

Direct Solid-State Fermentation of Soybean Processing Residues for the Production of Fungal Chitosan by *Mucor rouxii*

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Abstract

The feasibility of utilizing soybean-processing residues such as soybean meal and hulls as substrates for chitosan production by the fungus *Mucor rouxii* ATCC 24905 via solid-state fermentation (SSF) was investigated. The effects of the type of soybean-based substrate, length of cultivation period, substrate moisture content, substrate pH, incubation temperature and extraction conditions on chitosan yield were determined. The results showed that a maximum fungal chitosan yield of up to 3.44% by dry substrate weight (34.4 g/kg) could be achieved using a pure soybean meal substrate with an initial moisture content of 50% (w/w) and pH of 5 - 6 incubated for six days at 25°C. A more severe heat treatment (autoclaving vs. refluxing) resulted in higher chitosan extraction yields regardless of the strength of extraction reagents. Fourier transform infrared (FTIR) analysis of the fungal chitosan revealed its degree of deacetylation (DDA) to be between 55% and 60%.

Keywords

Solid-State Fermentation, Fungal Chitosan, Biopolymers, Bioconversion, Agro-Industrial Residues

1. Introduction

Chitosan is a natural biopolymer that has gained increased attention in recent years due to its interesting and beneficial biological and molecular characteristics, which enable its application in a wide variety of industrial and biotechnological fields. These fields range from agriculture, environmental remediation, pulp and paper

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coatings and barrier films, food preservation, and cosmetics to more advanced technology emerging fields such as functional materials in flexible printed organic electronic devices, protein separations, drug delivery, and tissue engineering [1]. Chitosan is a heterogeneous polysaccharide containing β -(1-4)-linked 2-amino-2-deoxy-D-glucose (glucosamine) and *N*-acetyl-2-amino-2-deoxy-D-glucose (*N*-acetyl glucosamine) units, with the former consisting of more than 60 % of the copolymer [2]. It is obtained through deacetylation of chitin, which contains mostly *N*-acetyl glucosamine units. Its unique and beneficial properties enabling its numerous applications include its cationic nature, chelation and ion-binding ability, protein immobilization, film- and gel-forming characteristics, chemical reactivity, amenability to modification, biocompatibility, and antimicrobial activity [3]. These properties are a consequence of its heterogeneous molecular structure and the presence of highly reactive amine groups at the glucosamine residues [4]. Fields and applications that can be served by utilizing chitosan and exploiting its properties include biomedical engineering, food and agriculture, environmental remediation, and polymers, films, fibers and coatings [1]-[3].

Chitosan is traditionally produced by subjecting crustacean shells to severe alkali, acid, and/or enzymatic treatments to isolate chitin, by removing impurities such as proteins, minerals and pigments, and by deacetylating of chitin to chitosan—that is, the removal of the acetyl groups in the *N*-acetyl glucosamine units comprising the majority of chitin to increase the proportion of glucosamine units in the polymer. Harsh reaction conditions are required due to the complex organization of chitin with other components in the crustacean exoskeleton [5]. Thus, the conventional process results in high labor and processing costs. Additionally, these raw materials are seasonally and geographically limited and have highly variable characteristics, which can lead to inconsistent product yield and molecular characteristics. This is undesirable for high purity applications such as in the pharmaceutical and biomedical fields [3] [6]. On the other hand, chitosan is also found as a major fungal cell wall component particularly of fungal strains belonging to the order Mucorales of the Zygomycetes class [6] [7]. These fungal species produce chitosan directly unlike crustacean-derived chitosan, which has to be produced via isolation and chemical conversion of chitin from the exoskeletons of these organisms. Fungal chitosan is more advantageous than crustacean-based chitosan, because the former can be produced in a controlled environment all year round independent of the seasonal seafood industry [8]. Fungal growth and chitosan productivity and properties can be controlled through the manipulation of fermentation and extraction conditions to give chitosan more consistent and desired physico-chemical properties compared with chitosan obtained from crustacean sources [9]. Moreover, the extraction process for isolating chitosan from fungal cell walls is relatively simpler and milder than obtaining chitosan from crustacean shells, thus producing less waste [9]. In terms of chitosan properties, chitosan produced by most of the identified producer fungal strains has roughly the same degree of deacetylation, and relatively lower viscosities and molecular weight than crustacean-derived chitosan, which improves its potential for processing and modification for its numerous applications particularly in agriculture and biomedical engineering [10].

