Protein Oxidation in Plant Protein-Based Fibrous Products: Effects of Encapsulated Iron and Process Conditions

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ABSTRACT: Plant protein-based fibrous structures have recently attracted attention because of their potential as meat replacers. It is, however, unclear how the process conditions and fortification with micronutrients may affect the chemical stability of such products. Therefore, we aimed to investigate the effects of process conditions and the incorporation of iron (free and encapsulated) on protein oxidation in a soy protein-based fibrous product. First, the physicochemical stability of iron-loaded pea protein particles, used as encapsulation systems, was investigated when exposed to 100 or 140 °C. Second, protein oxidation was measured in the iron-fortified soy protein-based fibrous structures made at 100 or 140 °C. Exposure to high temperatures increased the carbonyl content in pea protein particles. The incorporation of iron (free or encapsulated) did not affect carbonyl content in the fibrous product, but the process conditions for making such products induced the formation of carboxyls to a fairly high extent.

KEYWORDS: protein oxidation, plant protein, fibrous structures, iron, encapsulation

1. INTRODUCTION

Nowadays, there is a global trend to reduce meat consumption and move towards a more plant protein-based diet for environmental, health, and animal-welfare reasons. To help the population move towards such a transition, research has focused on developing products with fibrous structures similar to those in meat. At Wageningen University & Research, a range of shearing devices have been developed to make fibrous structures using plant proteins by applying a simple shear flow and high temperatures (100–140 °C). Another technique widely applied to make meat analogues is extrusion. Here, materials are processed using a similar temperature range, giving this study potentially broader relevance. So far, most of this research has been done using soy protein ingredients. Although the structure formation in such fibrous products has already been well described, it is still unclear how the process conditions may affect the chemical stability and thus the sensory and nutritional quality of these products.

Oxidation is often associated with deterioration in food products. Lipid oxidation in foods has been largely studied over the past decades, whereas protein oxidation has been considered to a much lower extent and mainly in meat. Protein oxidation results in a number of chemical modifications affecting both amino acid side chains and the peptide backbone. Such changes include thiol oxidation, aromatic hydroxylation, and formation of carbonyl groups. Moreover, oxidation can induce additional cross-links, backbone fragmentation, and conformational changes in the secondary and tertiary structure of the protein. The formation of cross-links results in aggregation and reduces solubility.

Despite the relevancy of such chemical modifications for food quality, protein oxidation in plant protein-based products is a rather new research topic that is now highly relevant given the increasing interest in the development and application of such products.

In fact, it can be hypothesized that different factors may induce protein oxidation in plant protein-based fibrous structures: first, the process conditions, which often involves a thermomechanical process; and second, the incorporation of nutritionally relevant micronutrients, such as iron, which have a pro-oxidant activity. For instance, ferrous sulfate has commonly been used for food fortification because of its high bioavailability and low cost, but its presence in food products can lead to lipid and protein oxidation. Besides, ferrous sulfate has an unpleasant metallic taste. To mitigate those undesirable effects, iron encapsulation could be a solution. Previous research has shown that encapsulating ferrous sulfate in pea protein spray-dried particles is a promising method, considering the iron bioaccessibility and the potential to mask the metallic taste. Moreover, the incorporation of such ferrous sulfate pea protein spray-dried particles (here referred to as iron-loaded pea protein particles) in cooked black beans and in a banana candy showed good consumer acceptability. It should be noted, though, that the iron-loaded pea protein particles were incorporated after the food was processed, which means that the iron-loaded pea protein particles were not exposed to further food process conditions. Therefore, it would be relevant to assess if additional processing can lead to undesirable chemical
reactions, such as protein oxidation, when such particles are exposed to process conditions such as high temperatures, which can be necessary for making a food product.

In this work, we investigated protein oxidation in a plant-protein-based fibrous product. We aimed to assess the effect of the process conditions used to make the fibrous product, and we quantified the effect of incorporation of iron-loaded pea protein particles on protein oxidation.

