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Purity Assessment of Commercial Zein Products After Purification

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Abstract Successful utilization of commercial zein products for certain food, pharmaceutical, cosmetic and medical applications requires a decolorized/deodorized zein of high purity that can be achieved by column filtration of commercial yellow zein solutions through Zeolite 5A and activated carbon. The objective of this investigation was to devise a combination of methodologies to assess purity and degree of deodorization and decolorization. Off-odor removal is defined by a UV spectroscopic ratio of 280:325 nm where diferuloylputrescine is the major contributor. Removal of yellow color, attributed to xanthophylls in zein, was followed by visible spectroscopic assays of a series of dilutions at 448 nm. SDS-PAGE analysis demonstrated removal of β-zein in combination with diminished sulfur content by sulfur analysis. Zein purity was assessed by Dumas nitrogen and FTIR of commercial zein before and after column filtration. Spectral differences were observed in the amide I (1,650 cm^{-1}) peak, amide II region (1,530 and 1,550 cm^{-1}) and the amide III peak at 1,240 cm^{-1}, where ratio of the dominant peaks were strongly dependent on purity of sample. Circular dichroism (CD) analyses validated the FTIR results by showing increased α-helical content for the column purified zeins. Combinations of these methodologies can be used to define zein products as a quality control measure for a commercial operation.

Keywords Corn zein · Protein purification · Column filtration · UV spectral analysis · Visible spectral analysis · FTIR · Circular dichroism · SDS-PAGE

Introduction

In 2007, Congress passed the Energy Independence and Security Act that mandates production of 36 billion gallons (1 US gallon $\approx$ 3.79 L) of biofuels by 2022 where corn ethanol is capped at 15 billion gallons/year. Corn gluten meal (wet milling) and distillers dried grains (dry grind) are co-products of the bioethanol industry. The alcohol soluble proteins from corn consist of α-zein, constituting 75–80% of the total zein, β-zein making up 10–15% of the total zein and γ-zein contributing 5–10% of the total zein [1]. To make ethanol production from corn economically feasible without government subsidy, it is imperative to give value to the co-products of that industry. The current focus on bioethanol production from corn has created much interest in zein as a high value co-product due to its excellent film forming and coating properties [2], its adhesive properties [3], and its ability to be melt-processed [4]. However, a major deterrent for its use in certain food, pharmaceutical, cosmetic and medical applications is its yellow color and off-odor [5, 6].

Activated carbons not only decolorize and deodorize zein, but also adsorb considerable amounts of protein thereby lowering overall yield of a decolorized/deodorized zein product [5, 7]. The zein purification process used in this investigation [8] was based on using a combination of molecular sieve with an activated carbon [7, 8] that yields a

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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white, odorless, ultra-pure zein to fulfill the requirements for certain food, pharmaceutical, cosmetic and medical applications. An additional benefit of this column filtration method is that zein products with different degrees of color removal and protein purity can be generated by recycling the column eluents.

Our objective is to design a combination of spectroscopic methodologies with UV and visible spectroscopic methodologies to assess color and odor removal, along with nitrogen analysis, SDS-PAGE analysis, FTIR and circular dichroism (CD) to determine protein purity. These methods can be readily adapted for use as a diagnostic tool in a scaled-up commercial operation.

Experimental Procedures

Materials

Freeman zein, designated FZ, lots F40008064C, F40009051C1, F40009101C13, were purchased from Flo-Chemical Corp., Ashburnham, MA. Japanese white zein, labelled JWZ, lot 040315-1, was obtained from Showa Sangyo, Tokyo, Japan. The percent protein (dry basis) for these commercial zeins was as follows: FZs averaged (88.5%) and JWZ (99.3%) based on % Dumas N × 6.25. Proximate analyses were performed by Agricultural Experiment Station Chemical Laboratories, University of Missouri-Columbia, Columbia, MO. Molecular sieve Zeolite 5A was purchased from Sigma-Aldrich Corp., St. Louis, MO, as 8–12 mesh beads; Norit GAC 830+, acid-washed coal, Darco 12 × 20, lignite coal, and Norit Rox 0.8, extruded plant materials, were obtained from Norit America Inc., Marshall, TX.

