin pK7GWIWG2(II).[19] Potato cultivar Dinamo was transformed using *Agrobacterium tumefaciens* strain AGLO

harboring the RNAi construct. Transformation, regeneration, and selection of lines were performed as described previously[20] with the modification that 50 mg/L kanamycin was used as the selection agent. Potato plants were field grown between May and October 2012, and the starch was processed in an industrial pilot plant (Lyckeby Starch AB, Kristianstad, Sweden). Characterization of the modified and native type starches included amylose content according to Chrastil,[21] and chain-length distribution using gel permeation chromatography after debranching according to Bertoft et al.[22] Pasting characteristics of the two starches were determined using a rapid viscoanalyzer (Newport Scientific Method 1 as defined by the manufacturer; Newport Scientific, Warriewood, NSW, Australia), and granule shape and size were investigated using a scanning electron microscopy (Hitachi TM-1000-micro, Hitachi, Tokyo, Japan).

Sample Preparation and Extrusion. Wheat gluten and MPS blends were hand mixed with ratios of 70/30, 50/50, and 30/70 (wt %), and glycerol content of either 30 or 45% (wt % of the total weight of the sample) were produced. For some samples, the addition of 20% water was tested. The sample composition and abbreviations are presented in Table 1 (Supporting Information). Efforts were made to obtain protein-based control samples from as received WG protein (45% glycerol). The WG-glycerol extrudates resulted in disrupted and nonhomogeneous samples and therefore were only used for studying WG protein molecular size distribution by size exclusion high-performance liquid chromatography (SE-HPLC). The WG-MPS composites were extruded at 110 and 130 °C using a Haake Minilab twin screw extruder (feeding rate was less than a kg/h) (Thermo Scientific Corporation, Germany) at 30 rpm in the form of strips 3.9–4.1 mm wide and 1.1–1.6 mm thick. A single temperature zone was used and the temperature was kept constant across the extruder barrel until the die exit. At least ten strips each with a minimum length of 70 mm were used for each specimen.

Protein Solubility Analysis by SE-HPLC. The protein amount and size distribution analysis by SE-HPLC has been performed according to a three step extraction procedure, for example, extraction with a buffer followed by a two-step extraction by both buffer and sonication, according a method adopted from Newson et al.[23] To measure both the soluble amount of protein and evaluate the size distribution of protein each of the extractions were evaluated by SE-HPLC, and protein solubility/extractability was calculated on the basis of total extractable protein.

The samples were prepared by cutting the material into pieces of approximately 0.2 mm in size using a scalpel. For protein extraction, 16.5 mg (±0.05 mg) of protein-starch composite was mixed with 1.4 mL buffer solution (0.5% SDS, 0.05 M NaH2PO4, pH 6.9) in a 1.5 mL Eppendorf tube. The samples with buffer solution were vortexed for 10 s in Whirli Vib 2 (Labassco, Sweden) and shaken for 5 min in IKA-VIBRAX VXR (IKA, Germany) at 2000 rpm, and later centrifuged (Legend Micro 17, Sorvall, Germany) for 30 min at 12 500 rpm. This supernatant was designated as the first extraction (1Ex). For the second extraction (2Ex), 1.4 mL buffer solution was added to the pellet from 1Ex, sonicated for 30 s at amplitude of 5 μm using a Sanyo Soniprep 150 Ultrasonic Disintegrator (Tamro, United Kingdom), and centrifuged for 30 min as 1Ex. For the third extraction (3Ex), 1.4 mL buffer solution was added to the pellet from the 2Ex and sonicated for 30 + 60 + 60 s followed by centrifugation. The samples were left to cool at room temperature during each sonication interval in the 3Ex step to avoid overheating. After sonication, the amount and size distribution of proteins in centrifuged samples from 1Ex, 2Ex, and 3Ex were analyzed with a Waters 2690 Separation Module and Waters 996 Photodiode Array Detector (Waters, U.S.A.) according to Newson et al.[23] For analysis, 20 μL of each sample was injected onto an SE-HPLC column (Biosep-SEC-S 4000, Phenomenex, U.S.A.) at an isocratic flow of 0.2 mL/min (50% acetonitrile, 0.1% TFA; 50% H$_2$O, 0.1% TFA). Empower Pro software (Waters, U.S.A.) was used for analysis of data (at 210 nm) and chromatograms representing the amount and size distribution of proteins were integrated and divided into two groups of proteins, polymeric (retention time 7–14 min) and monomeric (14–28 min). The obtained data were corrected for the initial protein content for each individual sample.
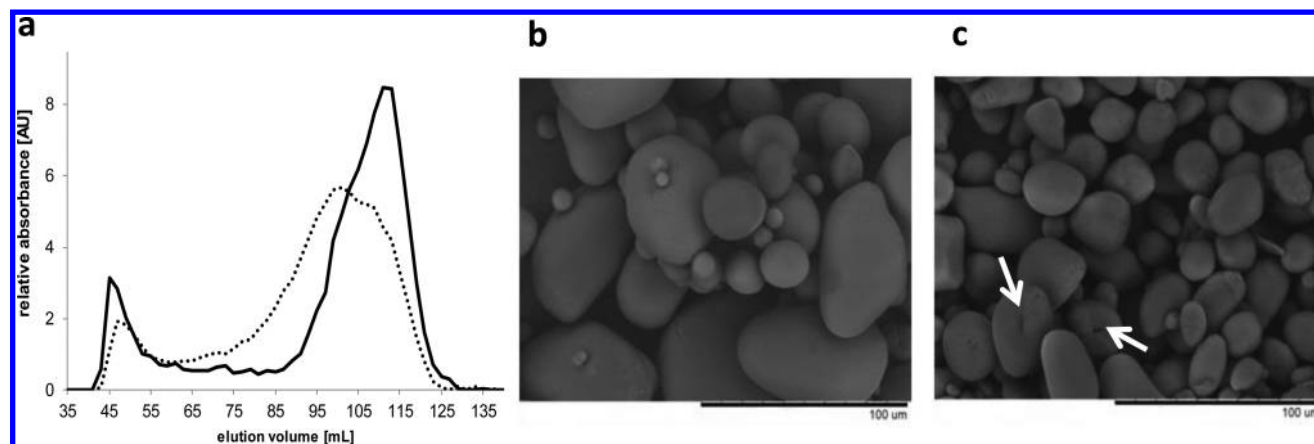
Figure 1. Gel permeation chromatogram after debranching of (a) Dinamo starch (solid line) and MPS (pointed line), and SEM micrographs of Dinamo (b) and MPS (c) starches, respectively.