2. MATERIAL AND METHODS

2.1. Materials. Pea protein concentrate (PPC, 81% protein, N × 6.25) was obtained from Nutralys (S85F, Roquette). Soy protein concentrate (SPC, 63.20% protein, N × 5.71; Alpha 6 ZP) was obtained from Solae. Food-grade ferrous sulfate heptahydrate, 2-propanol, sodium chloride (NaCl, S9625), diaminoethane tetraacetic acid (EDTA), tris(hydroxymethyl) aminomethane (Tris), potassium chloride (KCl), 2,4-dinitrophenylhydrazine (DNPH), trichloroacetic acid (TCA), sodium dodecyl sulfate (SDS), and guanidine hydrochloride (CH₅N₃HCl) were obtained from Sigma-Aldrich. Hydrochloric acid (HCl, 4 N) was purchased from AVS Titrinorm (VWR Chemicals), and solvents such as ethanol (ACS 99%) and ethyl acetate (ACS 99%) were purchased from Emsure (Merck Millipore). A Bicinchoninic Acid (BCA) Protein Assay kit was obtained from Thermo Scientific (Pierce). Demineralized water was used to make the feed solution and to prepare the soy protein matrix. Ultrapure water obtained from a Millipore Milli-Q system was used for all the other experiments.

2.2. Methods. 2.2.1. Production of Iron-Loaded Pea Protein Particles. Iron-loaded pea protein particles were produced according to Bittencourt et al. with a few modifications. A feed solution (6.34% solid content) with PPC as the wall material and ferrous sulfate heptahydrate as a core material was prepared. First, 10% (w/v) PPC was slowly added to distilled water, and the pH was adjusted to 7.0 with 1 M HCl. Then, the feed solution was heated to 80 °C for 30 min and left overnight in a shaking water bath. The feed solution was then homogenized with an IKA T18 Ultra-Turrax (Thermo Fisher Scientific, Inc.) at 8000 rpm for 5 min and left overnight in a shaking water bath at 40 °C and 100 rpm. The next day, the feed solution was diluted to 9% (w/v) and homogenized at 9000 rpm for 1.5 min. The pH was adjusted to 6.5 with 1 M HCl. A 1 M ferrous sulfate solution was prepared with demineralized water and was slowly stirred in the feed solution to obtain a final concentration of 22.5 mM. Subsequently, the feed solution was homogenized at 9000 rpm for 2 min. A final dilution was made to 7% (w/v) and stirred. The feed solution was then spray-dried using a Büchi B-290 Mini Spray Dryer (Büchi Labortechnik AG) with an inlet temperature of 180 °C and an outlet temperature of around 100 °C. The aspirator was set at 90%, the pump was set at 20%, and the feed rate was 6 mL/min. The samples were stored in plastic vials in the dark and frozen prior to further analysis. Spray-dried particles without iron were prepared following the same procedures as described above, except that the ferrous sulfate solution was replaced with demineralized water. The yield defined as the ratio of solids in particles and solids in the feed solution was 54.5 ± 2.4% for samples prepared with iron and 62.3 ± 0.9% without iron.

2.2.2. Exposure of Iron-Loaded Pea Protein Particles to High Temperatures. Iron-loaded pea protein particles were exposed to different temperatures in a HeraTherm thermostat oven (Thermo Fisher Scientific, Inc.). Two grams of sample was evenly spread as a thin layer in an aluminum tin, after which the tin was put in the oven at 100 or 140 °C for 30 min. These temperatures were chosen according to the temperatures applied to make plant protein-based fibrous products (Section 2.2.4). Pea protein particles samples without exposure to temperature were considered as controls. Afterward, the samples were analyzed for physicochemical characterization.

2.2.3. Physical Characterization of Iron-Loaded Pea Protein Particles. The morphology of the iron-loaded pea protein particles was analyzed by scanning electron microscopy (SEM). Samples were deposited on carbon conductive tape on aluminum SEM stubs and analyzed using a Phenom G2 Pure Semsro microscope (Thermo Fisher Scientific, Inc.). SEM pictures were analyzed with Imagej software (version 1.40g, National Institutes of Health) to calculate the content of broken particles.

The particle-size distribution (PSD) was determined by a laser-diffraction particle-size analyzer (Mastersizer 2000, Malvern Instruments Ltd.) using 2-propanol as the continuous phase, a particle refractive index of 1.45, a particle-absorption index of 0.001, and a dispersant refractive index of 1.39. Samples were gently agitated to ensure homogeneity before measurements.

Particles were analyzed for morphology and PSD just after spray-drying and after being exposed to temperature.