Zein Purification

FZs from each of the three lots and JWZ, one lot run in triplicate, were each individually purified using the column filtration (CF) method described by Sessa [8]. The purified samples are designated FZ-CF1 and JWZ-CF1, where the number 1 following CF indicates one cycle through the column series. Higher numbers after CF represents the number of cycles the eluents were passed through the combined columns. The recorded data, with their respective standard deviations, represents the means, where data for FZs represent replicates from the three different lots, while data for JWZ represent the means from triplicate analyses from the one lot. Those columns, one with dimensions 120 cm × 28 mm (i.d.), the second with dimensions 60 cm × 28 mm (i.d.), were hooked in tandem. The first column was packed with 70% ethanol washed and equilibrated Zeolite 5A, whereas the second, smaller column was packed with either 70% ethanol washed and equilibrated Norit GAC 830+, Darco 12 × 20 or Norit Rox 0.8 such that the ratio of the zeolite to activated carbon was 3:1 (w/w). Samples of zein were prepared at 8% solids in 70% ethanol (w/v) to make 300 mL. The column eluate was controlled by a peristaltic pump set at 20–30 mL/h. Zein sample eluents were either recirculated through the two columns for a total of 24 cycles, designated CF24, and collected as one batch or for CF1, 10 mL eluent samples were collected and pooled. All test tube eluents were pooled when eluents gave a 280-nm reading of 1.0 or higher. Purified zein samples were precipitated by dialysis through Snake Skin Pleated Dialysis Tubing (Thermo Scientific, Waltham, MA) with a 10,000 MW cut-off against de-ionized water for 2 days. Dialyzed samples were then freeze-dried.

Spectral Analyses

Ultraviolet and visible spectroscopies of zein samples, each dissolved in 80% ethanol, before and after column filtrations, were performed over the wavelength region 250–400 nm. A ratio of absorbances at 280 and 325 nm was used to assess the removal of diferuloylputrescine from purified zein product [6].

Zein yellow color, due to xanthophylls, was assessed by dissolving 1 g of sample in 10 mL glacial acetic acid. A series of six dilutions from 1:2 (v/v) to 1:20 (v/v) were prepared. Readings were taken of each dilution at wavelength 448 nm against a glacial acetic acid blank. Results were plotted as natural log of absorbance versus natural log of the dilution factor. FTIR spectra were collected using a single bounce Durascope from Sens IR in a Thermo Nicolet Avatar 370 Fourier Transfer Infra Red spectrometer to obtain a midrange ATR spectrum of zein samples. Using a diamond accessory, commercial zein samples and lyophilized purified zein powder were subjected to 64 scans per sample and 1.9 cm^{-1} resolution from 4,000 to 400 cm^{-1}. The infrared spectra were analyzed with the Omnic software package where peak maxima were determined manually after examination of absorbance values. The peak ratios within a given FTIR spectrum, were calculated by dividing the absorbance value at one wavelength by the absorbance value at a second wavelength. Baseline corrections were performed so that calculated ratios between separate spectra could be compared.

For circular dichroism (CD), zein samples were completely dissolved in 90% ethanol at 0.5 mg/mL. Samples were passed through a 5-μm Whatman nylon syringe filter. Zein far-UV CD spectra were collected on a circular dichroism spectrometer (Aviv Model 215, Aviv Biomedical, Lakewood, NJ). Samples were tested in quartz cells with a path length of 0.1 cm. Spectra were obtained at 260–200 nm with a 1-nm bandwidth and temperature

controlled at 25 ± 1 °C. Each zein spectrum was collected three times and averaged for the final result, while the solvent spectrum was collected ten times and averaged. The CD data is expressed in terms of mean residue ellipticity, $[\theta]_\lambda$, where the units are mdeg cm^2/dmol. It is calculated as $[\theta]_\lambda = \text{MRW}\ \theta_\lambda/10\ dc$, where mean residue mass (MRW) is 110 g/mol; θ_λ is measured at the specific wavelength in m deg; c is the concentration of protein in grams (dry basis)/cm^3 and d is the path length in cm [9].