Tensile Testing. The strips of the extruded samples were cut into a minimum length of 70 mm for tensile testing. The samples were conditioned for 48 h at 50% (±2) relative humidity (RH) and 23 °C before testing and thereafter the test was performed in the same conditions. Width and thickness of the samples were measured using Mitutoyo IDC 112B at five different points. A 100 N load cell, clamp separation distance of 40 mm and crosshead speed of 10 mm/min was used for all samples on an Instron 5566 universal test machine using Bluehill software (Instron AB, Danderyd, Sweden).

Fourier Transform Infrared Spectroscopy. FT-IR spectroscopy analysis was carried out on all the extruded samples and WG powder using a Spectrum 2000 FTIR spectrometer (PerkinElmer inc., U.S.A.) equipped with single reflection ATR (Golden Gate, Speac Ltd.). Prior to analysis, the samples were dried for at least 72 h in a desiccator using silica gel.

Scanning Electron Microscopy. The scanning electron microscope (SEM) (LEO 435VP, Cambridge, U.K.), acceleration voltage of 10 kV and secondary electron detection was used for the evaluation of the WG-MPS/MPS materials. The WG-MPS-45gly (70/30), WG-MPS-30gly-20W (70/30), and MPS-45gly samples were analyzed for the cross-sectional microstructure. The cross-section of the sample was prepared by cutting using a scalpel. Samples were coated with Au/Pd 3:2 coating (JFC-1100, JEOL, Tokyo, Japan).

Small-Angle X-ray Scattering. All the extruded samples were analyzed by small-angle X-ray scattering (SAXS). The SAXS experiments were carried out at the MAX-IV Synchrotron Laboratory, Lund, Sweden, using the beamline I911-4 with a wavelength of $\lambda = 0.91$ Å.[24] The scattering vector range $\mathbf{q}$ ($\mathbf{q} = 4\pi/\lambda \sin(\theta)$, where 2θ is the scattering angle) was 0.0082–0.47 Å^{-1}. A bidimensional hybrid pixel X-ray detector (Pilatus 1M, Dectris) was used to obtain two-dimensional images with sample to detector distance of 1901.71 mm and an exposure time of either 2 or 5 min. The SAXS data were analyzed using the software bli9114.[24] The obtained data were normalized with respect to integrated intensity incident on the sample during the exposure time and corrected for sample absorption and background.

To get additional details on the structure of WG-MPS composites, SAXS measurements at different temperatures were performed for the WG-MPS-45gly 70/30 (110 °C). The temperatures used were 25, 35, 45, 55, 60, 75, 80, and 90 °C with an exposure time of 60 s. The temperature at each step was kept constant for 5 min before a SAXS image was taken.

Wide-Angle X-ray Scattering. Wide angle X-ray scattering analysis (WAXS) was carried out at the MAX-IV Synchrotron Laboratory, Lund, Sweden, using beamline I911-2[25] with a wavelength of $\lambda = 1.04$ Å and a sample to detector distance of 149.8 mm. An area-CCD detector was used for the measurements. Silicon powder was used as a standard for calibration. The data was analyzed using Fit2D software.[26]

Oxygen Permeability. The samples for oxygen permeability were made by cutting the strips of extruded samples into small pallets (2−4 mm) using scalpel. The pallets were placed in a metal frame of a 1 mm thickness and 70 × 70 mm opening to control the size and thickness of the film. The pallets were then pressed in a hot compression molding machine (Polystat 400s, Servitech, Germany) between two aluminum plates for 10 min at a constant pressure (200 bar) and temperature (130 °C). The samples were conditioned at 23 °C and 50% RH for 16 h prior to the test. Oxygen transmission rate analysis was carried using Ox-Tran 2/21 (Mocon Inc., MN, U.S.A.), according to ASTM F1927-07. The test was carried out on a measured area of 5 cm^2 and gas flow of 10 mL/min. The oxygen transmission data were normalized to the oxygen pressure and the thickness of the samples to get the oxygen permeability values. Two replicates for each sample were tested.