Iron retention in the particles was determined after spray-drying and after being exposed to temperature using inductively coupled plasma–optical-emission spectroscopy (ICP-OES, iCAP 6500 duo, Thermo Fisher Scientific, Inc.). First, 1% (w/v) samples were suspended in 10 mL of aqua regia (7.5 mL of 37% HCL and 2.5 mL

of 65% HNO₃), and the organic phase was digested in a microwave digestion system (Milestone Srl). After microwave digestion, the samples were diluted 200X. A calibration curve was prepared with an iron stock solution in aqua regia within a range that went up to 150 mg/L. The samples were measured in the axial direction, and the wavelength of 240.88 nm was used for iron quantification. Iron retention was calculated with the following equation:

$$
\text{iron retention} (\%) = \frac{\text{iron content in pea protein particles (g per 100 g of solids)}}{\text{iron content in feed solution (g per 100 g of solids)}} \times 100\%
$$

(1)

2.2.4. Preparation of a Plant Protein-Based Fibrous Product. The fibrous product was prepared using a high temperature shear cell (HTSC, Wageningen University & Research) with SPC as the protein source. Samples were prepared with iron-loaded pea protein particles, with ferrous sulfate (considered free iron) solubilized in the water used to make the fibrous product, or without iron. The fibrous product was composed of 45 wt % SPC, 54 wt % demi water, 1% NaCl, and 3.5 mg of elemental iron per 100 g of product (except for the control with no iron). The iron content was based on 25% of the Nutrient Reference Value for female adults between 19 and 50 years, which is a high risk group for iron deficiency.14 Per run, 90 g of total weight were used. The model system was prepared according to Grabowska et al.15 at 100 or 140 °C and 30 rpm for 15 min; this was followed by a 5 min cooling step at 25 °C. After preparation, samples were prepared by cutting small pieces of 8 mm out of the product. The pieces were mixed to obtain a homogeneous distribution. Then, 9 g of samples were placed in a sous-vide bag and sealed using a V.300 Premium Line vacuum machine (Lava). Bags were placed in the oven at 35 °C for accelerated storage-stability analysis and analyzed after 1 and 7 days.

2.2.5. Quantification of Protein-Bound Carbonyls: DNPH Method. We first measured the carbonyl contents in pea protein particles prepared with and without iron after exposure to 100 and 140 °C. To investigate the effects of processing and iron incorporation, we quantified the carbonyl contents in fibrous products prepared with iron-loaded pea protein particles, with ferrous sulfate solution, or without iron. As a reference, the carbonyl content was measured in a 6 wt % protein suspension of PPC and SPC.

The carbonyl content was measured using the method described by Soglia et al.,16 with modifications to account for the low protein solubility of the samples (pea protein particles and fibrous products). Therefore, the samples were sequentially dispersed in buffers, which resulted in three different protein fractions: in fraction 1, the most-soluble proteins were dissolved; in fraction 2, the salt-soluble proteins were dissolved; and in fraction 3, some less-soluble proteins were dissolved (Figure 1).17

The following procedure was applied to the pea protein particle samples. First, 2 g of sample was dispersed in 20 mL of buffer 1 (100 mM Tris and 5 mM EDTA, pH 7.5) and left stirring overnight at 300 rpm at 4 °C. The samples were then centrifuged at 18 000g at 2 °C for 20 min (Brova Lynx 4000, Thermo Fisher Scientific, Inc.). The supernatant was collected as protein fraction 1. Next, buffer 2 (100 mM Tris, 50 mM NaCl, and 5 mM EDTA; pH 7.5) was added to the pellet (1:3, w/v) and vortexed for 30 s at 2500 rpm. The homogenate was centrifuged at 18 000g at 2 °C for 20 min. The supernatant was collected as protein fraction 2. Further, 0.15 M KCL solution was added to the pellet (1:3, w/v) and homogenized as described before, forming a suspension that constituted protein fraction 3.