Gel Electrophoresis

SDS-PAGE was performed with a Novex NuPAGE 4–12% gradient bis–tris gel (Invitrogen, Carlsbad, CA). Samples of 0.15 mg, dissolved in 0.055 M Tris buffer containing 2.0% SDS, 7.0% glycerol, 4.3% mercaptoethanol and 5 M urea were each loaded into the gel wells. Broad range molecular weight standards were obtained from Bio-Rad Laboratories (Hercules, CA). The gel was stained with Coomassie blue.

Results and Discussion

Purification of Zein by Column Filtration

The column filtration pattern, shown in Fig. 1, for elution of FZ through Zeolite 5A/NoritRox 0.8 (3:1, by wt.) with one passage yielded pooled zein products (FZ-CF1) with a UV spectral ratio 280:325 nm of 7.50, where the initial starting materials had a ratio of 2.48 (±0.02). A spectrophotometric method based on UV absorbances at 280 nm for protein and 325 nm for diferuloylputrescine, a contributor to off-odor of zein, was used to monitor its removal by column filtration [6]. Recycling the column eluents

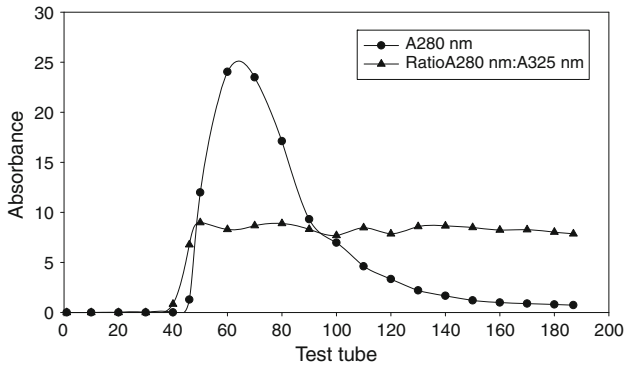

Fig. 1 Column filtration elution pattern for Freeman zein (FZ) through Zeolite 5A and NoritRox 0.8 (3:1, by wt.). Columns were eluted with 70% aqueous ethanol. Test tube eluates (10 mL each) were monitored at wavelength 280 nm along with a ratio of *A* @ 280:*A* @ 325 nm. The ratio indicates removal of the odor-causing contaminant in FZ

proved to be an effective process for more complete removal of the off-odor components. Initially, FZ ethanol solutions possessed a 280:325 nm mean ratio of 2.48 (±0.02), where one cycle, that represents the means of three replicates, yielded a ratio of 7.59 (±1.71) with data shown in Table 1. A higher ratio is indicative of removal of the off-odor contaminant. When eluents were recycled 24 times through columns packed with Zeolite 5A and activated carbon columns packed with either Norit GAC 830+ or Darco 12 × 20 (each 3:1, by wt.), the UV ratios for the means of those eluents were 15.49 (±2.06). When JWZ, run in triplicate and passed once through the two columns, the ratio changed from 5.03 (±0.12) to 10.20 (±1.32). Gelation of the Zeolite column eventually occurred when eluents were recycled due to a build- up of a residual layer on the Zeolite beads. No difference was observed in the proximate analysis (data not shown).

Based on proximate analysis of zeins before and after column filtration (Table 1), that process [8] generated high purity zein products. The column filtration process significantly reduced crude fat and ash contents of the FZ samples, based on their respective standard deviations. The sulfur content in FZ-CF24 was slightly reduced. Even with one passage of the eluents through the columns, a high purity zein product can be achieved. Mean recoveries of purified zein products with no recycling were 55.6 (±10.4) %, based on 14 replicates that included data from prior runs. With recycling, mean recoveries were 51.0 (±7.9)%, based on 9 replicates. The column filtration process yielded a moderate reduction in sulfur content for JWZ products, based on their respective standard deviations. Further investigation is needed to prove the significance of this reduction. Sulfur content was measured by inductive coupled plasma-optical emission spectroscopy (performed by Agricultural Experiment Station Chemical Laboratories, University of Missouri–Columbia, Columbia, MO).