Agro-industrial crops, by-products and residues can be used as inexpensive carbon sources for growing the fungi, which can help alleviate some environmental concerns on the disposal of these waste materials [11]. These materials are particularly suited for use as substrates and microbial growth support in solid-state fermentation (SSF) processes, which exploit the natural growth conditions of filamentous fungi and their capabilities to biosynthesize and excrete hydrolytic enzymes to directly ferment solid biomass substrates. SSF methods have been applied previously for the production of fungal chitosan using a variety of agro-industrial residues and fungal strains, though not as prevalent as using submerged liquid fermentation (SLF) cultures. Potato chip processing wastes [12], cottonseed hulls and corn residues [13], sweet potato [14], and residues from the manufacture of soymilk and mungbean noodles [15] were utilized as fermentation substrates for growth and chitosan production of various filamentous fungal strains. The filamentous fungus *Mucor rouxii*, a Zygomycete strain, has been well known to produce high amounts of chitosan using SLF [8] [16] [17]. The possibility of utilizing this fungus for SSF of agro-residues for chitosan production has not been tested previously.

Soybean processing residues, such as soybean meal and soy hulls, are excellent options for use as fermentation media/substrates for the growth of fungi that can produce chitosan due to their abundance and high nutrient content. According to the American Soybean Association, one bushel (60 lbs) of soybeans contain on a mass basis approximately 18.3% oil, 80% meal, and 1.7% of other residues such as the hulls and mill runs [18]. With a total annual soybean production of 89.5 million metric tons, the amount of soybean meal production is approximately 71.6 million metric tons while that of soybean hulls is estimated to be 1.5 million metric tons [19]. The conventional use of soybean meal includes supplementation of animal feed due to its high carbohydrate and protein

content. However, such use is limited due to the presence of oligosaccharides that are indigestible by non-ruminant livestock and poultry [20]. To some extent, soy hulls can be used as animal feed fillers. But due to their relatively low protein and high fiber content, they are not as widely used as soymeal for this application. Instead, they are used as a boiler fuel additive. These characteristics, however, can make both soybean meal and hulls an ideal solid-state fermentation (SSF) substrate for the growth and cultivation of fungi that can be used to produce chitosan. The fungal chitosan product, with all its interesting molecular properties and applications, can be used as a renewable and “green” biobased alternative not only for crustacean chitosan, but also for petroleum based polymers.

The goal of this study is to investigate fungal chitosan production via solid-state fermentation of soybean processing residues using *Mucor rouxii*. Experiments were conducted to determine the optimum cultivation and extraction conditions for maximum chitosan yield. The physicochemical characteristics of the produced chitosan were then determined using analytical methods.

2. Experimental

2.1. Materials

Mucor (Amylomyces) rouxii ATCC 24905, the fungal strain used in this study, was purchased from the American Type Culture Collection (ATCC). The freeze-dried culture was rehydrated with sterile deionized (DI) water and stock cultures were generated by cultivation of the rehydrated fungal cells on potato dextrose agar (PDA) slants at 25°C for five days. The stock cultures were then stored and maintained at 4°C until use. Soybean meal and hulls provided by three local soybean processors was tested as a solid-state fermentation substrate. These were then stored in airtight plastic containers in a refrigerator at 4°C until use. Representative samples of the soybean meal and hulls from the different sources were sent to Northland Labs, Inc. (Northbrook, IL) for analysis of carbohydrates, proteins, moisture, and ash using standard AOAC methods. The soymeal were analyzed and used as received while whole soy hulls were homogenized to granular form using a Waring blender. Composite samples consisting of equal amounts from each of the three suppliers were used for the fermentation process. Commercial grade sodium hydroxide pellets and Optima grade glacial acetic acid were purchased from Fisher Scientific (Pittsburgh, PA, USA). Low molecular weight chitosan purchased from Sigma Aldrich (St. Louis, MO, USA) was used as the reference chitosan for fungal chitosan characterization. The chemicals and standards were used as received.

