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ARTICLE

Composition and Molecular Weight Distribution of Carob Germ Protein Fractions

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Biochemical properties of carob germ proteins were analyzed using a combination of selective extraction, reversed-phase high-performance liquid chromatography (RP-HPLC), size exclusion chromatography (SEC) coupled with multiangle laser light scattering (SEC-MALS), and electrophoretic analysis. Using a modified Osborne extraction procedure, carob germ flour proteins were found to contain ~32% albumin and globulin and ~68% glutelin with no prolamins detected. The albumin and globulin fraction was found to contain low amounts of disulfide-bonded polymers with relatively low M_w ranging up to 5×10^6 Da. The glutelin fraction, however, was found to contain large amounts of high molecular weight disulfide-bonded polymers with M_w up to 8×10^7 Da. When extracted under nonreducing conditions and divided into soluble and insoluble proteins as typically done for wheat gluten, carob germ proteins were found to be almost entirely (~95%) in the soluble fraction with only (~5%) in the insoluble fraction. As in wheat, SEC-MALS analysis showed that the insoluble proteins had a greater M_w than the soluble proteins and ranged up to 8×10^7 Da. The lower M_w distribution of the polymeric proteins of carob germ flour may account for differences in functionality between wheat and carob germ flour.

KEYWORDS: Carob germ flour; gluten; celiac disease; gluten-free; protein; light scattering; caroubin;

INTRODUCTION

Celiac disease, an autoimmune disorder affecting the upper regions of the small intestines, is gaining increased attention worldwide. With 1−3% people afflicted with celiac disease in certain populations, this disease is considered to be the most common genetic disease of humans (*1*, *2*). The basis of the disorder is an inflammation of the intestinal villi that occurs upon the ingestion of gluten proteins from wheat, rye, barley, and possibly oats (*2*). With the ever-increasing awareness and diagnosis of this disease, gluten-free food alternatives are needed to enhance the quality of life of individuals with celiac disease. One means to address the gluten-free initiative is by identifying food ingredients with functional and quality attributes similar to those of wheat and associated proteins.

Carob, *Ceratonia siliqua*, is a leguminous shrub native to the Mediterranean region. Extracts from its seeds and pods of the shrub have been traditionally used as a food thickener and sweetener. In recent times, carob's primary use has been in the production of carob bean gum (locust bean gum), molasses, and chocolate substitutes. With large quantities of carob bean gum being produced annually, an appreciable amount of carob germ flour is coproduced as a result and marketed as a byproduct of gum production (*3*).

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Carob germ flour was first described for use in the production of wheat-free pasta and baked goods in a 1935 patent (*4*). Following this initial research, several other studies on carob germ flour and proteins have been conducted. In 1953 carob germ proteins were analyzed for use in high-protein cereal products for diabetics (*5*). Plaut et al. (*5*) also reported that the composition of carob germ proteins was 14.5% albumin, 50.0% globulins, 3.4% prolamins, and 32.1% glutelins. Rice and Ramstad (*6*) compared the amino acid composition of gluten to carob proteins washed from ground carob germ in a manner similar to washing gluten out of wheat. These authors found that there were significant differences in the amino acid composition between the two proteins, with carob germ proteins having less cysteine, glutamic acid, and phenylalanine but more of the charged amino acids, arginine, aspartic acid, and lysine. Feillet and Roulland (*7*) isolated proteins from carob germ flour as conducted by Rice and Ramstad (*6*) and designated these proteins "caroubins." These authors compared wheat gluten and caroubin using size exclusion chromatography (SEC) and SDS-PAGE. Unreduced caroubin was found to have large polymeric proteins with SEC chromatograms similar to that of wheat gluten, which led to the speculation that the large polymeric proteins of caroubin might have functional properties similar to those of wheat gluten (*7*). Rheological studies indicated that caroubin had viscoelastic properties; however, Feillet and Rolulland (*7*) pointed out that due to caroubin's low levels of cysteine, the mechanism of this

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viscoelastic behavior may be different from that of wheat gluten. Wang et al. (*8*) used Fourier transform infrared (FTIR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, scanning electron microscopy (SEM), and differential scanning calorimetry (DSC) to characterize the properties of hydrated caroubin and wheat gluten. These authors reported that hydrated caroubin was capable of forming sheets and fibrils. The caroubin was found to be more hydrophilic than gluten and, when exposed to water, exhibited fewer changes to protein structure than did gluten. Bengoechea et al. (*9*) isolated carob germ proteins using an alkali extraction followed by isoelectric point precipitation. The protein isolates were characterized using a combination of amino acid analysis, SDS-PAGE, and DSC. They reported that carob germ proteins were composed of aggregates formed both by disulfide bonds and through noncovalent interactions.