The diminished sulfur content may be attributed to the removal of β-zein during the purification scheme. Fig. 2 shows the SDS-PAGE of one of the three FZ replicates and one of the JWZ triplicates before and after purification by column filtration. The two major bands are those of the 19 and 22 kDa α-zeins. Below those bands, is a weak protein band that represents the β-zein component, mainly in lane 5 for commercial JWZ. The α-zein dimers are apparent at ~40 kDa. One difference is the absence of the β-zein band in the purified samples. β-zeins are high in cysteine and therefore can easily form disulfide linkages. It is possible that the β-zein could crosslink in solution and bind to the molecular sieves in the column filtration system thereby removing them from the purified zein samples. As mentioned previously, JWZ, when recycled, caused coating of the Zeolite beads with a fluff layer that eventually generated gelation in that column. Another significant difference

Table 1 Proximate analysis (d.b.) and ratio 280:325 nm of zein before and after column filtration (CF)

%	FZ	FZ-CF1	FZ-CF24	JWZ	JWZ-CF1
Crude protein[a]	88.5 (1.2)	99.7 (1.3)	100.1 (0.5)	99.3 (0.6)	101.5 (0.9)
Crude fat	1.86 (0.18)	0.00	0.00	0.07 (0.00)	0.00
Ash	2.48 (0.02)	0.11 (0.00)	0.02 (0.00)	0.18 (0.02)	0.10 (0.00)
Sulfur	0.68 (0.03)	0.61 (0.01)	0.55 (0.03)	0.82 (0.02)	0.64 (0.08)
280:325 nm[b]	2.48 (0.02)	7.59 (1.71)	15.49 (2.06)	5.03 (0.12)	10.20 (1.32)

Data represent the means (±SD) of one replicate from three different lots of Freeman zein (FZ) and one lot of Japanese white zein (JWZ) run in triplicate, where CF1 is one cycle and CF24 is 24 cycles through tandem columns

[a] Dumas N × 6.25

[b] Ratio 280:325 nm is indicative of removal of diferuloyputrescine, the major contributor of odor, where a higher number is indicative of removal

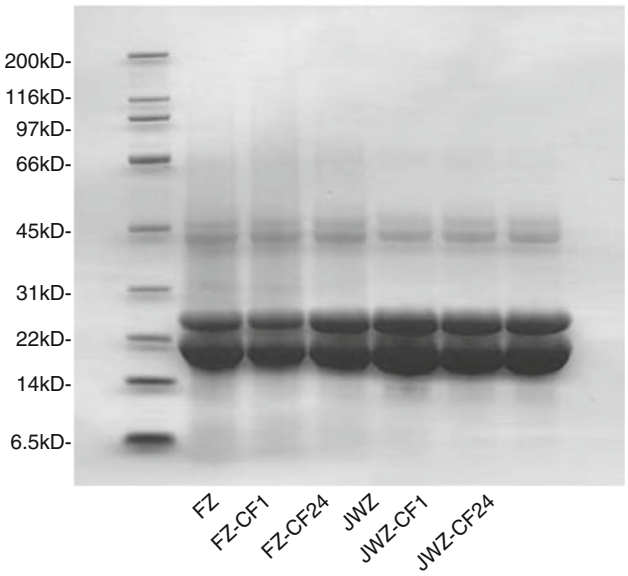

Fig. 2 SDS-PAGE of Freeman zein (*FZ*) and Japanese white zein (*JWZ*) before and after column filtration (*CF*) where the number following CF indicates the number of cycles through the Zeolite and activated carbon columns

