Note

Interaction of Ternary Biopolymers Obtained from Microwave Dry-heated Mixtures of Gluten, Whey Protein Concentrate and Kaolinite

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Received December 5, 2016; Accepted February 21, 2017

Gluten, whey protein concentrate and kaolinite mixtures were irradiated at microwave oven for different period of time. Based on these powders, ternary gluten, whey protein, kaolinite biopolymer composites were obtained by dispersing in water and heating for 30 min at 80°C. Produced biopolymer composites were dried in a thermostatic cabinet. The optimal irradiation time was determined by measuring small strain rheological properties using an oscillatory rheometer. The mixture of gluten and whey protein concentrate produced several times stronger gels than the singular proteins. It suggests that microwave heating propagated interactions between gluten and whey proteins. The biggest hardness was noted for composites obtained from powders irradiated for 30 s. SEM and elements mapping showed good dispersion of kaolinite particles in the gluten/whey protein matrix. Dry-heating of the mixed gluten, whey protein and kaolinite at 30 s caused interactions (probably mostly disulphide bridges) between unfolded protein molecules. Longer irradiation time than 30 s probably led to highly crosslinked network which negatively affected the properties of bioplastics.

Keywords: biopolymer, gluten, kaolinite, microwave heated, whey protein

Introduction

Kaolinite is a layered aluminosilicate with chemical formula Al2Si2O5(OH)4. Each layer consists of one tetrahedral siloxane surface and an octahedral gibbsite (Al(OH)3) sheet. The layers are bound together by hydrogen bonding between hydroxyl groups on the gibbsite basal plane and silicon tetrahedral sheet. Kaolinite has a heterogeneous surface charge with different surface structures between base and edge planes. Charge on the edges of kaolinite depends on the pH of the solution due to the protonation/deprotonation of hydroxyl groups and basal surfaces have a constant structural charge due to the isomorphous substitution of Si4+ by Al3+ (Barral et al., 2008).
Wheat gluten is a relatively low-cost by-product of wheat starch industry. It can be a material for different, sometimes nonfood applications. It contains single-chain polypeptides - gliadins and multiple-chain polymeric proteins interlinked by intermolecular disulfide and hydrogen bonds - glutenins. Wheat gluten can be biodegraded within 36 days in aerobic fermentation and within 50 days in soil without toxic products release (Patni et al., 2014).

Whey protein concentrates are the main and the most valuable products obtained from cheese production waste – whey. They have excellent nutritional value and the cost of production is not so high.

Proteins lose their secondary, tertiary and quaternary structure by denaturation. It can be caused by a thermal process or chemical agents. In food technology the most popular method is heating, because no supplementary, possibly sensory properties decreasing ingredient is added. There is a difference between wet and dry heating methods because of the differences in possible interactions in aqueous environment and changes of the viscoelastic properties of dry and wet mixtures. Due to low water content of powders, proteins are more stable with regard to heat unfolding. In comparison to the denaturation temperature of whey proteins close to 70°C, the denaturation of proteins in dry isolate is about 160°C (Gulzar et al., 2011). Above the denaturation temperature, proteins are unfolded, and hydrophobic patches and sulphhydryl groups are exposed. It enables protein to form aggregates. Gulzar et al. (2011) observed that, even if the denaturation temperature of the proteins in powdered state was higher than the dry heating temperature, an increase in the accessibility of sulphhydryl groups initially buried inside the protein structures was observed. Dry heating was accompanied by a loss of native-like β-lactoglobulin and α-lactalbumin and the formation of aggregated structures. The size of the aggregates increased at higher pH (Gulzar et al., 2011). The formation of dry-heat soluble aggregates enhanced the gelling properties of whey proteins. Gelling properties enhanced as pH and water activity of powders subjected to dry heating increased (Gulzar et al., 2012).