2.2. Fungal SSF Cultivation and Chitosan Production

The fungal spore suspension inocula used in the SSF cultures were prepared as follows: ten milliliters of sterile DI water was transferred aseptically into a *M. rouxii* ATCC 24905 stock PDA slant. The slant tube was then gently inverted ten times to suspend the fungal spores in water. After this, 2-mL of the spore suspension was immediately transferred aseptically into a new PDA plate. The spore suspension was distributed onto the PDA surface using a sterile disposable L-spreader. The inoculated plates were incubated at 30°C for five days. After the incubation period, the entire mycelial growth on the agar surface was scraped using a sterile metal spatula and transferred into a 250-mL flask containing 100 mL of sterile DI water. The mixture was gently shaken to suspend the fungal spores in the water. After this, the suspension was filtered through a sterile coarse filter paper into a new sterile collecting flask to remove the large agar and fungal debris.

The soybean residue substrates were prepared and inoculated as a single batch in a sterile autoclavable plastic bag. Sterile DI water was added to the filtered fungal spore suspension to attain the desired initial moisture content setting (30%, 50%, or 70% w/w). The pH of the sterile culture dilution water was previously set according to the desired initial pH setting of the substrate (pH 4, 5, or 6). The wet soymeal mash was shaken and mixed thoroughly inside the bag and then distributed in 10-gram portions (wet basis) in sterile polystyrene deep petri dishes. The petri dishes containing the soybean meal fungal cultures were then incubated at the desired temperature setting (25°C, 30°C, or 35°C) for the set cultivation period (3, 6, 9, 12, or 15 days). All experimental treatments were tested in triplicate petri dish cultures.

2.3. Measurement of Fungal Growth

The extent of growth of *M. rouxii* in the soybean meal cultures was determined indirectly by spectrophotometric

measurement of glucosamine in samples of the fermented soymeal substrate according to a method published elsewhere [21].

2.4. Chitosan Extraction

Fungal chitosan produced in the soybean residue cultures was extracted and isolated using an approach based on previously published methods [12] [22] [23]. The entire contents of each petri dish soy residues culture were processed first by extraction of residual proteins and other alkali soluble materials with NaOH solution with the desired concentration (1 M or 46% w/v) and volume-to-solids ratio (30:1 or 10:1, mL/g dry mass). Two different heated extraction methods were also tested: autoclaving at 121°C, 15 psi for 15 min or refluxing at 46°C for 8 h. Alkali insoluble materials (AIMs) were recovered by vacuum filtration and washed to neutral pH with DI water. The washed AIMs were transferred and weighed into tared glass petri dishes and dried in an oven at 60°C overnight or until a constant weight was achieved. The dry weight and moisture content of the AIMs were then calculated using the wet and dry weights measured. Chitosan was extracted from the AIMs with 2% (v/v) aqueous acetic acid. Two different volume-to-solids ratios (40:1 or 10:1, mL/g dry mass AIMs) and heat treatments (autoclaving at 121°C, 15 psi for 15 min or refluxing at 95°C for 8 h) were tested. After the extraction period, the slurries were transferred into tared centrifuge tubes and centrifuged at 3000 RPM for 20 minutes, after which the insoluble fraction was discarded. The pH of the supernatant was adjusted to pH 10 by the dropwise addition of 4 M NaOH in order to precipitate the chitosan out of solution. The suspension was then centrifuged at 3000 RPM for 20 minutes, after which the supernatant was discarded. The chitosan pellets were then washed successively with DI water, 95% ethanol, and acetone; frozen at -20°C overnight; freeze-dried; and weighed to calculate the chitosan yield (% w/w dry mass of initial soymeal substrate).

2.5. Characterization of Fungal Chitosan by FTIR Spectroscopy

The Fourier transfer infrared (FTIR) spectra of the product fungal chitosan from soymeal fermentation were obtained by analyzing solid powder samples with an Alpha-E FTIR spectrometer (Bruker Optics Inc., Billerica, MA, USA). Sixty-four scans were conducted per sample between wavelength number of 4000 - 700 cm^{-1} at a resolution of 2 cm^{-1} . The degree of deacetylation (DDA) of the chitosan samples was estimated according to the method of [24]. The absorbance peak at wavelength number $\approx 1560 \text{ cm}^{-1}$ (amide II) was used as the characteristic signal while the peak at wavelength number $\approx 1070 \text{ cm}^{-1}$ (C-O bond stretching) was used as the reference signal. DDA values were calculated using the absorbance ratio A_{1560}/A_{1070} and the corresponding calibration curve relating the absorbance ratio to DDA.