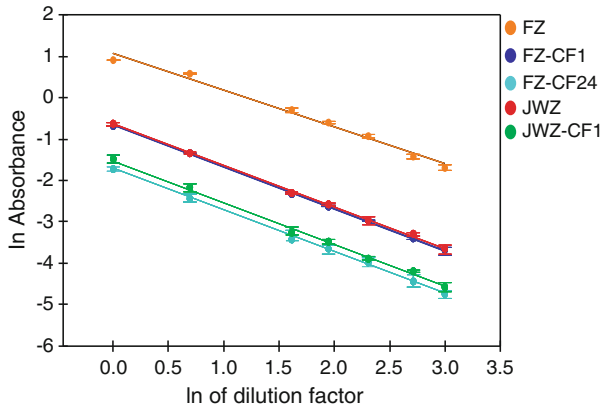

Fig. 3 Visible spectra of Freeman zein (*FZ*) and Japanese white zein (*JWZ*) at wavelength 448 nm before and after column filtration (*CF*) where the number following CF indicates the number of cycles through Zeolite and activated carbon columns. Plot is natural log of dilution factor versus natural log of absorbance at 448 nm

between the commercial and purified FZs is the reduced smearing observed between the 23 kDa α-zein and zein dimers at ~40 kDa, mainly with FZ-CF24. Commercial JWZ contains very little smearing. With JWZ-CF1, the only visible difference is the removal of the β-zein band at 14 kDa.

The yellow color of commercial zeins, particularly when dissolved, is caused by xanthophylls. A simple colorimetric analysis was devised [5], whereby a series of dilutions of zein dissolved in glacial acetic acid were assessed at wavelength 448 nm with glacial acetic acid as the blank. Plots of three FZ replicates and triplicate runs of JWZ, with their respective natural log of absorbances versus natural log of dilutions, are shown in Fig. 3. As can be observed in that figure, FZ possessed the most yellow color, JWZ had moderate amounts of color. FZ-CF1 possessed similar amount of yellow color as the original unprocessed commercial JWZ. JWZ-CF1 had diminished yellow color compared with commercial JWZ. FZ-CF 24 had the least yellow color. All the trend lines of the data points for each zein sample are parallel and are reproducible based on their respective error bars. This method can be used as a comparative, qualitative diagnostic tool to demonstrate degree of yellow color removal.

FTIR Spectral Analysis

FTIR spectroscopy is a useful tool to study the secondary structure of proteins. The secondary structure of zein is predominantly α-helical, with additional structure provided by β-sheet, turn and random coil segments [9–11]. A typical FTIR spectrum of zein (Fig. 4a) possesses an amide I peak at ~1,650 cm^{-1}, amide II peaks at ~1,530 and ~1,515 cm^{-1}, amide III peak at ~1,240 cm^{-1}, and a C–N stretch peak at ~1,445 cm^{-1}. Since the protein side chains have little effect on the signal, the amide I peak is the most