■ RESULTS AND DISCUSSION

Molecular Characterization of Potato Starch. The potato variety Dinamo was genetically modified in order to produce a starch with decreased degree of branching and increased length of glucan chains, by targeting two starch branching enzyme genes, *SBE1* and *SBE2*. The MPS showed a significant increase in total amylose content from 18 to 27% compared to the parental variety (Table 2, Supporting Information). Gel permeation chromatography after starch debranching showed that the chain-length distribution of MPS was altered compared to the parental variety. Long chain amylose molecules eluted first (between 40 and 50 mL) and shorter chains from debranched amylopectin eluted later in the chromatogram (between 75 and 100 mL) (Figure 1a). The MPS showed a shift in the chromatographic profile seen as an earlier second eluting peak (pointed line) indicating a higher amount of long chains from debranched amylopectin compared to the parental variety Dinamo (Figure 1a). The pasting properties of the MPS differed significantly from Dinamo, indicating higher gelatinization temperature, final viscosity, and set back but a lower peak viscosity and breakdown for the MPS (Table 2, Supporting Information). A higher amylose content and longer chain-length of amylopectin are known to affect pasting properties.[27] The results in this study confirmed the altered molecular structure seen by gel permeation chromatography. Scanning electron microscopy of MPS showed different starch granule morphology compared to Dinamo where the MPS granules were smaller in size, had irregular shapes, and a slightly fissured phenotypic appearance (Figure 1b,c; indicated by arrows). The uneven granule shape has previously been
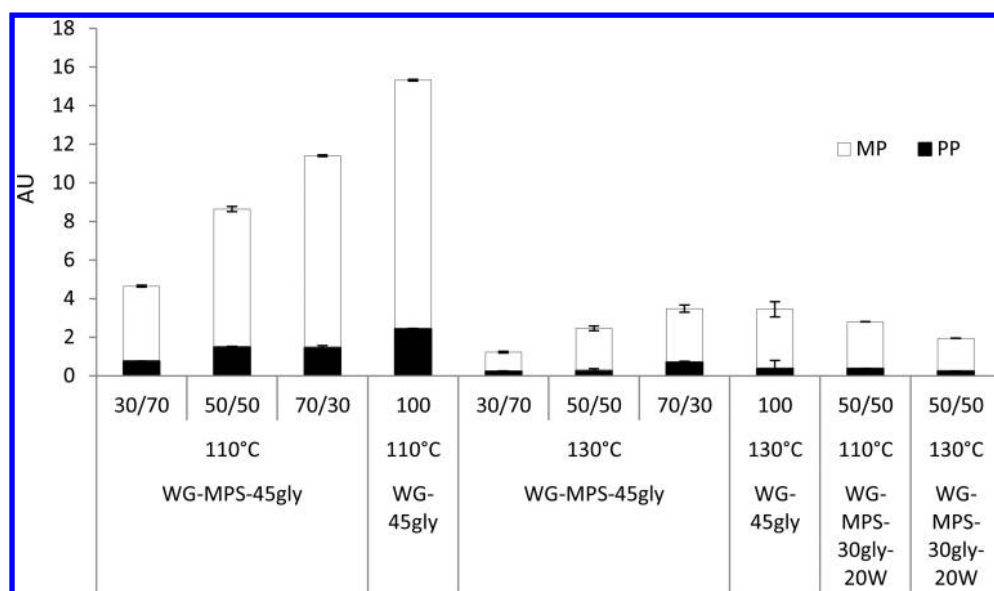
Figure 2. Solubility of polymeric (PP) and monomeric proteins (MP) of WG-MPS-45gly and WG-MPS-30gly-20W composites extruded at 110 and 130 °C by SE-HPLC; WG-45gly samples extruded at 110 and 130 °C were used as controls. Arbitrary units (AU) correspond to normalized protein solubility.
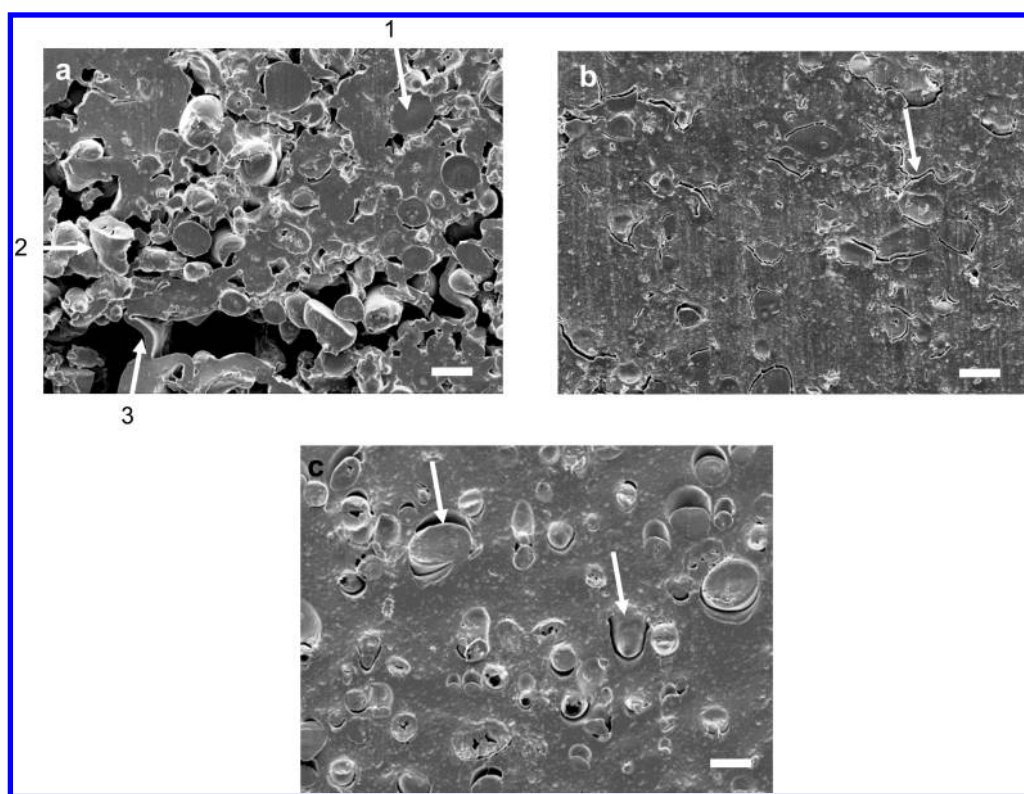

Figure 3. SEM micrographs of WG-MPS and MPS samples (a) vertical cross-section in the extrusion direction of WG-MPS-45gly 70/30 extruded at 130 °C; (b) WG-MPS-30gly-20W 70/30 extruded at 110 °C, and (c) MPS-45gly extruded at 130 °C. Bars correspond to 10 μm.

shown to be related to the change in chain-length of amylopectin and amylose.[28,29]

Temperature Effect on Protein Polymerization in Protein–Starch Composites. The WG-MPS composites containing two plasticizers combinations one with 45% glycerol and other 30% glycerol with 20% water were extruded at 110 and 130 °C and were analyzed for the soluble amount and size distribution of protein by SE-HPLC (Figure 2). A significant decrease in protein solubility was observed in all the WG-MPS-