For the fibrous product, 9 g (9.5%, w/v, protein) of sample was homogenized with buffer 1 (1:3, w/v) using an Ultra Turrax at 13 600 rpm for 1 min in an ice bath. Samples meant to determine the effect of 7 days of storage were taken 1 day before measurement and soaked in buffer 1 at 4 °C overnight. The dispersion was centrifuged at 18 000g at 2 °C for 20 min. The supernatant collected as protein fraction 1. Next, buffer 2 (1:3, w/v) was added to the pellet and homogenized at 13 600 rpm for 30 s in an ice bath. Afterward, the sample was centrifuged as described before. The supernatant was collected as protein fraction 2. Further, 0.15 M KCL solution was added to the pellet (1:3, w/v) and homogenized as described before, forming a suspension that constituted protein fraction 3.

We attempted to accordingly analyze those three protein fractions from pea protein particles and fibrous product suspensions. However, the soluble-protein concentration was less than 1 g/L in protein fraction 3 for pea protein particles and in protein fraction 2 for fibrous products. For this reason, for the quantification of protein-bound carbonyls, only protein fractions 1 and 2 were considered for pea protein particles, and only protein fractions 1 and 3 were considered for fibrous products. The soluble-protein concentration was determined in each fraction by the BCA method. Protein fractions were filtered with a 0.22 μm syringe filter (Millipex PES, Merck Millipore). Then, 1 mL of working reagent (50:1 reagent A/B) was added to 30 μL samples, which were incubated in a Thermomix at 37 °C and 300 rpm for 30 min. After incubation, the samples were cooled down and kept at 4 °C for 5 min and at room temperature for 10 min. Calibration curves (0 to 1 g/L) with bovine serum albumin were prepared in the buffers used to separate the protein fractions and were prepared under the same conditions as the samples. Then, the absorbance was measured at 562 nm with a UV−visible spectrophotometer (HACH Lange DR 3900) in polystyrene cuvettes. To calculate the percentage of soluble-protein concentration in each fraction, we assumed that all proteins in the fibrous product and pea protein particles were solubilized (Figure 1).18

The carbonyl content was determined with the DNPH method adapted from Soglia et al.,16 Vuorela et al.,19 and Levine et al.20 First, 0.8 mL of protein fraction 1 or 1 mL of protein fraction 2 from pea protein particle samples were precipitated with 40% TCA (1:1, v/v). For the fibrous product, 1 mL of protein fractions 1 and 3 were precipitated with 20% TCA (1:1, v/v). Then, the samples were centrifuged at 15 000g for 5 min. The supernatant was removed with a Pasteur pipet, and 400 μL of 5% (w/v) SDS was added to the pellet. The samples were heated in a thermomixer (Eppendorf Thermomixer C and Grant QBT4) at 99 °C for 10 min and then put in an ultrasonic bath (Elmasonic P and Branson 5210, Elm) at 40 °C for 30 min at 80 kHz with 90% sweep. Afterward, 0.8 mL of 0.3% (w/v) DNPH in 3 M HCl was added to the samples, and 0.8 mL of 3 M HCl was added to the blanks. Then, the samples were incubated at room temperature in the dark for 60 min and vortexed at 2500 rpm for 5 s every 10 min. After incubation, 400 μL of 40% (w/v) TCA was added to precipitate the proteins, and the samples were centrifuged at 15 000g for 5 min. The supernatant was removed, and the samples were washed three times with 1 mL of ethanol/ethyl acetate (1:1) and centrifuged at 15 000g for 5 min every time. After the washing steps, the pellet was dissolved in 1.5 mL of 6 M guanidine hydrochloride and vortexed at 2500 rpm for 5 s. Samples were incubated in a thermostainer at 37 °C overnight.

Then, the samples were centrifuged at 5000g for 10 min, and the absorbance of the supernatant was measured at 370 nm using a UV−visible spectrophotometer and polystyrene cuvettes. Samples with absorbance outside the range of 0.12−0.55 were diluted in 6 M guanidine hydrochloride. The soluble-protein concentration in 6 M guanidine hydrochloride was determined by the BCA method, as described previously. The carbonyl content was calculated with the following equation:

$$
carbonyls (\text{mmol/kg}) = \frac{\text{ABS}_{\text{sample}} - \text{ABS}_{\text{blank}}}{\epsilon \times \text{soluble-protein concentration}} \times \text{protein concentration}
$$

where \( \text{ABS} \) is the absorbance of the sample and of the blank at 370 nm, and \( \epsilon \) is the molar absorptivity coefficient of carbonyls, set as 22 000 M⁻¹ cm⁻¹.