Microwave irradiation is applied for heat-processing in the food and feed as it require lower energy input and shorter processing time. Khan et al. (2015) showed that microwave irradiation for 5 min was as effective as moist heating at 120°C for 60 min in protecting flaxseed protein against ruminal changes. Lately some nonfood applications of whey proteins were investigated (Tomczyńska-Mleko et al., 2015). Biopolymers based on whey protein concentrate and montmorillonite and gluten, whey protein concentrate and kaolinite were obtained (Kawecka-Podlaski, Poland). Kaolinite - KAO (Lot# BCBM2772V) was purchased from Sigma-Aldrich, Co. (St. Louis, MO, USA).

\begin{enumerate}
\item \textbf{Materials and Methods} Wheat gluten - G powder was supplied by Massive (Czechowice-Dziedzice, Poland). According to the provider, the powder consisted of 84.1% protein, 7.8% starch, 1.51% fat, 0.69% ash and 5.5% water. Whey protein concentrate - WPC with 80.0% protein and 3.4% ash was purchased from SM Spomlek (Radzyń Podlaski, Poland). Kaolinite - KAO (Lot# BCBM2772V) was purchased from Sigma-Aldrich, Co. (St. Louis, MO, USA).
\item \textbf{Samples preparation} Gluten, whey protein concentrate and kaolinite were mixed in plastic bags in proportions to obtain in final solutions: 15% (w/w) protein from gluten, 7% (w/w) protein from whey protein concentrate and 5% kaolinite. The above composition of the powders mix was based on the previous research findings for montmorillonite biopolymer mixtures and preliminary research on biopolymers with kaolinite (Wesołowska-Trojanowska et al., 2016). The above concentration of the ingredients allows for production of biopolymer with the textural properties similar to natural clay. Mixtures were heated at microwave oven (Samsung Electronics, Surrey, UK) for 30 s, 60 s or 90 s at 850 W power. To investigate the influence of microwaves on the proteins, G, WPC and their mixtures were heated in microwave oven for 30 s. After heating samples were mixed in distilled water using a magnetic stirrer for 30 min. Dispersions were heated in water bath for 30 min at 80°C. After heating the samples were immediately cooled down with cold tap water for 10 minutes and were subjected to the evaluation of their rheological properties.
\item \textbf{Dynamic Oscillatory Measurements} were performed using RS300 (ThermoHaake, Karlsruhe, Germany) rheometer with a serrated parallel steel plate geometry (35 mm diameter, 2 mm gap size) to limit the potentiality of sliding effects. Samples were cut by a surgical scalpel (35 mm in diameter and 2.5 mm high) and analyzed by frequency sweeps at 0.1 – 10 Hz range in linear viscoelastic region (at 0.01 strain evaluated previously by strain sweeps). All the measurements were performed at the temperature 21°C.
\item \textbf{Preparation of dry biopolymers} To obtain dry biopolymer
material, the samples were dried in a thermostatic cabinet ST3/3 (Pol-Eko-Aparatura, Wodzisław Śląski, Poland) for 24 h at 45 °C. Their texture was evaluated using puncture test and their microstructure was analyzed by scanning electron microscopy.

**Puncture Tests** were performed using a P/2N needle probe (2 mm diameter). The samples were analyzed on a 15-mm thick metal plate with a 10-mm diameter hole in the center and a crosshead speed of 1 mm s⁻¹. The upper surface of a dried sample was fixed by a 3 mm steel plate with a 10-mm diameter hole in the center to prevent the warping of the sample. The thickness of the samples was 2.0 mm at the test area and it was measured using a micrometer with an accuracy of 0.01 mm. Three measurements were carried out to obtain each result.

**Scanning Electron Microscopy (SEM)** Dried biopolymers were fixed by immersion in 2.5% glutaraldehyde solution in 0.1 M sodium cacodylate buffer. The samples were dehydrated in serial dilutions of ethanol and acetone and dried at the critical point in liquid carbon dioxide. Preparations were coated with gold using a vacuum evaporator EMITECH K550x (Emitech, Ashford, United Kingdom). Samples were viewed and photographed using a scanning electron microscope JEOL JXA-8230 (Tokyo, Japan). The elemental mapping of the samples was performed by detecting the X-rays emitted on excitation by a focused electron beam.

**Statistical Analysis** was performed using the statistical program STATISTICA 5.0 PL (StatSoft Polska, Warsaw, Poland). The significance of differences between means was determined using the Tukey’s test at confidence level of p ≤ 0.05 based on the least significant difference.