45gly and WG-MPS-30gly-20W composites compared to the WG-45gly samples produced at the studied temperatures (Figure 2). Significantly smaller amounts of both polymeric and monomeric proteins were extracted in the WG-MPS-45gly composites extruded at 130 °C compared to those extruded at 110 °C. For the WG-MPS-45gly samples 30/70 and 50/50 extruded at 130 °C, the lowest amounts of proteins were extracted, that is, with nearly no polymeric proteins and only small amounts of monomeric proteins being solubilized (Figure
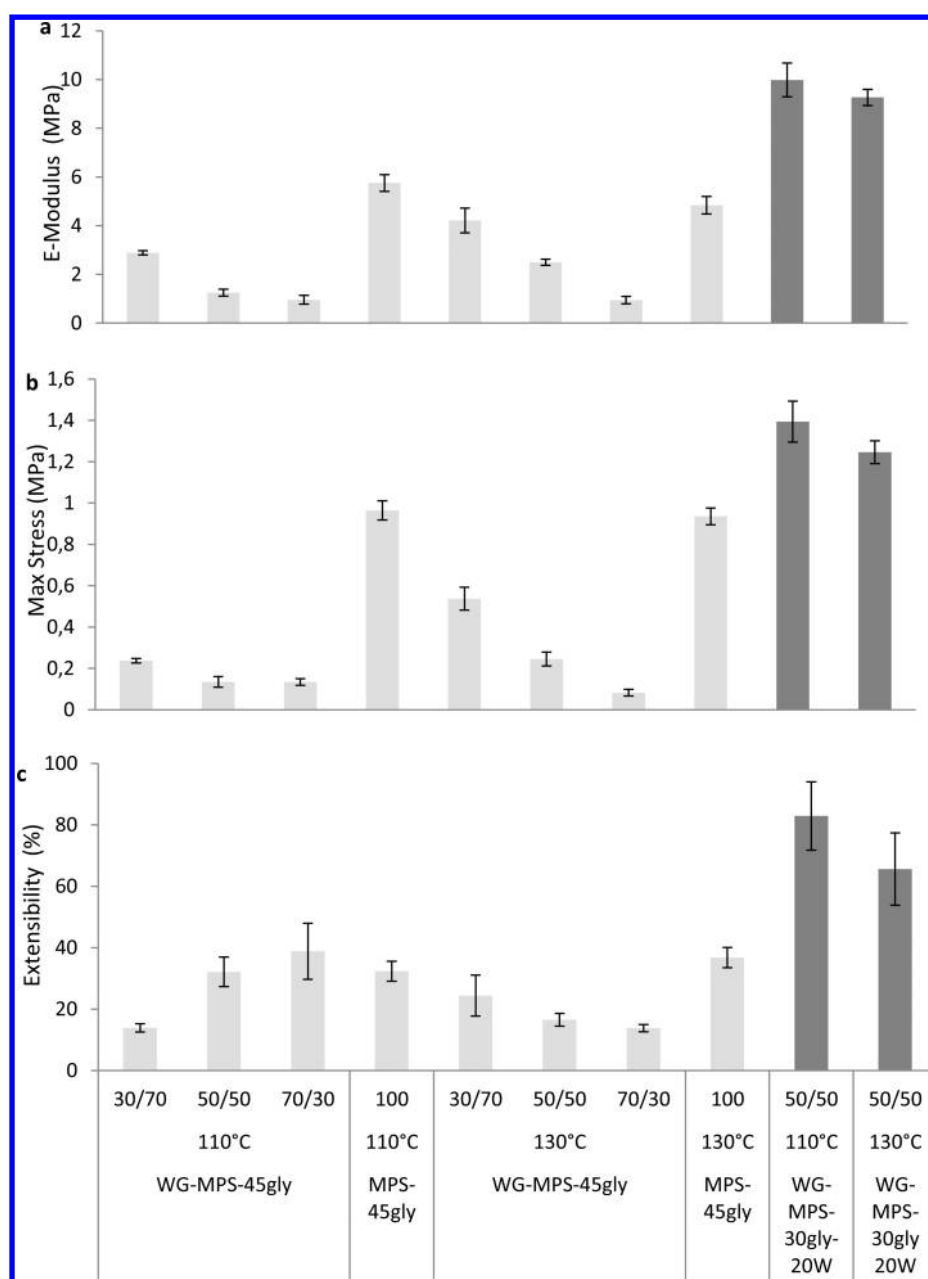
Figure 4. Mechanical properties of WG-MPS-45gly and WG-MPS-30gly-20W composites extruded at 110 and 130 °C: (a) E-modulus, (b) maximum stress, and (c) extensibility.

2). Consequently, this suggests a combination of plasticizers, for example, glycerol and water, was much more favorable for increased polymerization of the proteins. This was observed for the composites extruded at 110 °C than those produced at 130 °C, where nearly the same amounts of proteins were extracted (WG-MPS-45gly vs WG-MPS-30gly-20W both at 130 °C). For WG-MPS composites containing glycerol and water besides a greater total amount of plasticizer in the blend (50% vs 45%), which played an important role, an interactive plasticizing effect of water and glycerol on the molecular chain mobility of WG proteins was also apparent. This effect on WG proteins seemed to favor the final protein−starch composite system, where the amount of starch did not seem to affect the polymerization/cross-linking behavior of the protein (Figure 2).

A low extractability of the polymeric WG proteins observed in WG-MPS-45gly composites produced at 130 °C indicates

that protein polymerization was more favorable at the higher extrusion temperature (compared to 110 °C). Furthermore, a low degree of extractability of monomeric proteins in WG-MPS-45gly and WG-MPS-30gly-20W composites at 130 °C (vs 110 °C) indicates the disruption of intramolecular bonds and their reorganization into more stable intermolecular disulfide linkages.[30] These linkages seemed to be strong enough also to "capture" starch in the protein−starch composites. Furthermore, extrusion provided shear mixing and mechanical energy into the protein−starch blend, which also induced protein aggregation in the composite samples, especially those extruded at 130 °C, as observed in previous studies of WG and glycerol blends.[10,31,32]

From the SE-HPLC analysis of the WG-45gly sample extruded at 130 °C (Figure 2), a blend of both aggregated polymeric proteins and monomeric proteins similar to the case

of the WG-MPS-45gly 70/30 sample extruded at 130 °C was observed. This agrees with similar protein extrusion studies[32] indicating that mixing during extrusion may result in severe aggregation inside the extruder barrel. In our study, this aggregation decreased the protein solubility especially at higher temperatures (130 °C) during the actual extrusion step.

Microstructure of Protein–Starch Composites by Scanning Electron Microscopy (SEM). The composites with higher protein versus starch ratio were selected for microstructural study in order to evaluate morphology of the protein matrix and MPS when glycerol and glycerol–water were used. The selected composites were studied by SEM at a 70/30 protein/starch ratio, that is, the WG-MPS-45gly (130 °C), WG-MPS-30gly-20W (110 °C), and MPS-45gly (130 °C) (Figure 3). A large variation in the microstructural pattern was observed between the samples studied (Figure 3). The WG-MPS-45gly (70/30) (130 °C) showed an in-homogeneous structure with a relatively high amount of intact starch granules, which were either incorporated in the protein–starch blend (arrow 1) or were separated from the protein matrix (Figure 3a, arrow 2) together with some voids present (Figure 3a, arrow 3). The WG-MPS-30gly-20W (70/30) sample extruded at 110 °C showed a microstructure, which was more uniform, that is, with a protein–starch matrix being rather homogeneous with some intact starch granules present (no voids, with a few small cracks in the matrix) (Figure 3b). This result indicates that the added water plasticized (and also hydrated WG protein) while improving starch incorporation into the blend due to plasticization effect of water on proteins and favorable environment for the homogenization of the sample.