3. Results and Discussion

3.1. Characterization of Soybean Processing Residues

Following analysis, the soybean meals were found to contain almost 50% by weight proteins, which is roughly five times that of the soy hulls. In terms of the carbohydrate content, soy hulls had around 79% by weight of carbohydrates, which is almost twice that of the carbohydrates in soymeal. Both meal and hulls contain similar residual fats while the meal has slightly higher moisture and ash contents than the hulls. The high carbohydrates and protein content of these residues could serve as carbon and nitrogen sources for direct fungal growth under solid-state cultivation and support the rationale behind the selection of these agricultural residues as fermentation substrates for chitosan production.

3.2. Effect of Substrate Type

Pure soybean meal, soy hulls, and 1:1 meal-hull mixtures were tested as SSF substrates for growth and chitosan production by *M. rouxii* ATCC 24905. The results (Figure 1) show that fungal growth (as measured by glucosamine in the culture) under solid-state cultivation conditions in a pure soymeal culture or in a 1:1 meal/hull mixture were not significantly different, while growth in pure soy hull was the lowest. This result was expected as the substrates containing the soymeal contained higher protein (nitrogen) contents than pure soy hulls, resulting in the increased biosynthesis of fungal mycelial materials. On the other hand, chitosan yield was highest from the pure soymeal cultures ($1.63\% \pm 0.16\%$ w/w dry substrate) while those from the pure soy hull ($1.26\% \pm 0.15\%$) and 1:1 meal/hull mixtures (0.97 ± 0.15) were not significantly different. The chitosan yield displayed a

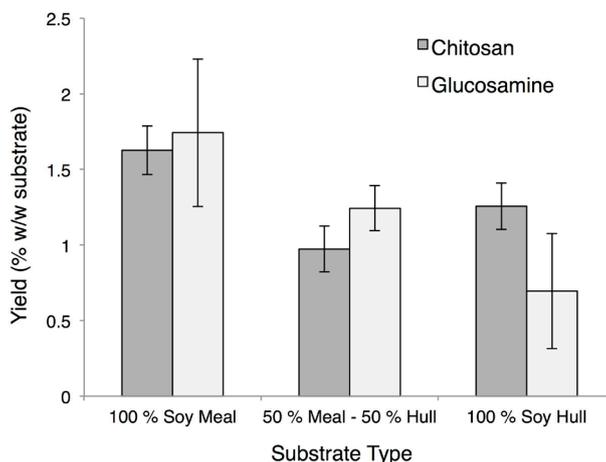


Figure 1. Growth and chitosan production by *Mucor rouxii* ATCC 24905 on different soybean processing residues. Incubation temperature—30°C, initial pH unadjusted, initial moisture content—50% (w/w), and cultivation time—six days.

positive correlation with fungal growth levels for both the pure soymeal and the 1:1 meal:hull mixtures. Being a fungal cell wall component, the production of chitosan is considered to be growth-associated (*i.e.*, production rates correlate with growth rate) under carbon- and nutrient-rich condition. However, under a condition of high carbohydrate-to-protein proportion such as in soy hull cultures, the chitosan yield displayed an opposite trend relative to fungal growth between the pure soy hull and 1:1 meal/hull cultures. It appears that despite the lower fungal growth level in the soy hull culture, the fungal cell wall mass generated contained a relatively higher composition of chitosan. Based on these results, pure soymeal substrates were utilized in the succeeding fermentation tests.

3.3. Effect of Incubation Period

The effect of the length of the cultivation period on fungal growth and chitosan yield using pure soymeal SSF cultures is shown in **Figure 2**. All other fermentation variables were kept constant at the following levels: 50% (w/w) initial substrate moisture content, 30°C incubation temperature, and unadjusted initial substrate pH. The fungal growth pattern (as glucosamine) showed a possible diauxic pattern, with a first stationary period attained between 6 to 12 days of cultivation followed by a sharp increase at day 15. It is possible that during the first stationary period, the fungal cells utilized the readily available adhered carbon and nutrients in the soymeal particle surfaces for partial growth and assembly of hydrolytic enzymes (cellulase, proteases, etc.). During the stationary period, the synthesized enzymes were utilized by the fungal cells for the degradation of the polymeric carbohydrates and proteins into more readily assimilable carbon and nutrients, which were then made available for a succeeding exponential growth phase. Despite the observed increase in growth, the results show that maximum chitosan yield was achieved after only six days of cultivation after which chitosan yields fluctuated (not significantly different). Previous studies have indicated that during the attainment of the stationary growth phase, the fungal cell walls may have begun to lose their fluidity and potential for chitosan formation due to consolidation by chitin crystallization and bond formation with other cell wall components, which could lead to reduced extraction yields [22] [25]. Thus, six days was considered to be the optimum length of cultivation period for maximum chitosan yield and was maintained in the succeeding experiments.

