Soybean meal nano scales resistant starch- model carbohydrates for encapsulation of bioactive compounds

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Abstract

Nano particles, because of their release efficiency and reduced toxicity, are widely used as encapsulating materials. Resistant starch serves as a particularly good source of encapsulating materials; it is resistant to enzymatic digestion by pancreatic enzymes in mono gastric animals like human. This RS can be used to encapsulate substances that are unstable to light, heat, oxygen and upper intestinal physiological environment. This study researched the use and benefits of RS as an encapsulating material. The resistant starch (RS) fraction of soybean meal was isolated using enzyme assays with α-amylase and amyloglucosidase. The isolated RS was sonicated at 40 °C for 5 hours. The resulting particles sizes ranged from 60 to 80 nm. This material was then coated on a glass bead; this served to construct a model system for targeted delivery to colon. The chemical digestion mimicked the small intestine with pH 4.5 and 8.5 and the stomach with pH 1.5 and 6.5. The percentage of dissolution in both the stomach and the intestine showed 15-30%. The enzymatic digestion with pancreatic α -amylase and amyloglucosidase showed less than 30% digestion. The thermal stability of the encapsulated system was studied with differential scanning calorimetry (DSC). The DSC showed the stability of the material of up to 101 °C and an enthalpy of 277.3J/g. This study demonstrated that soybean meal RS can be used to encapsulate minerals, micronutrients, antioxidants, and drugs. Encapsulation using RS as the nano based materials could provide means to increase chemical and physical stabilities of the encapsulated material

Introduction

Agricultural by-products are extensively used as animal feed, despite their high nutritive value for human food and other applications. Soybean meal, a by-product from soybean oil processing, is an is widely used as animal feed.[1,2,3] The annual North American soybean production 50 million tons annually and an approximately half is used as animal feed after soybean oil extractions.[4]. Soybean oil extraction also results in increase meal carbohydrates to 40%[5]. Starch is the main storage polysaccharides in soybean meal.[1] A fraction of this starch is resistant to enzymes in the upper gastrointestinal tract due to physical inaccessibility, retrogradation, or chemical changes.[6] This resistant starch has several health benefits including reduced risk of colon cancer, the ability to modify lipid metabolism, and the production of butyrate upon fermentation by gut micro-flora.[7] The soybean meal is a good source of resistant starch, and could prove to be beneficial if its use were extended beyond animal feed.

Resistant starch could be useful for encapsulation because it can withstand the acidic gastric environment and pancreatic enzymes in the small intestine.[9] The encapsulation of bioactive compounds with resistant starch can increase the bioavailability of the nutrient, effectively deliver the drug to the target area, and release the probiotics in a viable and metabolically active state.[10,11] Furthermore, these resistant starch-like compounds can not only act as a good delivery vehicles or carriers, but they can also act as prebiotics.[9] The prebiotic nature of resistant starch selectively increases the beneficial micro-flora in the gut and suppresses the pathological bacteria.[9] Therefore, the incorporation of resistant starch as a carrier for substances like drug, nutrient, and probiotics has the dual benefit in target delivery and physiological enhancement due to its prebiotic properties.

Nnano particles have capabilities in delivering bioactive components.[11] Nano particles used as carriers can range from 10-1000 nm.[12] The smaller-sized nano particles possess several advantages when used as carrier materials.[12] The reduced size of the particles enables delivery of the encapsulated materials into cells; the facile delivery of the nano particle is favorable due to the thermodynamic stabilities of the reduced particle size. Nano particles can homogeneously and effectively distribute the encapsulated material to the target region of the organism.[13] The slow release and reduced volume of the encapsulated substances can improve the efficacy of the encapsulated material.[13] Furthermore, nano scale carriers can achieve targeted delivery of bioactive material to the target region in the digestive tract of the organism; these nano scale carriers contain non-toxic, biodegradable, and stable compounds that could withstand the conditions in the digestive tract.