Although research has shown that carob germ flour contains large polymeric proteins (*7*), to date no research has been conducted to investigate the molecular weight distribution of carob germ proteins. In wheat, it is not only the presence of high molecular weight protein polymers but their molecular weight distribution (MWD) that is important in determining gluten functionality. Likewise, no research has been conducted to identify which classical Osborne fraction contains the polymeric proteins of carob germ flour. The only thing known about their solubility is that they are apparently not water-soluble and are soluble in neutral SDS solutions (*7*). Understanding which class of proteins the polymeric proteins of carob germ belong to and their solubility may help to explain some of the functional differences between caroubin and gluten.

As pointed out by Feillet and Roulland (*7*), carob germ flour proteins provide an opportunity to not only better understand the functionality of carob germ proteins but also to learn more about wheat gluten functionality. Thus, the goals of this project were to determine the molecular weight distribution of carob germ flour proteins using methods commonly applied to characterize wheat polymeric proteins and to determine which traditional Osborne class the large polymeric carob proteins were in.

MATERIALS AND METHODS

Carob germ flour (10% moisture, 48% protein, 21% carbohydrates, 6% fat, 7% ash) was graciously donated by Danisco Foods (Kansas City, MO). Additional chemicals were obtained from Sigma-Aldrich unless specified otherwise.

Osborne Extraction. For basic characterization of the proteins in the carob germ flour, the following Osborne fractionation scheme (*10*) was used to divide proteins into the following solubility classes: water- and salt-soluble proteins (albumins and globulins), aqueous alcohol soluble (nonreduced) proteins (prolamins), insoluble aqueous alcohol soluble (reduced) proteins (cross-linked prolamins), and alkali-soluble proteins (glutelins). Two different aqueous alcohol extractions, with and without reducing agent, have been widely used to investigate cereal protein solubility and provide information on the nature of protein cross-linking (*11*−*13*). Initially, 20 mg of carob germ flour was extracted twice with 1 mL of appropriate solvent for 15 min with continuous vortexing (at an instrument setting of 6). After each extraction, samples were centrifuged for 5 min at 9300*g* and the supernatants pooled in a 1:1 ratio. The albumin/globulin fraction was extracted with a 50 mM Tris-HCl buffer containing 100 mM KCl and 4 mM EDTA, pH 7.8 (*14*). Upon completion of the albumin/globulin extractions, the supernatants were removed and the residue was washed with 1 mL of deionized water to eliminate excess salts left by the extraction buffer. The water was discarded. Next, the soluble prolamin fraction was extracted using 1 mL of 50% *n*-propanol as described above. After this extraction step, 1 mL of 50% *n*-propanol containing 2% dithiothreitol (DTT) (w/v) was added to the remaining pellet and extracted as above to extract the insoluble (reduced) prolamins. Finally, the pellet was extracted with 12.5 mM sodium borate, pH 10.0, containing 2% SDS (w/v) and 2% DTT (w/v) to extract the glutelins. Samples extracted as described above were analyzed by microfluidics as described later. On the basis of the results from the experiments described above, in some cases the 50% *n*-propanol and 50% *n*-propanol plus DTT steps were omitted and only the albumin/globulin and glutelin fractions were extracted.

For SEC analysis, it was necessary to extract the glutelin fraction without reducing agent. Thus, glutelins were extracted using the pH 10 SDS buffer described above, but in place of reducing agent, sonication (10 W for 30 s) was used to solublize the proteins without the need for a reducing agent as is commonly done to extract polymeric wheat proteins. All extracts were divided into two aliquots, one of which was used "as is" (i.e., unreduced) for the SEC-MALS analysis, whereas the second set of aliquots was reduced by adding β-mercaptoethanol (β-ME) (to a final concentration of 2%) to aliquots of the nonreduced extractions and allowed to sit for 30 min before analysis by RP-HPLC and SEC.

Soluble and Insoluble Polymeric Protein Extraction. Proteins were extracted (unreduced) into "soluble" proteins (SP) which, at least in wheat, typically include all monomeric proteins and smaller polymeric proteins. Following extraction of SP, the "insoluble" proteins (IP) that would hypothetically contain the largest polymeric proteins were extracted. In wheat, these IP are known to be correlated to dough strength (*15*−*17*). To accomplish the extraction, a sequential procedure was carried out. Soluble proteins were first extracted from 20 mg of carob germ flour with 15 min of continuous vortexing in 1 mL of 50 mM sodium phosphate, pH 7.0, buffer containing 1% SDS (w/v). After 5 min of centrifugation at 9300*g*, the supernatant was collected and the extraction procedure was repeated. The supernatants from both SP extractions were pooled in a 1:1 ratio. Insoluble proteins were extracted from the remaining residue using sonication (10 W for 30 s in 1 mL of 50 mM sodium phosphate, pH 7.0, buffer containing 1% SDS (w/v)). Two extractions were made, and supernatants were centrifuged and pooled as described above. Residue proteins (RP) were extracted with the 50 mM sodium phosphate, pH 7.0, buffer containing 1% SDS (w/v) plus 2% DTT (w/v) from the residue remaining after the IP extractions and pooled as above.