2.3. Statistical Analyses. Pea protein particle samples were prepared in duplicates. Samples exposed to temperatures were prepared in duplicate and used immediately. The sample was measured twice with an average of three readings from the Mastersizer to determine the particle-size distribution. Fibrous products were prepared in duplicates, and the DNPH method was done in triplicate per sample and per blank. The BCA assay was done in triplicate.
3. RESULTS AND DISCUSSION

In this section, the properties of pea protein particles obtained after spray-drying are first characterized, followed by a description of the chemical stability of the particles. Then, the effect of particle addition to the fibrous product and the effect of thermal treatment on protein oxidation are presented and discussed.

3.1. Characterization of Iron-Loaded Pea Protein Particles. The iron retention of the iron-loaded pea protein particles after spray-drying and exposure to 100 and 140 °C were 59.86 ± 0.91, 61.45 ± 0.5, and 61.71 ± 0.58%, respectively. Our iron-retention results were lower than the value of 67% described by Ferreira but higher than the value of 43.9% described by Bittencourt et al. The differences are probably due to adaptations in the spray-drying settings.

The morphology of iron-loaded pea protein particles was monitored just after particle preparation and after exposure to 100 and 140 °C for 30 min (Figure 2). Iron-loaded pea protein particles presented more invaginations, were less spherical, and had smoother surfaces compared with pea protein particles without iron. Pea protein particles exposed to 100 and 140 °C looked more broken than the control samples. The roughness and invaginations found in our pea protein particles have been described by other authors as typical for particles made with PPC as encapsulation material. The authors argued that a high protein content can produce spray-dried particles with less invagination, suggesting that the presence of carbohydrates in protein concentrates can result in more invaginations. In the present work, we can see that the presence of iron also affected the particle morphology.

Directly after spray-drying, the PSD of pea protein particles with and without iron (Figure 3A) showed 3 peaks around 1, 0.18, and 10 μm. This result shows that pea protein particles were polydisperse, independent of the presence of iron. After exposure to high temperature, larger particle sizes were measured (Figure 3B,C). Especially in case of iron-loaded pea protein particles, aggregation was observed after heating at 140 °C. Several physical and chemical mechanisms, including protein oxidation induced by iron and heat, could cause protein aggregation seen in Figure 3C. The average particles sizes, d1,2 of iron-loaded pea protein particles exposed to 140 °C (1.69 ± 0.17 μm) or 100 °C (0.78 ± 0.32 μm) were larger than that of the sample not exposed to heat (0.37 ± 0.03 μm). The span values also showed an increase in polydispersibility from 8.33 ± 0.14 (no heat) to 35.84 ± 14.38 (iron-loaded pea protein particles at 140 °C).

3.2. Chemical Characterization of Iron-Loaded Pea Protein Particles: Protein Oxidation. Carbonyl content was determined as a measure of protein oxidation to assess the chemical stability of iron-loaded pea protein particles being exposed to 140 and 100 °C for 30 min (Figure 4A,B). Here, we present the results of carbonyl contents in protein fractions 1 and 2 only, because the soluble-protein concentration was too low in fraction 3.

In both protein fractions (Figure 4A,B), the carbonyl content was higher after spray-drying than in the starting PPC suspension: for the latter, values of about 12.7 mmol of carbonyl per kilogram of soluble protein (protein fraction 1) and 9.7 mmol of carbonyl per kilogram of soluble protein (protein fraction 2) were obtained, represented as dotted lines in graphs Figure 4A,B. After exposure of the pea protein particles to high temperature (100 or 140 °C for 30 min), there was an increase in carbonyl contents for both protein fractions, without significant differences between the heat treatments (Figure 4A). The presence of iron in the pea protein particles clearly decreased protein solubility, which in fact could be due to protein aggregation as a result of protein oxidation. However, we could not see any increase in carbonyl content compared with that of the pea protein particles without iron. Considering that only a small fraction of the protein in the iron-loaded pea protein particles was soluble in guanidine hydrochloride, most probably there were more carbonyls formed than in particles without iron. For protein fraction 2, iron-loaded pea protein particles exposed to 140 °C had a significantly higher carbonyl content compared with those of the samples exposed to 100 °C and the control (Figure 4B). However, there was no effect of temperature on carbonyl content for the pea protein particles without iron.
Protein oxidation values for iron-loaded pea protein particles were only significantly different from those of the pea protein particles without iron in protein fraction 2 at 140 °C.

