

Microwave-Assisted Rapid Modification of Zein by Octenyl Succinic Anhydride

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ABSTRACT

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Corn is one of the most important food and industrial crops in the United States. Zein constitutes about half of the endosperm proteins in corn. Potential applications of zein include use in fibers, adhesives, inks, cosmetics, textiles, and chewing gum. Recently, attempts have been made to utilize zein for food coatings and biodegradable materials. The new applications of zein require it to be resistant to water. Thus, we are interested in new routes for chemical modification of zein (Biswas et al, *unpublished*). 2-Octenyl succinic anhydride (OSA) is extensively used in modifying food starches. Our objective was to take advantage of OSA hydrophobicity, flexibility, and compatibility with nonpolar groups and in-

corporate OSA onto zein. This OSA-modified zein would possess the best of both OSA and zein. A zein solution in dimethyl formamide (DMF) was reacted with various levels of OSA to give modified zein. In a microwave reactor, the reactions proceeded tremendously fast and they were over in 5 min. The anhydride group of OSA reacted with the hydroxyl/amine group of zein to form ester/amide of zein. The reaction of OSA-modified zein was confirmed by proton nuclear magnetic resonance (NMR) and IR spectroscopy. The preliminary evaluation showed that zein and OSA-modified zein had comparable mechanical properties.

Zein is unique among the commercially available proteins by virtue of the fact that it is one of the few cereal proteins extracted in a relatively pure form and it is soluble in many organic solvents (Lawton 2002). Its applications (Cheryan 2001) in the past have included inks, label and overprint varnishes, epoxy curing agents (Howland 1962), medicinal tablets, food coatings (Baldwin et al 2003), and biodegradable materials (Isobe et al 2003a,b). Zein has also found use as a film former. The notable characteristics in zein films are high gloss, microbial resistance, and edibility. However, zein-cast films are too stiff and brittle to be used for packaging. Zein film becomes weaker when it absorbs water. One method to prepare zein acetate (Veatch 1941) was invented to increase the water resistance, strength, and flexibility of zein films and coatings. However, this method is useful for making acetyl derivative only. To improve flexibility and reduce moisture pick up, we modified zein with 2-octenyl succinic anhydride (OSA). OSA is widely used to modify food starch by reacting it with starch under controlled pH conditions (Tarvainen 2003). These OSA-modified starches (Wurzburg) have lipophilic (oil-loving) properties and are used in emulsions (Tesch 2002) and encapsulation (Trubiano 2000) and are also useful as flavor carriers because the long octenyl chain is compatible with the flavor and oil molecules. Despite the widespread use of OSA in starch, there has not been any report of utilizing OSA to modify zein. OSA-modified zein, like OSA-modified starch, could be potentially used for food application if it contains <3% OSA by weight. The long chain of methylene groups of OSA could improve water resistance and flexibility of films. Thus, by adjusting the OSA amount, one may control the flexibility and other properties of zein film. In thermal reactions recently, microwave technology (Adam 2003) has shown a pronounced advantage over conventional heat sources where the energy must first be conducted through the walls of the vessel containing the reactants. Microwaves heat the contents directly, allowing the temperature to rise much faster and boosting reaction rates and eliminating side reactions (Loupy 2002). In fact, in many cases, the immense

increase in reaction rate cannot be explained by just the rapid supply of heat, and the cause remains unexplained. In this work, we have applied the benefits of fast reaction of microwave technology in chemically modifying zein.