The microstructure of the MPS-45gly sample revealed a structural pattern consisting of the highest amount of intact starch granules compared to the protein–starch counterparts in this study (Figure 3c). It is important to note that some of the starch granules were poorly imbedded in the starch matrix (Figure 3c, arrows). In summary, the WG-MPS-30gly-20W showed the favorable processing conditions for extruding such protein-modified potato starch composites. Therefore, more focus should be directed to incorporating all the intact starch granules into a more uniform blend of the components. For the extruded starch samples, a larger processing window with extrusion temperatures higher than 130 °C extrusion temperatures and the use of water as plasticizer could be explored. Our study on MPS samples produced by extrusion is in agreement with similar studies on potato starch films produced by compression molding, film blowing, and casting,[8] where a complete gelatinization of the initial semicrystalline structure of the starch was observed to be difficult. In order to disrupt all the intact starch granules, various forms of mixing (i.e., premixing) as well as much higher temperatures, for example, 140 °C and higher, are necessary in order to improve blend component homogenization. In fact, a premixing step of the components before extrusion in this study was not enough to achieve a homogeneous sample as also found by Thunwall et al.[9]

Mechanical Properties of Protein–Starch Composites: The Effect of Extrusion Temperature and Composition of the Blend. Mechanical properties of the WG-MPS-45gly, WG-MPS-30gly-20W composites, and MPS-45gly extrudates are shown in Figure 4. An increase in extrusion temperature (from 110 to 130 °C) resulted in a significant increase in the E-modulus and maximum stress in WG-MPS-45gly composites. A significant increase in extensibility of WG-MPS-45gly compo-

sites correlates with an increase in the protein content for 30/70 and 50/50 samples at 110 °C, no such correlation was observed at 130 °C (Figure 4). An increase in the protein content (from 30 to 70%) in the composites resulted in a significant decrease in E-modulus and maximum stress at 130 °C (Figure 4). The MPS-45gly samples extruded at both temperatures studied showed the highest E-modulus and maximum stress with similar extensibility compared to the WG-MPS-45gly samples (Figure 4).

In this study, the main purpose was to produce WG-MPS samples using glycerol as the plasticizer; however water was added to several samples in order to improve the processability of the blend during extrusion. The WG-MPS-30gly-20W samples showed the highest tensile properties in terms of modulus, maximum stress, and extensibility when compared to all the WG-MPS-45gly samples produced without water (Figure 4). Hence water was acting as a plasticizer here (modulus increased in its presence) but primarily led to increased degree of gelatinization of starch granules. In biopolymer systems, for example, proteins, water acts as a plasticizer and contributes in developing the hydrogen bond interactions. These interactions has been reported to improve the strength and elasticity of WG-based films.[33] In this study, we used potato starch with a slightly increased amylose content (27%) also containing modified amylopectin structure, which, as expected, showed that a careful selection of plasticizer (in this case combination of glycerol and water) together with optimal extrusion temperature (such as 130 °C or even higher) is needed in order to obtain a homogeneous composite. High amylose potato starch (amylose content 86%) is known to have a higher viscosity compared to normal potato starch.[9] The potato starch used in this study contained only slightly more amylose than ordinary potato starch but showed 1.7 fold higher viscosity. In addition, the added water seemed to improve gelatinization of the starch. The WG-MPS (30/70), extruded at 130 °C in the present study, showed a higher maximum stress and lower extensibility in comparison with compression molded WG/potato starch based composites (with 37.5% plasticizer content).[16] The WG-MPS showed similar extensibility and slightly higher E-modulus than observed in soy protein/acetylated high amylose corn starch composites with plasticizer content of 30%.[34] Slightly lower modulus and maximum stress values in the WG-MPS originate from the fact that a significant portion of starch was still present as granules/intact material. This in turn suggests that an improved adhesion between the WG protein and MPS, as well as increased starch gelatinization, could further improve the tensile properties. In a similar study by Verbeek et al.,[1] the excessive aggregation of WG proteins was found to cause the formation of dense associations in the extruder barrel, resulting in an increase in the viscosity of the blend.[1] In our study, this increase in viscosity, was most likely related to an increase in retention times during extrusion in some cases up to 30 min that resulted in degradation or excessive aggregation of the proteins (WG-gly45 control samples). However, in this study we were able to show that extensible composites with relatively high maximum stress and extensibility (ca. 90%) could be obtained with WG protein–potato starch blends containing glycerol and water as plasticizers. The overall behavior indicates that the composites containing glycerol and water have a potential processing window for further improvement of their mechanical properties.

Protein Secondary Structure. FT-IR spectroscopy results in the amide I region (1600−1700 cm^{-1}) of WG-MPS-45gly and WG-MPS-30gly-20W samples, extruded at 110 and 130 °C, are shown in Figure 5. It is possible to assess the relative
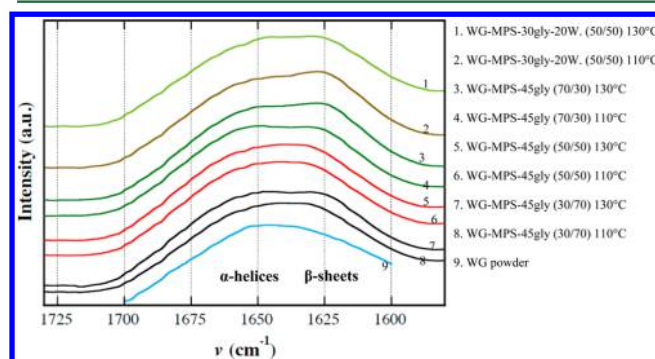

Figure 5. FT-IR spectra of all WG-MPS-45gly and WG-MPS-30gly-20W composites extruded at 110 and 130 °C. Spectra range 1645−1660 cm^{-1} corresponds to α-helices and 1620−1635 cm^{-1} to β-sheets.

amount of α-helices/random coils and β-sheets by comparing the relative size of the IR spectrum in the 1645−1660 cm^{-1} region to that of the 1620−1635 cm^{-1} region.[13] To begin with, the relative sizes are not large; the curves are relatively flat in the 1620−1660 cm^{-1} region. All extruded samples were, however, as expected, more aggregated than the pristine WG powder, the latter having the highest intensities in the 1645−1660 cm^{-1} region (Figure 5). This indicated that intensive shear mixing at elevated temperature (extrusion) contributed in transforming the WG protein from a pristine powder with a less organized structure to a more organized aggregated structure.