As described above, spray-drying temperature (180 °C) alone induced protein oxidation in the PPC used as a wall material to encapsulate the iron. Costa et al. showed that spray-drying reduced free sulphydryl contents in pea protein isolates, which could be an indication of protein oxidation. Comparable to our findings, Tang et al. described a similar increase in carbonyl content when heating soy proteins at 100 °C for 30–90 min compared with that of the no-heat treatment. However, the values found were much lower than ours: ~3 mmol of carbonyl per kilogram of soluble protein for proteins not heated and ~6 mmol of carbonyl per kilogram of soluble protein for soy proteins heated for 90 min. In our study, PPC itself was already oxidized and then exposed first to the spray-drying temperature and later to further heat treatment, which also increased protein oxidation.

These results showed that spray-drying leads to less chemically stable iron-loaded pea protein particles and that the presence of iron reduces the soluble-pea protein concentration. Some improvement might be necessary to increase the particles’ oxidative stability; the chemical status of the starting protein material also seems important because the PPC powder was already oxidized, which indicates that protein oxidation started either during the protein fractionation process or during storage of the powders.

3.3. Chemical Stability of Fibrous Products with Iron Incorporation: Protein Oxidation. Plant protein-based fibrous products prepared with a HTSC at different temperatures were used as model products to study fortification with iron either as free iron or as encapsulated iron (iron-loaded pea protein particles), as described in the previous sections. Sequential suspensions of the fibrous products were prepared in different buffers, and three protein fractions were obtained. Protein fractions 1 and 3 had more soluble proteins than fraction 2, and for this reason, carbonyl contents were only measured in fractions 1 and 3.

Figure 4 shows the carbonyl contents in protein fractions 1 and 3 of fibrous products prepared at 100 or 140 °C. The presence and form of iron (free versus encapsulated) did not seem to substantially affect carbonyl formation in the fibrous product: the only significant difference was found in protein fraction 1 with samples prepared at 140 °C. Carbonyl content increased significantly over storage time (up to 168 h) in protein fraction 1 of the samples prepared without iron (only at 100 °C) and with encapsulated iron (at both temperatures). The effect of temperature was only pronounced in protein fraction 1 for samples prepared with encapsulated iron after 24 h of storage.

Protein fraction 3 showed higher carbonyl contents compared with those of protein fraction 1 of samples prepared at both temperatures. Our results show that the process used to make the fibrous products increased carbonyl content in this fraction for both temperatures tested. Interestingly, protein oxidation did not increase with the incorporation of free or encapsulated iron into the product. This result was not expected, because studies have shown that even low iron
temperatures applied in the HTSC increased the carbonyl content of the most oxidized protein fraction, and that both of the proteins had substantially higher carbonyl content compared with that of the SPC suspension. Conversely, protein fraction 3 had a lower carbonyl content than the SPC suspension: the latter showed values about 15 mmol of carbonyl per kilogram of soluble protein for protein fraction 3. For protein fraction 1 at day 0, 5.78 mmol of carbonyl per kilogram of soluble protein for protein fraction 3. The carbonyl contents in myo-brillar proteins in meat products are often measured in only one protein fraction using a salt-treatment procedure. In this study, iron was applied without such synergistic pro-oxidant components, which could be a sign of oxidation because protein oxidation induces protein aggregates as a reason for decreased myosin solubility from chicken muscle. Even though the modifications in the DNPH method increased the detection of carbonyls, this method has some points of attention. The DNPH method measures total carbonyls in a small fraction of the total proteins, and the extent of protein oxidation in the insoluble-protein concentrate-based materials has been shown to be a relevant parameter for controlling the quality and safety of food products. In the current study, the addition of iron did not result in clear additional oxidation.

3.4. On the Interpretation of the DNPH-Method

Results. The protein-oxidation values reported above should be interpreted carefully, because of the low protein solubilities of most samples. The average soluble-protein concentration was 4.2 ± 0.01 g/L in buffer 1 (4.4%) and 2.9 ± 0.04 g/L in buffer 3 (3.1%, Figure 1) for the fibrous products tested. However, the soluble-protein concentration in 6 M guanidine hydrochloride for protein fraction 1 was reduced to half (Figure 6). Protein loss during the washing steps of the DNPH method is reported as expected, which increases the standard deviation and lowers the reproducibility. In samples with very low solubility, such as with our fibrous product, we only measured carbonyls in a small fraction of the total proteins, and the extent of protein oxidation in the insoluble-protein fraction (i.e., the majority of proteins) remained unknown. Fibrous products were already quite insoluble. That fact alone could be a sign of oxidation because protein oxidation induces protein aggregation and can decrease solubility as a result of that.