MATERIALS AND METHODS

Materials and Equipment

Zein was obtained from Freeman Industries (Tuckahoe, NY). The OSA, dimethyl formamide (DMF), and solvents were obtained from Aldrich Chemical (St. Louis, MO). Nuclear magnetic resonance (NMR) solutions were prepared by warming a mixture of 10 mg of zein esters and 10 mg of 40% sodium deuterioxide in 1 mL of deuterium oxide (NaOD and D₂O obtained from Cambridge Isotope Laboratories, Andover, MA). An advanced microwave labstation reactor (ETHOS 1600, Milestone, Monroe, CT) was used in conjunction with a fiber optic temperature probe and EasyWAVE operating software. NMR spectra were obtained by using a spectrometer (DRX 400, Bruker Instruments). The pulse programs used are from the Bruker pulse program library (zg30 for the initial spectrum). The probe was a 5-mm broadband inverse probe. The proton 90° pulse width is 6.4 μsec. The acquisition time is 5.45 sec. Fourier transform infrared (FTIR) spectra samples of zein derivatives were prepared by pulverization under liquid nitrogen, mixing at 0.25% in potassium bromide, and pressing into transparent disks. FTIR spectra of samples pressed in KBr disks were measured on an FTIR spectrometer (FTS 6000, Digilab, Cambridge, CT) equipped with a deuterated triglycine sulfate (DTGS) detector. The absorbance spectra were measured at 4 cm⁻¹ resolution, signal-averaged over 32 scans, and baseline corrected. Spectra of zein derivatives were scaled to compare specific peak ratios with corresponding regions in spectra of unreacted zein.

Zein samples were blended with 15% triethylene glycol as plasticizer using a torque rheometer (Haake Rheocord 90) equipped with high-shear roller blades. Zein samples were ground and compression molded into ASTM D 638 type V bars. Tensile bars were stored for one week at 50% rh. Mechanical properties of zein samples were measured with an Instron universal testing machine (model 4201) with a crosshead speed of 1 cm/min.

Experimental Procedure to Prepare OSA-Modified Zein

A 25% (w/w) solution of Zein F 4000 in DMF was prepared, then 2-octenyl succinic anhydride (OSA) was added. OSA was reacted in five different concentrations (3, 5, 8, 15, and 25% with respect to zein weight). Each mixture was poured into a cross-linked fluopolymer reactor with a stir bar and placed in a

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Milestone Ethos synthesis microwave. Using the EasyWAVE operating software and the fiber optic temperature probe, the mixture was irradiated to 75°C for 10 min. After the reaction, the mixtures were poured into water and water-soluble DMF was washed away. These washings were done in a blender to ensure thorough removal of DMF.

The samples were dried in vacuum oven at 60°C. Dry samples were washed three times with ethyl acetate and filtered each time with a Buchner funnel to remove any unreacted free OSA. Later, we found that the reactions were completed in 5 min and microwave irradiation for 10 min was unnecessary.

RESULTS AND DISCUSSIONS

The OSA reactions were done with 25% zein solution in DMF. DMF was the preferred solvent because, unlike alcohol or water, it has no nucleophilic group and is inert to the anhydride group of OSA (Fig. 1). Thus in DMF, the amine and alcohol group of zein would be free to react with the anhydride group of OSA. OSA was used at five different levels (3, 5, 8, 15, and 25% by weight) with respect to the weight of zein in the microwave reactor. Reactions were performed for 5 min. When reaction time was increased

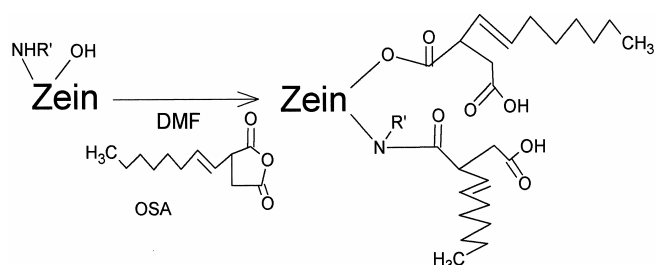


Fig. 1. Reaction of zein with 2-octenyl succinic anhydride (OSA).