A slightly more aggregated structure was observed in the WG-MPS-45gly (30/70, 70/30) samples extruded at the higher temperature, whereas no differences could be observed for those with a 50/50 WG/MPS ratio.

The WG-MPS-30gly-20W (50/50) composites contained a higher amount of β-sheets when extruded at 110 °C compared to at 130 °C (Figure 5). This suggests that the lower extrusion temperature (110 °C) in the presence of water favors sulfhydryl-disulfide interchange reactions[1,31] in the WG protein (more monomeric proteins, Figure 2) and formation of sizable amounts of hydrogen bonding (β-sheets).

Wide-Angle X-ray Scattering. The WAXS data of WG-MPS and MPS plasticized extrudates, and MPS powder is shown in Figure 6. For all the samples studied, a large number of peaks indicating the crystalline nature of MPS were observed (Figure 6a; peaks indicated by lines). From diffractograms of the WG-MPS extrudates compared to the MPS powder and crystalline starch structures previously reported,[35] it can be concluded that the B-type crystalline starch structure is present in the WG-MPS samples at both 110 and 130 °C. The main scattering reflections found in the MPS powder are indicated with their correspondent Miller index (Figure 6a).[35] At high protein concentrations in the blend (as 70/30), the starch peaks were smaller (at both 110 and 130 °C) (Figure 6a,b), whereas the impact of the amorphous morphology of WG[36] on the WAXS pattern was obvious. In addition, a decrease in starch crystallinity was observed for the WG-MPS blends at 130 °C compared to 110 °C, for example, starch peaks were less intense and broader (Figure 6a,b).

The addition of water in the WG-MPS-30gly-20W extrudates seemed to reduce the crystallinity of starch in the blend at both
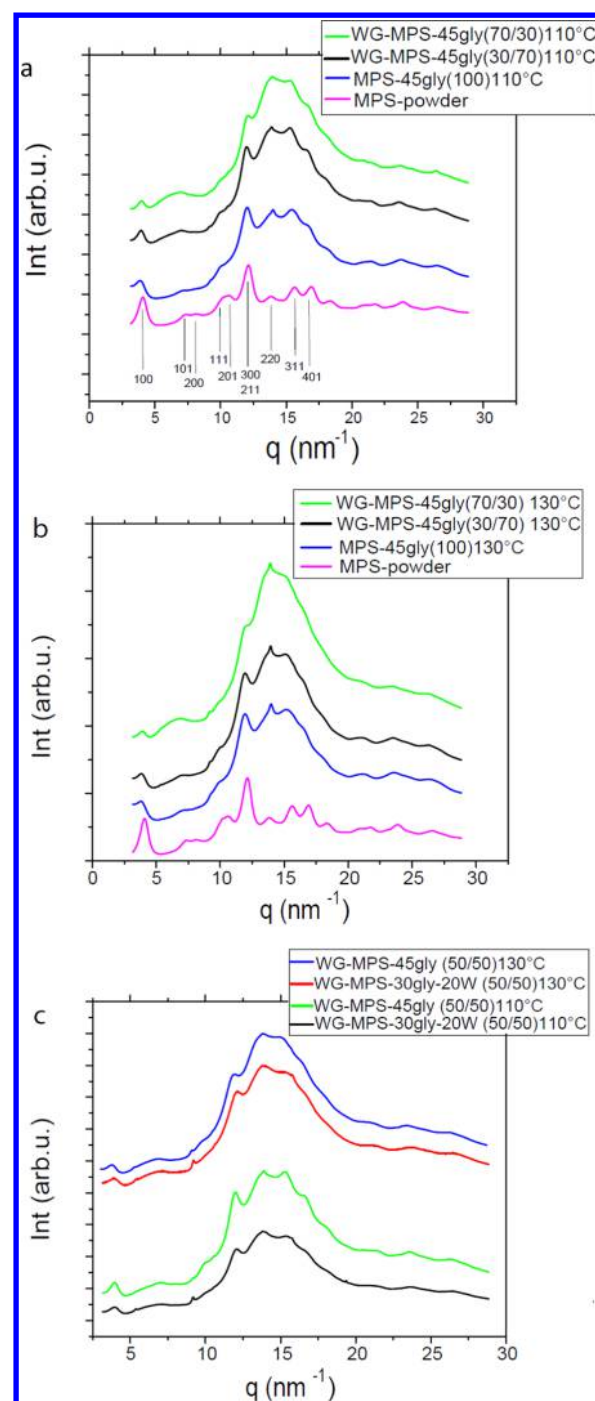

Figure 6. WAXS profiles of WG-MPS-45gly and MPS-45gly samples extruded at (a) 110 °C, (b) 130 °C and WG-MPS-30gly-20W samples extruded at (c) 110 and 130 °C, respectively. Curves are shifted vertically for clarity.