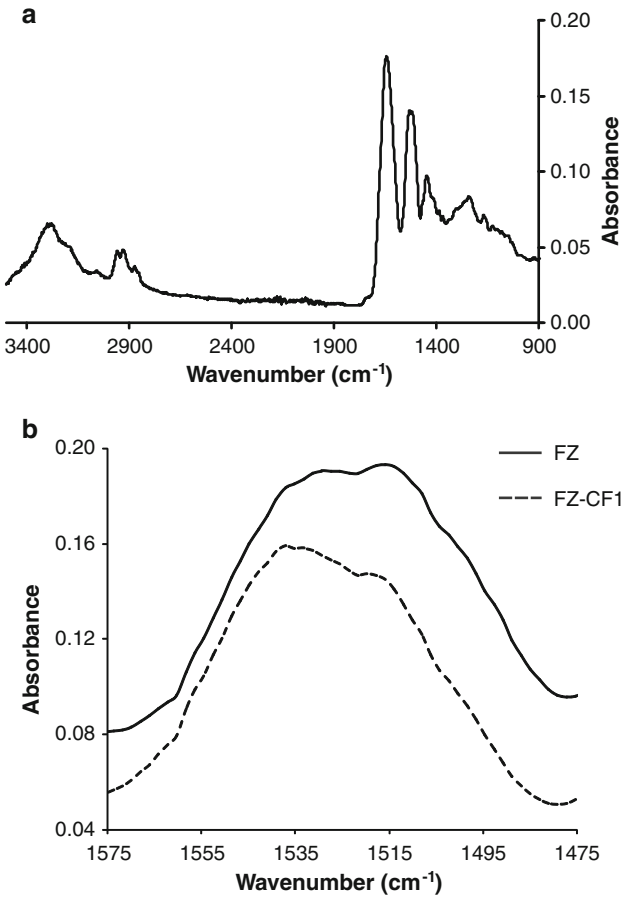

Fig. 4 FTIR of Freeman zein (*FZ*) (**a**); FTIR of FZ amide II peaks before and after column filtration (*CF*) where the number following CF indicates the number of cycles through Zeolite and activated carbon columns (**b**)

commonly used vibration for studying secondary structure [13]. The amide I peak at 1,650 cm^{-1} is the most prominent peak in the zein spectrum and can be attributed to the α-helical component of the secondary structure [14]. A shoulder at 1,630–1,640 cm^{-1} is due to β-sheet secondary structure in the zein [15]. Table 2 shows the amide I peak position for FZs and JWZs before and after column filtration. The standard deviations for the FZ peaks in Table 2 were the means from the three different lots used in this investigation. The amide I peak for FZ-CF-1 shifts from 1,643 to 1,645 cm^{-1}, FZ-CF24 from 1,643 to 1,649 cm^{-1} and JWZ-CF1 gave no shift on column filtration. The average position of the α-helix peak in pure proteins is 1,654 cm^{-1} [16]. Commercial zein samples can contain a mixture of several different α-zeins along with β-zeins. β-zein has significantly less helical secondary structure (and more β-sheet) than α-zein [11], so the peak at ~1,650 cm^{-1} in commercial zein samples shifted lower because of the β-sheet FTIR signal centered at ~1,630 cm^{-1} [15]. JWZ gave no shift in the amide I peak upon purification by column filtration because that commercial sample was originally 99.3% pure as purchased.

The position of the amide I peak in both FZ-CF1 and FZ-CF24 shifted to a higher wave number after purification (Table 2). This shift can be attributed to the removal of the contaminating proteins from that FZ product. From data in Table 1, proximate analysis of FZ-CF1 protein increased to 99.7% on column filtration, FZ-CF24 protein increased to 100.1%, while JWZ showed no significant increase in purity. With the exception of JWZ and JWZ-CF1, the FTIR data shows that the removal of contaminating proteins during purification by column filtration will yield a zein product with higher α-helical content than the original, commercially produced zein.

The amide II peaks at ~1,530 and ~1,515 cm^{-1} result from interaction between the N–H bending and C–N stretching of the C–N–H group [17]. For the FZ-CF1 and FZ-CF24 zeins, the 1,530 cm^{-1} peak shifted to a higher wave number while JWZ-CF1, for some unexplainable reason, shifted downward from 1,534 to 1,531 cm^{-1} (Table 2). The peak at ~1,515 cm^{-1} showed slight increases upon purification of both FZ and JWZ proteins. The bands in the amide III region, which are predominantly due to the in-phase combination of N–H in-plane bending and C–N vibrations, are sensitive to changes in secondary structure [18, 19]. The dominant peak in this region of a zein FTIR spectrum is located between 1,238 and 1,244 cm^{-1}, depending on the specific zein (Table 2). This peak results from a combination of random coil and β-sheet structures [20]. For the FZs, this peak occurs at 1,238 cm^{-1}

Table 2 FTIR of peak shifts for zeins before and after column filtration (CF)