3.4. Effect of Initial Moisture Content

Figure 3 shows the effect of the initial substrate moisture content on growth and chitosan yield of *M. rouxii* ATCC 24905 in SSF soymeal cultures. In these experiments, the incubation period was set at six days based on the optimum level obtained in the prior experiments, the incubation temperature at 30°C, and initial substrate pH was not adjusted. The lowest moisture level tested (30% by wt.) resulted in the lowest fungal biomass growth and chitosan production. However, fungal growth and chitosan yields were not significantly different between

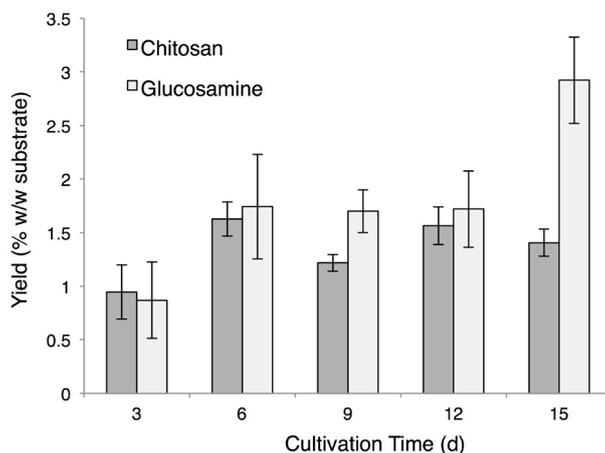


Figure 2. Effect of cultivation time on growth and chitosan yield by *Mucor rouxii* ATCC 24905 on pure soymeal substrate. Incubation temperature—30°C, initial pH unadjusted, initial moisture content—50% (w/w), and cultivation time—six days.

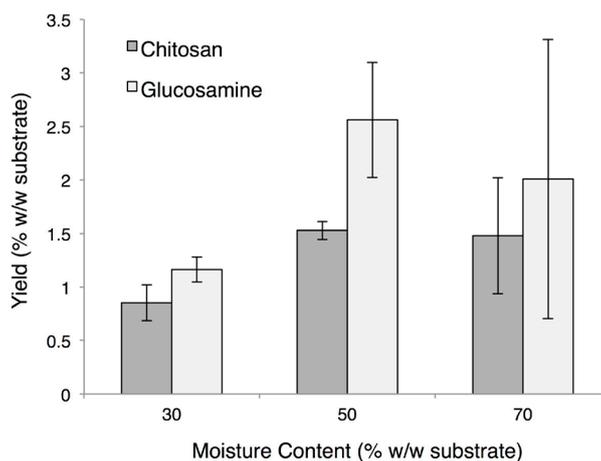


Figure 3. Effect of initial substrate moisture content on growth and chitosan production by *Mucor rouxii* ATCC 24905 on pure soymeal substrate. Incubation temperature—30°C, initial pH unadjusted, and cultivation time—six days.

the 50% and 70% initial moisture content treatments (chitosan yield is between 1% and 2% w/w dry substrate). The moisture content of the substrate is a critical parameter related to the water activity of the microorganisms in SSF processes where moisture levels are relatively low. The fungal cells need an appropriate level of water in which to exercise the various biochemical reactions for the enzymatic breakdown and uptake of the biomass substrate. The results in this study indicated that *M. rouxii* ATCC 24905 was able to tolerate a lower substrate moisture content of 50% for growth and chitosan production in SS unlike other fungal strains previously studied for chitosan production via SSF, that were grown in 60% - 70% moisture substrates [12] [22] [26]. Using less water to moisten the soybean meal substrate could mean less dilution of nutrients and growth factors due to water addition in the culture. Furthermore, using soymeal cultures with 70% initial moisture content appeared to produce high variability in the results. Thus, the initial soymeal substrate moisture content of 50% was considered to be the optimum level for maximizing fungal growth and chitosan production using the strain *M. rouxii* ATCC 24905.