110 and 130 °C temperatures (Figure 6c). This was particularly clear for the samples extruded at 110 °C with the diffraction pattern of small and poorly defined crystalline peaks compared with the pattern of water-free samples (Figure 6c). In this study, the greater transformation of the starch granules morphology occurred when the glycerol−water versus glycerol was used and at the higher extrusion temperature of 130 °C. The addition of water contributed to physicochemical changes in MPS with the partial gelatinization of starch granules, resulting in a loss of crystallinity.[37]
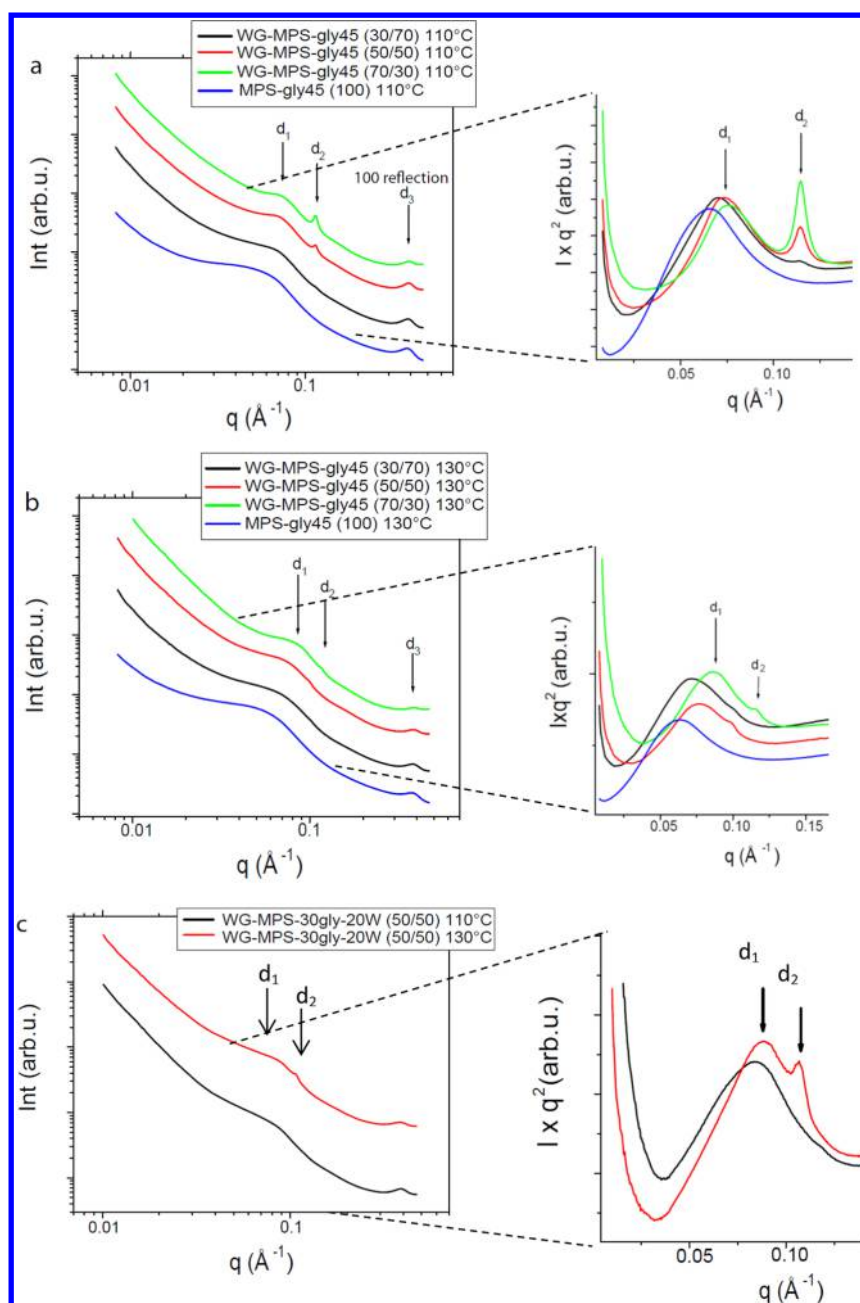
Figure 7. SAXS curves of WG-MPS-45gly, MPS-45gly samples extruded at (a) 110 °C and (b) 130 °C, and WG-MPS-30gly-20W extruded at (c) 110 and 130 °C. Curves are shifted vertically for clarity. $I \times q^2$ versus q plots in a reduced q-range are added to enhance the behavior of the d_1 and d_2 peaks.

The WAXS results showing a decrease in the crystallinity of MPS, especially due to the water used in the blends, explain the improvement in E-modulus, maximum stress, and extensibility of materials. The addition of water causes the swelling of the amorphous regions of the starch and the melting of amylopectin crystals.[38] Even in the presence of WG proteins, gelatinization of starch facilitated an improvement and greater homogenization of the blend during extrusion, which ultimately affected the final material properties.

Small-Angle X-ray Scattering. The SAXS curves of the WG-MPS blends and MPS-45gly samples extruded at 110 and 130 °C are shown in Figure 7. The MPS-45gly samples showed two peaks, a broad peak d_1 at low q angle and a more defined peak d_3 at high q value (Figure 7a, b; d_3 indicated by arrow).

The broad peak d_1 indicated a characteristic lamellar structure of starch granules with an average period estimated from the position of the peak maxima, for example, d_1 = 94.7 and 98.5 Å at 110 and 130 °C, respectively (Table 3, Supporting Information). According to the WAXS results, the d_3 peak, is the 100 reflection of the B-type crystalline structure of starch indicated the scattering distance between the two double helixes of amylopectin in a hexagonal unit cell,[35] estimated at 16 Å in this study (Table 3, Supporting Information).

Both the composition of the blend and the processing temperature contributed to changes in the morphology of the WG-MPS composites. The WG-MPS-45gly samples (30/70, 50/50, and 70/30) extruded at 110 °C showed a broad peak, d_1 and two well-defined peaks, d_2 and d_3, with distances of 89.4−
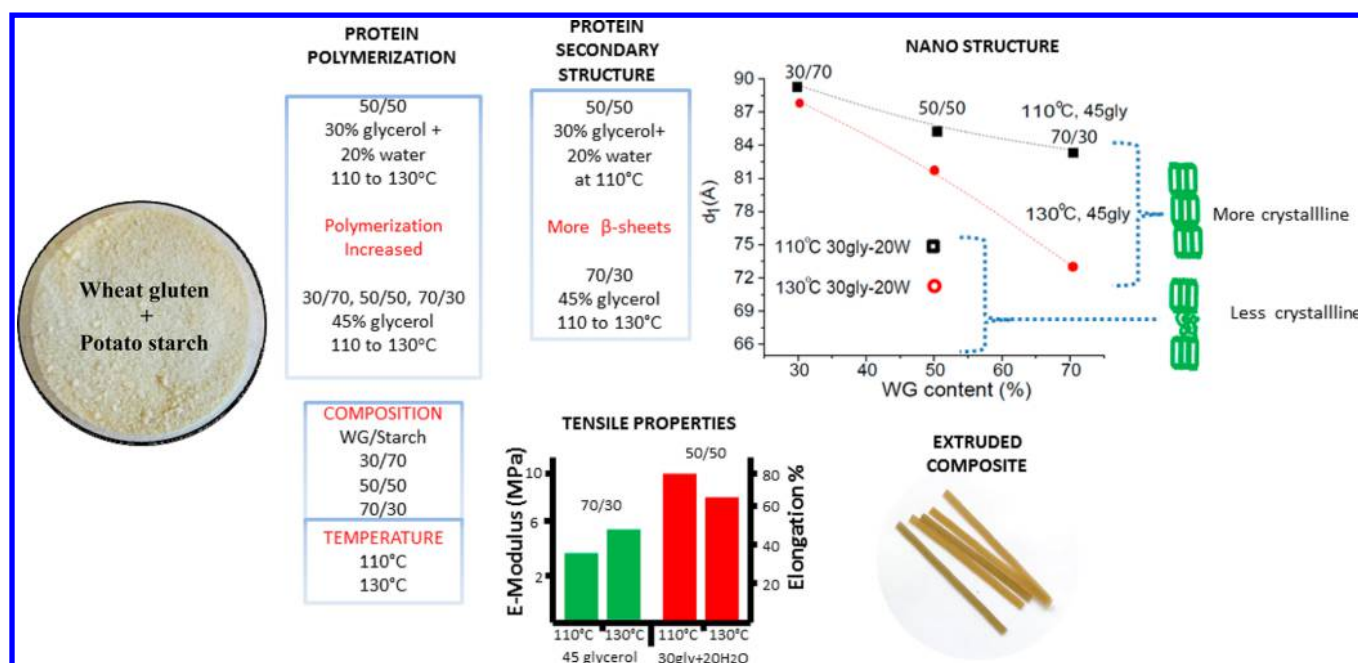
Figure 8. Schematic diagram of summarized effects of temperature, composition of the blend on the WG protein polymerization and secondary structure, as well as nanomorphology and mechanical properties of extruded WG-MPS composites.