**Results and Discussion**

**Rheological properties** Fig. 1 presents the influence of frequency on storage and loss moduli for biopolymers (before drying) obtained from microwave dry heated powders at different heating time. At 30 s of irradiation the biggest values of storage and loss moduli values were noted. Higher dry heating time resulted in less rigid biopolymer matrix. Fig. 2 shows the influence of frequency on storage and loss moduli for gels of gluten, whey protein concentrate and their mixture obtained from microwave dry heated powders at 30 s. The mixture of gluten and whey protein concentrate produced several times stronger gels than the singular proteins. It suggests that microwave heating propagated interactions between gluten and whey proteins, probably by disulfide bonds.

**Textural properties** Biopolymers were dried in a thermostatic cabinet for 24 h at 45 °C. Their texture was evaluated using puncture test. Fig. 3 shows a curve representing changes of puncture force with travel distance of needle probe for dried gluten/ WPC/KAO biopolymer composites obtained from powder microwave heated for 30 s. It can be noted from the shape of the curve, that the sample presents hard, quite homogenous and elastic material as there was close to linear change of force with travel distance of needle probe. After puncture to about half of the sample
thickness, the material broke. The biggest hardness was noted for biopolymers obtained from powders irradiated for 30 s (Fig. 4). It is in agreement with the values of the moduli and the value of viscosity × density.

Scanning Electron Microscopy Fig. 5 shows scanning electron microscopy of the biopolymer surface. Kaolinite is seen as lighter particles with sharp edges (Mackinnon et al., 1993). Fibers present in the biopolymer matrix are probably the hairs of wheat grain brush. Similar fibers were observed and recognized using SEM and fluorescence microscopy in our previous research (Wesołowska-Trojanowska et al., 2016).

Al, Si, S, O and C mapping was performed for the investigated samples. Fig. 6. shows sulfur and aluminum mapping for the biopolymer structure. It presents good dispersion of kaolinite particles and the sulphur in the proteins. A similar microstructure was observed by Mallakpour and Dinari (2012). Comparison of the SEM pictures obtained for different irradiation time did not reveal greater differences in microstructure of biopolymers (not shown). Barral et al. (2008) investigated the interactions between whey proteins and kaolinite by adsorption–desorption experiments performed at the isoelectric point (IEP) of the proteins and at pH 7.

They found that kaolinite is a strong adsorbent for proteins with the maximum adsorption capacity at the IEP. X-ray diffraction data for the protein–kaolinite complexes showed that protein molecules were not intercalated in the mineral structure, but immobilized at the external surfaces and the edges of the kaolinite. The protein is more likely to adsorb on the alumina side basal plane of kaolinite (Andersen et al., 2016).

Fourier transform IR measurements indicated the absence of hydrogen bonding between kaolinite surfaces and whey proteins and the adsorption of the proteins was explained by electrostatic interactions and steric effects (Barral et al., 2008; Duarte-Silva et al., 2014). Zhang et al. (2012) investigated the effects of dry heat on the enzymatic hydrolysis and structure of wheat gluten. FTIR method showed the alterations of its secondary structure by an increase in β-sheet content and concomitant disappearance of extended structure. In dry-heated gluten tertiary structures were also disrupted, which was demonstrated by the increases in
sulphydryl and in the decrease of hydration capacity (Zhang et al., 2012).

Elevated temperatures of the powders caused by microwave heating can lead to different chemical reactions: deamidation, hydrolysis, dehydration and aggregation (Gulzar et al., 2011). Intramolecular and intermolecular cross-links which are responsible for aggregation can enforce or weaken the gel protein matrix, depending on a balance between these two types of cross-links. The strong intramolecular interaction leads to more coagulate-like structure in comparison to a strong gel matrix when the forces are balanced (Gulzar et al., 2011). This is probably a cause of observed optimal heating time on the rheological properties of obtained biopolymers. At microwave heating time longer than 30 s the exposure of active sites is too strong which leads to strong intramolecular coagulate-forming interactions. Gulzar et al. (2012) noticed, that the gel strength and water holding capacity of dry heated proteins were improved up to a maximum point then they started decreasing due to excessive denaturation/aggregation of whey proteins leading to insoluble aggregates. At the process of plastic making, the wheat gluten proteins are process of plastic making gluten is heated from 80 to 170°C. Higher temperatures lead highly crosslinked network which negatively affect the properties of bioplastics (Ullsten et al., 2009). The same effect is probably observed for whey protein and gluten mixtures. Most whey proteins and gluten contain disulphide bridges and sulphydryl groups responsible for this kind of behavior.

References