3.5. Effect of Incubation Temperature

Figure 4 shows the effect of incubation temperature on fungal growth and chitosan production in solid-state

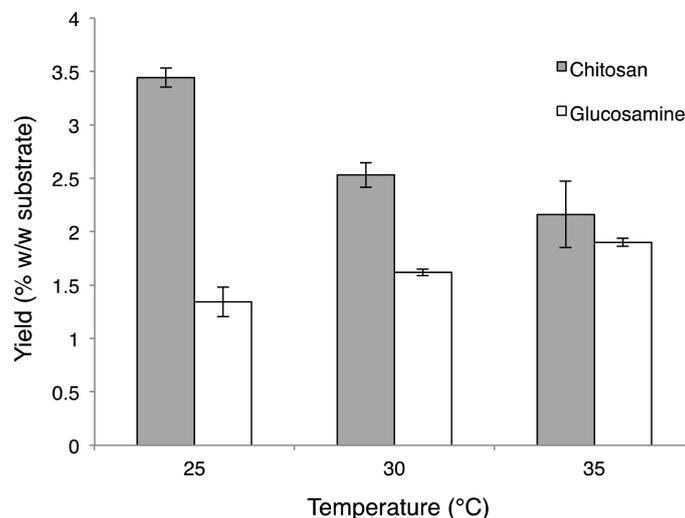


Figure 4. Effect of incubation temperature on growth and chitosan production by *Mucor rouxii* ATCC 24905 on pure soymeal substrate. Initial pH unadjusted, initial moisture content—50% (w/w), and cultivation time—six days.

cultures of *M. rouxii* ATCC 24905. In these experiments, the incubation period was set at six days and the initial moisture content of the soymeal substrate was 50% (w/w) based on the optimum levels obtained in the prior experiments. The initial substrate pH was not adjusted. The experiments demonstrated opposite trends of growth and chitosan yield with increasing incubation temperature. The highest chitosan yield of around $3.44\% \pm 0.09\%$ (w/w of total solid substrate) was achieved at 25°C and decreased considerably at incubation temperatures of 30 and 35°C. On the other hand, total fungal growth was shown to increase as the incubation temperature increased. A higher incubation temperature appeared to favor fungal growth in the solid soymeal culture but results in lower chitosan yields. A possible explanation is that the fungal cell walls might be losing their fluidity in favor of a denser and more consolidated structure dominated by chitin, which results in reduction of chitosan composition and/or extractability at incubation temperatures higher than 25°C. This could be a defense mechanism by the fungal cells to counteract the potentially detrimental effects of high temperatures on fungal growth and metabolism. On the operational standpoint, incubation at lower temperatures could lead to lower costs attributed to heating and humidification of fermentation chambers but could imply the necessary fermentation bed cooling mechanisms to counteract the buildup of temperature gradients across the substrate bed thickness.

3.6. Effect of Initial Substrate pH

Figure 5 shows the effect of the initial pH of the moistened soybean meal substrate on fungal growth and chitosan production in solid-state cultures of *M. rouxii* ATCC 24905. In these experiments, the incubation period was set at six days, the incubation temperature at 30°C, and initial substrate moisture content was at 50% (w/w) according to the results of the prior experiments. The results obtained demonstrate that there are no significant differences in terms of fungal growth and chitosan yields among the initial substrate pH levels investigated. It seems logical to select the unadjusted pH as the best condition from the operational cost standpoint, which would not require prior treatment of the soymeal substrate with acid and/or alkali for pH adjustment. However, an initial pH setting between 5 and 6 might be ideal for helping to minimize the variability of the product chitosan yields and characteristics. Additional tests will be conducted to confirm this hypothesis.

3.7. Effect of Extraction Conditions

In addition to testing the effect of the fermentation variables on fungal growth and chitosan yield, different extraction methods were also tested to determine their effects on chitosan recoveries. Different methods were selected from literature. The results of these tests are summarized in **Figure 6**. In these experiments, the fermentation conditions were kept constant at six days cultivation period, 50% initial substrate moisture content, unad-