83.6 Å for d_1, 55 Å for d_2 and 16.2−15.9 Å for d_3 (Figure 7a,b; Table 3 (Supporting Information)). The d_1 peak exhibited the superimposition of two contributions to the scattering, the lamellar structure of starch and the broad correlation distance between aggregated WG proteins previously observed in WG-glycerol films.[13,36] The scattering distance d_2 could potentially be interpreted as the first Bragg reflection of the hexagonal structure observed previously in WG-based materials.[36,39] In this study, we also performed complementary SAXS temperature experiments to examine the thermodynamic changes in the morphology (data not shown here). The Bragg peak (d_2 peak in this study) disappeared at 55−60 °C, which resembled the first peak from the hexagonally arranged structure from WG films[39] (Supporting Information, Figure S1), although in this study the other peaks associated with the hexagonal structure were not present. The d_2 values remained constant for all the composition of the blend extruded at 110 °C, while the peak intensity increased along with an increase in WG content of the blend (Figure 7a; enlarged area in the $I \times q^2$ versus q plot). The scattering distance d_3, indicated the presence of crystalline starch, and this distance was not affected by the change in composition of the blend.

The WG-MPS-45gly samples extruded at 130 °C showed smaller distances for the d_1 peak compared to the samples extruded at 110 °C (Figure 7b; Table 3, Supporting Information). The d_1 distance of 73.2 Å for the 70/30 (WG-MPS) composite was the smallest and might be attributed to the higher protein content in the sample, resulting in higher cross-linking of the proteins. Similar to the d_1 distance for extruded WG-30% glycerol films at 130 °C (74.8 Å) as shown in previous studies.[6]

The WG-MPS-30gly-20W (50/50) extruded at 110 and 130 °C showed smaller distances for d_1 (74.6 and 71 Å) and d_2 (53 and 58.6 Å), respectively compared to the WG-MPS-45gly 50/50 samples (Figure 7c; Table 3, Supporting Information). The addition of water resulted in significant decrease in d_1 distances probably due to strong plasticization of WG proteins or partial gelatinization of the starch.

Oxygen Permeability. The oxygen permeability (OP) of the selected protein-starch and MPS samples were measured at 23 and 38 °C (50 and 90% RH, respectively) are shown in Table 4 (Supporting Information). The lowest OP values were observed for the WG-MPS-45gly 30/70 composite, measured at 23 °C and 50% RH (Table 4). For the higher measurement temperature (38 °C) only the extrudate with the highest protein content was possible to measure (268 mm mL/m² 24h atm), which showed a 36 fold increase in comparison to 23 °C and 50% RH (Table 4, Supporting Information).

The OP of the WG-MPS-45gly 70/30 composite showed promising use of this material for example, as multilayer packaging films, compared to synthetic plastics such as polyethylene terephthalate (PET)[40] (Table 4, Supporting Information), although the mechanical properties would be compromised. In addition, the WG-MPS composite gas permeability can be compared with other WG based composites, that is, WG/nanoclay.[6] The WG-MPS composite (70/30) showed better barrier properties when a greater amount of protein and 45% glycerol were used in the blend.

■ CONCLUSIONS

Modified potato starch (MPS) consisted of longer glucan chains in the debranched amylopectin fraction and had a slight increase in amylose ratio with respect to native starch (27 versus 20%). This resulted in a higher gelatinization temperature and viscosity of the MPS compared to native starch. The WG proteins in the WG-MPS extrudates were more polymerized at 130 °C than at 110 °C. The secondary structure of WG proteins indicated a larger amount of β-sheets for the WG-MPS-45gly 70/30 (130 °C) and WG-MPS-30gly-20W 50/50 (110 °C).

The microstructural properties of the composites were greatly affected by the composition of the blend, extrusion temperature, and plasticizer used. The most favorable

processing conditions for extruding of WG-MPS composites have been found when 30% glycerol/20% water was used. This resulted into the most homogeneous microstructural pattern (with few intact starch granules) between the samples studied by SEM. The nanostructural morphology of all the composites studied indicated the MPS showing the B-type crystalline structure by WAXS and a characteristic lamellar arrangement of around 100 Å by SAXS. The WG proteins showed similar structures for the 50/50 and 70/30 WG-MPS blends at both processing temperatures by SAXS, indicating a morphology resembling to hexagonal structure previously found in WG proteins. A larger amount of WG protein in the blend resulted in an increase in extensibility at 110 °C and a decrease in both the E-modulus and maximum stress at 110 and 130 °C, respectively (see Figure 8).

The blend with 30% glycerol and 20% water had the most profound plasticization effect on the mechanical properties of the 50/50 WG-MPS materials, that is, the highest E-modulus, maximum stress, and extensibility between the composites studied. The oxygen barrier properties under ambient conditions were improved by the highest amount of protein in the sample (i.e., 70/30), which showed excellent barrier values. Because oxygen permeability is comparable with PET synthetic plastics, this material could be of further interest in exploring multilayer film packaging applications.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

All tables are included in the Supporting Information. The supplementary data for SAXS provides information about the d_2 peak observed in WG-MPS extruded samples. The d_2 peak disappeared between 50 and 60 °C, which resembled the hexagonally arranged wheat gluten protein structure as was found in previous studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENTS

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