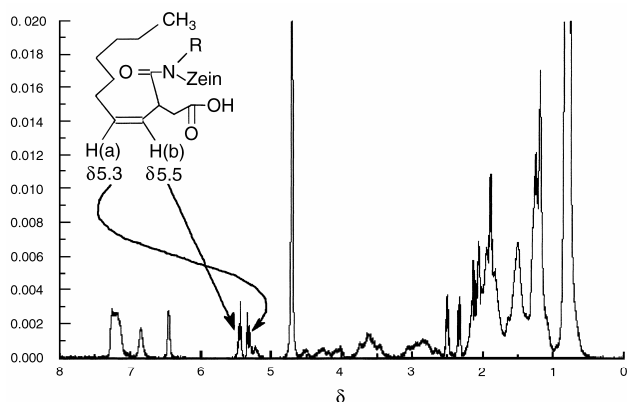


Fig. 2. Proton nuclear magnetic resonance (NMR) spectra of 2-octenyl succinic anhydride (OSA) (15%) modified zein.

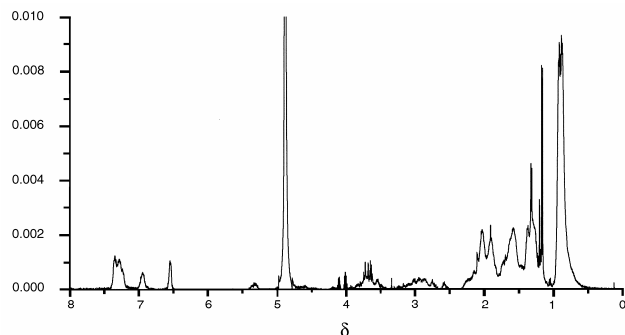


Fig. 3. Proton nuclear magnetic resonance (NMR) spectra native zein.

to 15 min, the extent of OSA reacted with zein remained the same, indicating that the reaction was over in 5 min or less.

The proton NMR of OSA-modified zein revealed the olefinic protons as two peaks at $\delta 5.3$ and $\delta 5.5$, which are indicative of the presence OSA attached to the zein (Fig. 2). As the derivative was thoroughly washed in a soxhlet extractor by ethyl acetate, any unbound OSA would have been removed. The level of OSA bound (reacted with zein hydroxyl/amine group) was determined by comparing the two olefin peaks of OSA at $\delta 5.3$ and $\delta 5.5$ with the $\delta 6.5$ zein aromatic peak (Fig. 3). The NMR of plain OSA in CDCl_3 shows the olefin peaks at $\delta 5.30$ and $\delta 5.63$. The slight shift from $\delta 5.63$ to $\delta 5.5$ for zein-bound OSA olefin is probably due to the opening of anhydride group and the use of D_2O as NMR solvent. The ratio of the area under the $\delta 5.5$ peak to the $\delta 6.5$ aromatic peak indicated the relative amount of OSA bound with zein. Figure 4 demonstrates that the more OSA is used as a reactant, the more OSA is bound to zein. OSA still reacted with zein when the reaction was conducted in 10% zein solution (alcohol-to-water ratio 90:10, v/v). The presence of two peaks at $\delta 5.3$ and $\delta 5.5$ indicated that the acylation reaction still occurred in the alcohol-water mixture. This product was comparable to OSA-modified zein prepared in DMF. Thus, use of DMF as a solvent is not essential for this reaction and it could be replaced by an alcohol-water mixture, which is a common solvent for zein. The yields for both DMF and water-based reactions were in the range of 70–80%. Thus, this process could become practical and commercially feasible if there is a demand for OSA-modified zein. However, when the reaction was conducted in DMF, more OSA reacted with zein, probably due to fewer side reactions in DMF. In addition, greater solubility of zein in DMF makes it possible to react more zein for a given amount of solvent (higher throughput for scale-up production).