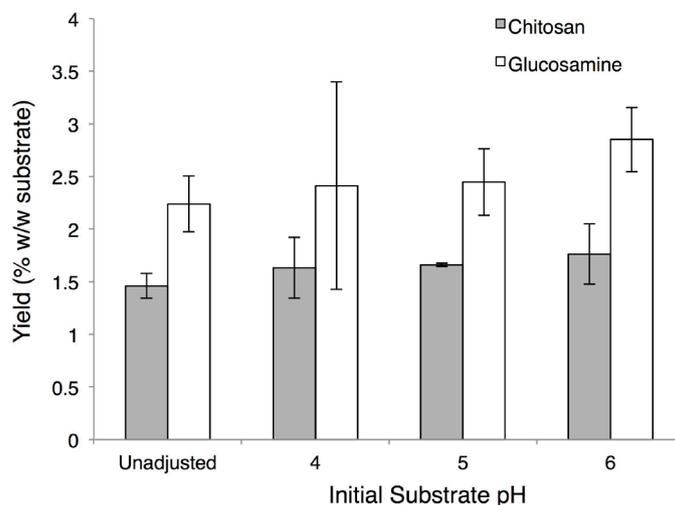


Figure 5. Effect of initial substrate pH on growth and chitosan production by *Mucor rouxii* ATCC 24905 on pure soymeal substrate. Incubation temperature—30°C, initial moisture content—50% (w/w), and cultivation time—six days.

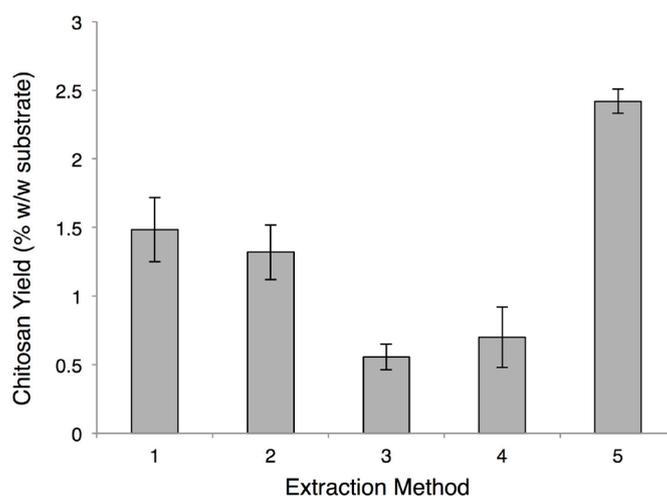


Figure 6. Effect of extraction method on growth and chitosan yield by *Mucor rouxii* ATCC 24905. Method 1—1 M NaOH at 121°C for 20 min (30 mL per g substrate) followed by 2% (v/v) acetic acid (40 mL per g substrate) at 95°C with refluxing for 8 h. Method 2—1 M NaOH at 121°C for 20 min (10 mL per g substrate) followed by 2% (v/v) acetic acid (10 mL per g substrate) at 95°C with refluxing for 8 h. Method 3—46% (w/w) NaOH at 46°C for 13 h (30 mL per g substrate) followed by 2% (v/v) acetic acid (40 mL per g substrate) at 95°C with refluxing for 8 h. Method 4—46% (w/w) NaOH at 46°C for 13 h (10 mL per g substrate) followed by 2% (v/v) acetic acid (10 mL per g substrate) at 95°C with refluxing for 8 h. Method 5—1 M NaOH at 121°C for 20 min (30 mL per g substrate) followed by 2% (v/v) acetic acid (40 mL per g substrate) at 121°C for 20 min.

justed initial substrate pH, and 30°C incubation temperature. **Figure 6** shows that chitosan recoveries differed significantly among the different methods investigated with regards to the concentration of reagents used as well as the severity of the heat treatment method used. For instance, Methods 1 and 2 utilized the same reagent concentrations but with differing volume ratios, while Method 2 used a lower volume of reagent. Although a higher volume-to-mass ratio for both the base and acid reagents (Method 1) resulted in a slightly higher chitosan re-

covery, the results were not significantly different. The same case was observed with Methods 3 and 4, which used a higher base concentration but with heating and refluxing instead of autoclaving. Compared with Methods 1 and 2, Methods 3 and 4 resulted in a significantly lower chitosan recovery. This finding implies the importance of the base-assisted deproteinization step for maximizing the chitosan yield. However, **Figure 5** also shows that when a more severe reaction condition (*i.e.*, autoclaving) was employed for the acetic acid extraction of chitosan step, the chitosan recovery was significantly higher than all the other extraction methods tested. Despite this, a more thorough economic and cost-benefit study should be made to justify the use of more severe extraction conditions and correlations must be made with the effect of fermentation conditions.