FTIR peak	FZ	FZ-CF1	FZ-CF24	JWZ	JWZ-CF1
Amide I	1643 (2)	1645 (2)	1649 (1)	1649 (1)	1650 (1)
Amide II, ~1,530 cm^{-1}	1529 (2)	1533 (1)	1535 (2)	1534 (1)	1531 (2)
Amide II, ~1,515 cm^{-1}	1516 (1)	1520 (2)	1518 (1)	1518 (1)	1519 (2)
C–N Stretch, ~1,445 cm^{-1}	1446 (2)	1448 (1)	1448 (1)	1448 (1)	1449 (1)
Amide III	1239 (1)	1242 (1)	1238 (2)	1244 (1)	1242 (2)

Data represent the means (±SD) of one replicate from three different lots of Freeman zein (FZ) and one lot of Japanese white zein (JWZ) run in triplicate, where CF1 is one cycle and CF24 is 24 cycles through tandem columns

Table 3 Comparison of ratios of FTIR peaks for zein before and after column filtration (CF)

FTIR peak/ratio	FZ	FZ-CF1	FZ-CF24	JWZ	JWZ-CF1
Amide I/amide II	1.24 (0.02)	1.45 (0.02)	1.48 (0.01)	1.47 (0.02)	1.49 (0.01)
Amide I/1,445 cm^{-1}	1.75 (0.03)	2.63 (0.04)	2.76 (0.02)	2.77 (0.01)	2.78 (0.01)
Amide II/1,445 cm^{-1}	1.41 (0.03)	1.82 (0.03)	1.86 (0.01)	1.88 (0.02)	1.87 (0.01)
Amide I/Amide III	2.02 (0.05)	3.43 (0.06)	3.61 (0.03)	3.59 (0.03)	3.64 (0.02)
Amide II/Amide III	1.62 (0.04)	2.37 (0.05)	2.43 (0.01)	2.43 (0.01)	2.43 (0.01)
1,445cm^{-1}/Amide III	1.15 (0.02)	1.30 (0.02)	1.31 (0.01)	1.29 (0.02)	1.31 (0.01)
1,530 cm^{-1}/1,515 cm^{-1}	1.02 (0.06)	1.07 (0.02)	1.06 (0.02)	1.08 (0.02)	1.06 (0.01)

Data represent the means (±SD) of one replicate from three different lots of Freeman zein (FZ) and one lot of Japanese white zein (JWZ) run in triplicate, where CF1 is one cycle and CF24 is 24 cycles through tandem columns

before purification. This peak shifted to 1,242 cm^{-1} for FZ-CF24. With JWZ and JWZ-CF1, the peak at 1,244 cm^{-1} showed no difference upon purification. The intensity of the amide III bands decreased relative to the amide I peak upon purification. This phenomenon suggests that some random coil structure is no longer present due to the removal of denatured contaminant proteins during purification.

Another prominent peak in the zein FTIR spectra is the peak at 1,445 cm^{-1}. This peak can be attributed to the amide C–N stretching mode [17]. As observed with the other FZ FTIR peaks, the peak shifts to a higher wave number in the purified samples (Table 2). After purification, FZs shifted to 1,448 cm^{-1}. The peak for JWZ increased to 1,448 cm^{-1} for the JWZ product after column filtration. With the exception of JWZ, the peak attributed to amide C–N stretching shifted two wave numbers higher.

Shifting peak positions are not the only notable change in the zein FTIR spectra after purification. The ratios of the various peaks are also altered. Table 3 shows the ratios of FTIR peaks for FZ and JWZ. Ratios were calculated by dividing the absorbance for one peak in a spectrum by the absorbance of another peak within the same spectrum. When calculating the ratio of the amide II peak to another peak, the absorbance at ~1,530 cm^{-1} was used as the amide II value. Within the standard deviations the ratios for JWZ and JWZ-CF1 were identical to each other. For FZ-CF1 and FZ-CF24, FTIR peak ratios tended to be higher for all except for the last one at 1,530/1,515 cm^{-1}. These differences may be attributed to the removal of contaminating β-zein from the commercial samples which should increase the α-helical content of the protein.