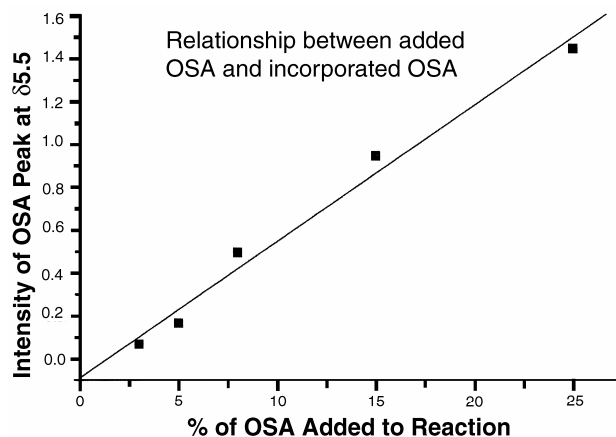


Fig. 4. Incorporated 2-octenyl succinic anhydride (OSA) is proportional to the amount of added OSA.

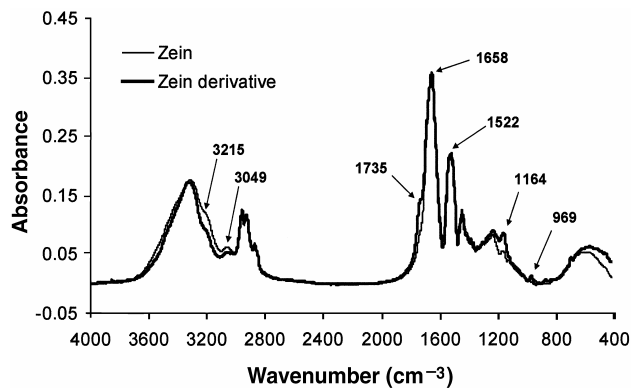


Fig. 5. Infrared (IR) spectra of native zein and OSA derivative.

The FTIR spectrum of the zein derivative (Fig. 5) indicated increased ester formation by the presence of a raised shoulder at $1,735\text{ cm}^{-1}$ on the amide I band ($1,658\text{ cm}^{-1}$) of the unreacted zein. The increase in the band at $1,164\text{ cm}^{-1}$ is indicative of the increase in unconjugated ester groups in the zein derivative. Evidence of the reaction of 2-octenyl succinic anhydride (OSA) with zein also appeared as a definite -COOH absorbance at 969 cm^{-1} in the spectrum of the derivative. A new pendant -COOH is consistent with the simultaneous formation of the zein ester linkage from the reaction of the cyclic anhydride with a hydroxyl group in zein. FTIR spectra showed significant decreases in hydroxyl -OH ($3,215\text{ cm}^{-1}$) and primary amine -NH_2 ($3,049\text{ cm}^{-1}$) bands as well as a small decrease in the secondary amine -NH ($1,522\text{ cm}^{-1}$) band, as would be expected if some of these groups were esterified. A concomitant increase in the amide I band ($1,658\text{ cm}^{-1}$) due to reaction of the cyclic anhydride with amine groups in zein was too small to be observed relative to the huge amide I band in unmodified zein. Figure 5 compares FTIR spectra of zein (Biswas 2003) with the product of the reaction of zein with OSA.

Although we have not thoroughly evaluated the properties of the OSA-modified zein coatings, preliminary studies indicated that its mechanical properties are comparable to zein itself where the zein control possessed $24.3 \pm 2.8\%$ elongation and a tensile strength of $23.9 \pm 0.2\text{ MPa}$ versus $22.1 \pm 2.4\%$ elongation and tensile strength of $28.5 \pm 1.4\text{ MPa}$ for the OSA derivative.

CONCLUSIONS

We have developed a simple microwave-assisted method to prepare OSA derivatives of zein. Zein was dissolved in DMF in 25% concentration and, in this solution, the free hydroxyl and amine groups of zein readily reacted with OSA in 5 min in a microwave reactor. This work demonstrates the use of OSA and application of rapidly expanding use of microwave technology in the area of zein chemistry.

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