3.8. Characterization of Fungal Chitosan Using FTIR

The chitosan extracted from *Mucor rouxii* ATCC 24905 fungal cultures in soybean meal was characterized by FTIR spectroscopy and compared with commercially available chitosan. The purpose of this analysis was to confirm the identity of the extracted chitosan pellets and to estimate the degree of deacetylation (DDA) of the fungal chitosan product, an important parameter that influences the activity of the chitosan polymer for its intended applications. The DDA of the fungal chitosan produced from solid-state fermentation of soybean meal was estimated using the absorbances of the FTIR signals at approximate wavelength numbers of 1560 and 1070 cm^{-1} (Shigemasa *et al.*, 1996) As shown in **Figure 7**, the signal at 1560 cm^{-1} corresponds to the characteristic band of the amide group (Amide II) in the N-acetylglucosamine residue of chitin, which accounts for more than 90% of the monomer residues in the polymer chain. Although definitions vary among different sources, the chitin chain is identified as chitosan if the polymer chain contains a significant proportion of the deacetylated glucosamine residue. Thus the intensity of the band at 1560 cm^{-1} is expected to decrease as the DDA increases. The signal at 1070 cm^{-1} corresponding to C-O bond stretching in the chitin/chitosan monomers was used as the reference band. Using the calibration curve developed by Shigemasa *et al.*, the DDA of representative samples of fungal chitosan from different fermentation and extraction conditions was estimated to be between 55% - 60%.

4. Conclusion

Solid-state fermentation (SSF) of soybean processing residues using the fungus *Mucor rouxii* ATCC 24905 for chitosan production was for the first time investigated. The optimum SSF conditions for maximum chitosan

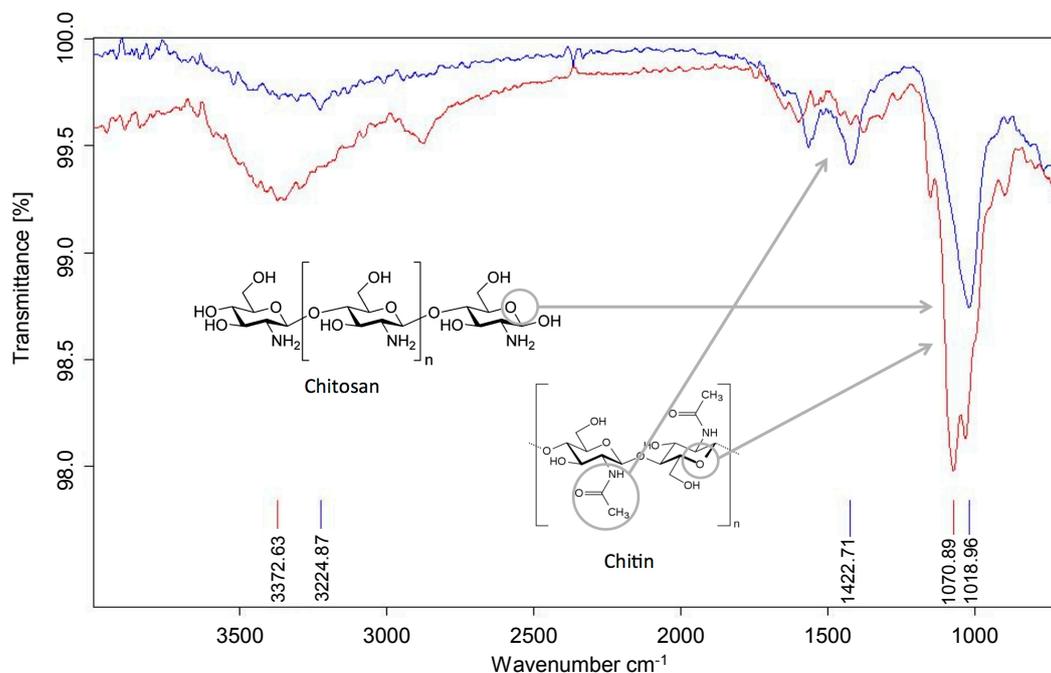


Figure 7. FTIR spectra of commercial chitosan (red) and *Mucor rouxii* ATCC 24905 chitosan from fermentation of pure soymeal substrate (blue).

yields were determined. FTIR analysis of the fungal chitosan derived from soybean meal fermentation confirmed its molecular identity and estimated its degree of deacetylation to be around 55% - 60%. Extraction conditions such as the concentration of acid and base reagents and severity of heat treatment were also shown to influence the chitosan yield.

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