The modifications included in the DNPH method have contributed to increased carbonyl detection, especially in protein fraction 3. The improvement in DNPH was described by Soglia et al. as beneficial for increasing protein solubility and the exposure of carbonyls buried within the protein structure, which resulted in a 3-fold higher carbonyl content compared with that from the traditional DNPH method. Ooizumi and Xiong described the formation of oxidized protein aggregates as a reason for decreased myosin solubility from chicken muscle. Even though the modifications in the DNPH method increased the detection of carbonyls, this method has some points of attention. The DNPH method measures total carbonyl content, and it is not possible to determine which amino acid was oxidized. According to Estévez, most of the carbonyls are derived from the oxidation of proline, threonine, lysine, and arginine, which are higher in soybeans (mg per 100 g of food) than in beef (except proline).

Protein oxidation in fibrous products and in plant protein-concentrate-based materials has been shown to be a relevant measurement for investigating the chemical stability of these protein-rich products when submitted to process conditions...
protein oxidation compared with that in the SPC powder, atures applied to make temperature (100 or 140 °C). (A) Protein fraction 1 of samples prepared at 100 °C, (B) protein fraction 1 of samples prepared at 140 °C, (C) protein fraction 3 of samples prepared at 100 °C, and (D) protein fraction 3 of samples prepared at 140 °C. Results are expressed as means, and bars are standard deviations (n = 2 independent replicates, 3 measurements per sample).

used to make meat analogue products. In the current study we found that spray-drying increased protein oxidation in pea protein particles, with or without iron. The presence of iron decreased pea protein solubility, which may be associated with protein oxidation. Moreover, the starting PPC powder obtained from the manufacturer presented a certain level of protein oxidation. Moreover, the starting PPC powder found that spray-drying increased protein oxidation in pea protein particles, with or without iron. The presence of iron did not increase protein oxidation at either process conditions. In the fractionation process and the storage conditions. In the obtained from the manufacturer presented a certain level of protein oxidation. Moreover, the starting PPC powder fractionation process and the storage conditions. In the obtained from the manufacturer presented a certain level of protein oxidation. Moreover, the starting PPC powder used to make meat analogue products. In the current study we found that spray-drying increased protein oxidation in pea protein particles, with or without iron. The presence of iron decreased pea protein solubility, which may be associated with protein oxidation. Moreover, the starting PPC powder obtained from the manufacturer presented a certain level of protein oxidation. Moreover, the starting PPC powder

Figure 6. Soluble-protein concentrations (g/L) in 6 M guanidine hydrochloride for protein fractions obtained from the fibrous products. Samples were prepared with no iron (No Fe, white bars), free iron (Fe, dashed bars), or iron-loaded pea protein particles (Fe–pea protein particles, black bars). (A) Protein fraction 1 of samples prepared at 100 °C, (B) protein fraction 1 of samples prepared at 140 °C, (C) protein fraction 3 of samples prepared at 100 °C, and (D) protein fraction 3 of samples prepared at 140 °C. Results are expressed as means, and bars are standard deviations (n = 2 independent replicates, 3 measurements per sample).

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ABBREVIATIONS USED
ABS, absorbance; BCA, bicinchoninic acid; DNPH, 2,4-dinitrophenylhydrazine; ε, molar absorptivity; EDTA, diami-noethane tetracetic acid; HCl, hydrochloric acid; HTSC, high-temperature shear cell; ICP-OES, inductively coupled plasma–optical-emission spectroscopy; KCl, potassium chloride; NaCl, sodium chloride; PPC, pea protein concentrate; PSD, particle-size distribution; SDS, sodium dodecyl sulfate; SEM, scanning electron microscopy; SPC, soy protein concentrate; TCA, trichloroacetic acid; Tris, tris-(hydroxymethyl) aminomethane

[Image of a protein oxidation graph]
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