The benefit of using nano particles as delivery systems in therapeutics include reduced side effects, targeted delivery, active cellular uptakes, and enhanced efficacy. Colorectal cancer is the third most common cancer diagnosed in 2012 in both males and females.[14] Therapies colon cancer treatment including surgery, chemotherapy, radiation therapy, immunotherapy, and nutritional supplement are less effective.[15] Curent treatments of colon cancer may cause side effects including bleeding, loss of appetite, headaches, and blood clots.[14] By using bio-based nano materials may provide means for effective delivering of the drugs to the colorectal region. Nano materials also prove useful in reducing the risk of colon cancer development. Currently, there are several supplements, minerals (calcium), and vitamins (folic acids) that can be provided.[14] The major difficulty associated in taking these supplements, vitamins, and minerals is that these compounds get metabolized during gastrointestinal transport due to pancreatic enzymes and the acidic environment.[8] Encapsulation of nutrients and drugs with resistant starch may provide means to deliver undigestible form.[9] Therefore, main objectives of the present study were to (1) isolate the resistant starch from soybean meal, (2) produce nano particles, (3) study the viability of such material to be used as encapsulating sources under human physiological conditions.

Experimental

Materials

The soybean meal samples were obtained from Northern Crop Institute (NCI), North Dakota State University, Fargo, ND. All chemicals, enzymes (α-amylase from *Aspergillusoryzae* and Amyloglucosidase from *Aspergillusniger*), and solvents for High Performance Anion Exchange (HPAE) analysis were purchased from Sigma Aldrich Co (St. Louis, MO), VWR International (Radnor, PA), EMD Serono Inc. (Rockland, MA) and J. T. Baker Chemicals Co (Phillipsburg, NJ) and were used without further purifications.

Methods

Soybean meal resistant starch nano particle preparation

Isolating of soybean meal resistant starch

Resistant starch isolation was adopted from a reported procedure.[16,17]

Soybean resistant starch nano particle preparation

The isolated resistant starch nano particles from soybean meal were subjected to mechanical agitation. The resistant starch was subjected to sonication using an ultra sonicator (Branson Inc, Chicago, Illinois) at 40 °C for 5 hours.

Chemical dissolution in stomach

Nearly 0.1g of the soybean meal resistant starch nano particles was exposed to buffers containing 0.1M hydrochloric acid at pH 1.5 and 6.5 for 3 hours.[18,19] The buffer solutions with the samples were subjected to 37 °C at 100 rpm. These conditions stimulated the stomach environment. The samples were withdrawn at 30-minute time intervals, centrifuged at 1500 rpm, dried at 40 °C overnight, and weighed. The dissolution was calculated as percent weight loss of the material. The analysis was carried out in duplicates.

Chemical dissolution in intestine

Approximately 0.1g of the soybean meal resistant starch nano particles was kept in buffer containing 0.1M phosphoric acid at pH 4.0 and 8.5 individually for 5 hours. The buffer solutions with the samples were subjected to 37 °C at 100 rpm to stimulate intestinal environment[18,19]. The aliquots of samples were withdrawn at 60-minute time intervals, centrifuged at 1500 rpm, dried at 40 °C overnight, and weighed. The dissolution was calculated as percent weight loss of the material. The analysis was carried out in duplicates.

Enzymatic digestion[17]

Nearly 0.1g of the soybean meal resistant starch nano particles was exposed to an enzymatic solution containing pancreatic α -amylase (30U/ml) and amyloglucosidase (300U/ml) for 16 hours at 37 °C at 100 rpm. These conditions stimulated the intestine environment. The samples were withdrawn at the end of the 16-hour period, centrifuged at 1500 rpm, dried at 40 ºC overnight, and weighed. The digestion was calculated as percent weight loss of the material. The analysis was carried out in duplicates.

Thermal analysis

The soybean meal resistant starch nano particles and soybean meal starch nano particles were exposed to differential scanning calorimetry (DSC Q 1000, Setaram Inc, NJ) at temperatures ranging from 0 ºC to 180 ºC at 10 ºC/ minute[20].

Resistant starch content

Resistant starch content in soybean meal was adopted from a reported procedure[17].

Results and discussion

The present study demonstrated the suitability of the soybean meal resistant starch nano particles to be as potential encapsulating materials for micronutrient and drug delivery to the colon. The nano scale particles of soybean meal resistant starch were evaluated by scanning electron microscopy. The nano particles were exposed to chemical and enzymatic digestion by stimulating the stomach and intestinal environment. The thermal stability of the nano particles were studied using differential scanning calorimetry.

Soybean meal nano particles

Sonication of the soybean meal resistant starch at 40 ºC for five hours gave rise to nano scale particles in the range of 60-95 nm (figure1). Ultrasonication is a physical treatment that uses ultrasound, generated from the piezoelectric effect or magnetization, to create high-energy vibration. This vibration can reduce the particles from several micrometers to less than 100 nm. Previous studies[22] have reported that subjecting starch particles to sonication for 75 minutes reduced the particle size to only about 200 nm. But as the time passes the particle size continues to decrease due to intense vibration experienced by the particles.