By visually examining the expanded scale shown in Fig. 4b, a significant difference occurred with the ratio of absorbance at 1,530 cm^{-1} to that at 1,515 cm^{-1}. For FZ (data not shown), the ratio changes from 0.94 to 1.06 upon purification. While the absolute value of these changes are not nearly as great as those noted in Table 3, this difference is most noticeable when visually analyzing the FTIR spectra. The difference between a commercial and purified sample could be quickly determined by looking at the profile of the two amide II peaks.

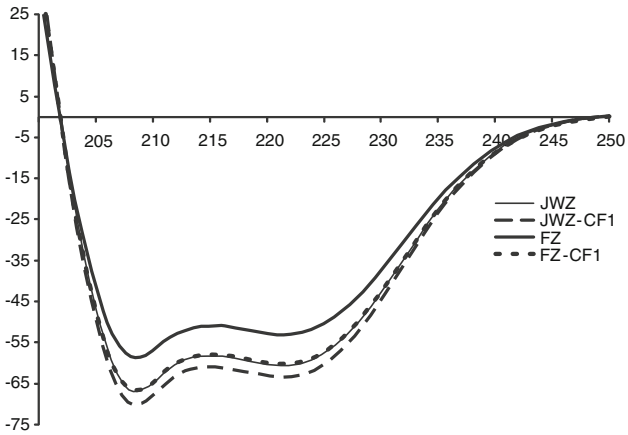

Fig. 5 Far-UV circular dichroism (CD) spectra of Freeman zein (*FZ*) and Japanese white zein (*JWZ*) before and after column filtration (*CF*) where the number following CF indicates the number of cycles through Zeolite and activated carbon columns. Samples analyzed were at 0.5 mg/mL in 90% aqueous ethanol at 25 °C

Circular Dichroism of Zeins Before and After Purification

CD is a common method for studying conformational changes in protein secondary structure. Zein has been previously studied by CD to analyze the influence of hydration [12], the effect of solvent [21] and temperature [21, 22] and for general structure prediction [10]. CD has also been used to differentiate between a mixture of α-zeins extracted from corn flour and a highly purified 19 kDa α-zein [23]. The present work used CD to identify different levels of zein purity and to confirm the FTIR data. Far-UV data was collected from 260 to 200 nm at 0.5 mg/mL in 90% ethanol. Figure 5 shows the CD spectra of FZ and JWZ before and after purification by column filtration. The two negative peaks observed at 208 and 222 nm indicate the strong presence of α-helical secondary structure. The

absolute magnitude of these peaks reflects the amount of α-helix present in the protein [24]. The purified fractions of all zein samples exhibited greater α-helical content than the commercial samples. This increase in helical content is attributed to the removal of denatured protein and β-zein during the purification process. The absolute magnitudes of the FZ-CF1 spectra at 208 and 222 nm are 6 and 8% greater, respectively, than FZ. The absolute magnitude of these two peaks in JWZ-CF1 was 5% greater than those in JWZ. While JWZ showed very little difference after purification by FTIR spectroscopy, CD is a more direct measure of α-helix content. The 5% difference in JWZ-CF1 can be attributed to the removal of the β-zein, which has significantly lower helical content than α-zein [11]. These CD results correspond well with the FTIR data showing a change in structural conformation of the purified sample compared to the commercial zein.

Conclusions

Column filtrations of commercial yellow and white zeins with a combination of zeolite and activated carbon effectively diminished odor (based on increased ratio of UV spectra at 280 vs. 325 nm) and color (based on diminished absorbance at 448 nm) and yields a highly purified zein product with diminished crude fat, crude fiber, ash and sulfur based on proximate analysis. The purified zein product by use of the column filtration process is mainly α-zeins based on SDS-PAGE analysis, FTIR amide I ratio shifts and CD analysis.

Commercial zeins are usually defined by proximate analysis, namely protein, crude oil, and moisture. Lot-to-lot and batch-to-batch variations from these commercial operations have a propensity to be problematic for users of zein in certain applications. The combination of column filtration to purify commercial zein and analytical methodologies based on visible and UV analysis, SDS-PAGE analysis and FTIR analysis provide simple, inexpensive methods for defining a consistent product for commercial operation.

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