The nano scale particle-size provides several advantages. With a higher surface area per mass due to particle size reduction allows the particle to become thermodynamically stable. These particles with reduced size especially, the nano scale particles are facile materials in delivery and easily absorbed in to the cell. Compared to macro and micro scale particles, the nano scale particles are relatively more homogenous.

Resistant starch could survive extreme gastrointestinal tract conditions. For this reason, resistant starch is a suitable material to encapsulate the colon-targeted materials like drugs, nutrients, genes, and probiotics. The nano scale particle size of this resistant starch isolated from an agricultural by product makes this material a potential source for targeted delivery in the colon.

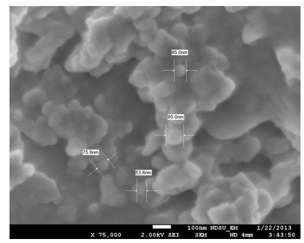

Figure 1. SEM images of nano particles of soybean meal resistant starch

Chemical and enzymatic digestion

The material developed in the present study was stable under simulated stomach and intestine environment. These results show that the material is a potential source to deliver micronutrients and drug materials to the colon. As shown in figure 2 and 3, the dissolution percentage of the soybean meal resistant starch nano particles were in the range of 15-30%. The percentage of dissolution with this material is low compared to the previous studies[23] performed with 20% resistant starch and high amylose corn starch. Also, exposure of this material to pancreactic amylase and amyloglucosidase showed that the percentage dissolution was only about 21%. The lower dissolution of this material when exposed to chemical and enzymatic environment in stomach and intestine shows that the soybean meal resistant starch in nano scale could survive human gastro intestinal envrionments.

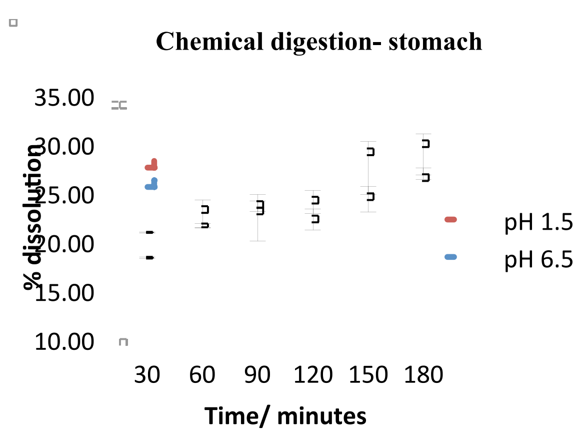

Figure 2. Chemical digestion of soybean meal nano particles at pH 1.5 and 6.5 at 37 ºC and 100 rpm for 3 hours (180minutes) stimulating stomach environment

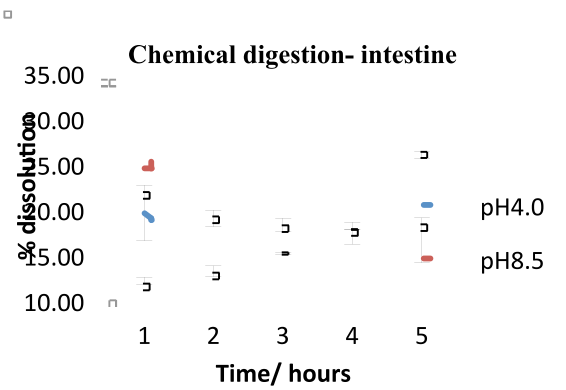

Figure 3. Chemical digestion of soybean meal nano particles at pH 4.0 and 8.5 at 37 ºC and 100 rpm for 5 hours stimulating the small intestine environment

Thermal stability by DSC

The stability of the soybean meal resistant starch nano particles under physiological temperature was studied by DSC. According to DSC studies the material has an enthalpy change of 277.3 J/g. This is comparatively higher than previous studies[24] done with native starch, which reported that the change of enthalpy is nearly 20 J/g. In addition to the higher enthalpy, the material is stable up to 100 ºC as compared to the previous studies[25] with the native starch, which is only stable up to 60 ºC. Soybean meal resistant starch nano particles are stable under human physiological temperatures.

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