Microfluidic Analysis. Molecular weights of reduced protein extractions were determined by microfluidic electrophoresis on an Agilent 2100 Bioanalyzer (Lab-on-a-Chip) (Agilent, Santa Clara, CA). Protein fractions for the Osborne extractions were analyzed with the Lab-on-a-Chip system as described by the protocols provided from the manufacturer. Briefly, 4.0 μL of sample for each fraction analyzed was mixed into 2 μL of Agilent denaturing solution in a 0.5 mL microtube. This mixture was vortexed, and proteins were denatured by exposing them to 95 °C for 5 min. Next, 84 μL of DI H_2O was added to the protein extraction/denaturing solution mixture and vortexed. Protein 230 chips with a molecular weight range of 4.5−240 kDa were prepared according to Agilent specifications; each well was filled with 6 μL of the extraction solutions from above. The prolamin and prolamin reduced extractions were run with the same conditions as above, but using a Protein 80 chip with a molecular weight range of 5−80 kDa to achieve better resolution.

RP-HPLC Analysis. Osborne fractions were analyzed via RP-HPLC on an Agilent 1100 HPLC system equipped with Poroshell SB300 C8 (Agilent, Palo Alto, CA) column and guard column. Separations were achieved using a linear gradient from 10% acetonitrile/0.1% trifluoroacetic acid (TFA) (w/v) to 90% acetonitrile/0.1% TFA (w/v) over 20 min with a flow rate of 0.7 mL/min and a column temperature of 50 °C. Sample detection was by UV at 214 nm, and 10 μL of sample was injected for all samples.

SEC-MALS. Soluble proteins, insoluble proteins, and residue protein samples were analyzed via SEC using an Agilent 1100 HPLC system equipped with a Biosep-4000 column (Phenomenex, Torrance, CA) and guard column. The mobile phase was a 50 mM sodium phosphate, pH 7.0, buffer containing 1% SDS (w/v) (*18*). Proteins were detected at 214 nm over a 30 min span with a flow rate of 1 mL/min and an injection volume of 20 μL. Column temperature was fixed at 40 °C. For characterization of the M_w distributions of SP and IP extracts, SEC-MALS was conducted using the SEC conditions above with the HPLC system connected to a Wyatt DAWN Helios II multiangle light scattering (MALS) detector and an Optilab Rex differential refractometer (Wyatt Technology Corp., Santa Barbara, CA). Scattering angles were normalized using bovine serum albumin. The temperature of the differential refractometer was maintained at 25 °C. Dn/Dc of 0.39 was used for all SEC separations of carob protein and was determined as described in Bean and Lookhart (*18*).

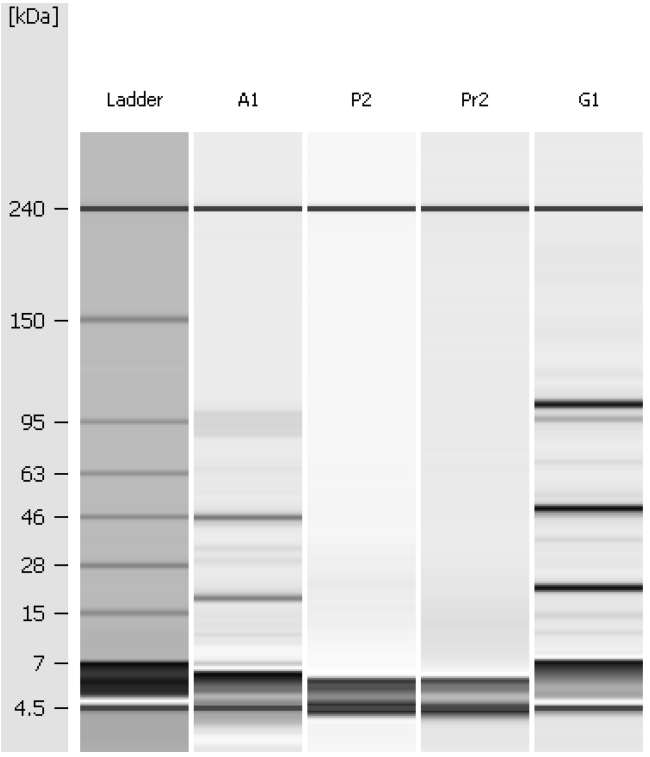

Figure 1. Electropherogram of (ladder) M_w standards, (A1) albumin/globulin, (P2) prolamin, (Pr2) reduced prolamin, and (G1) glutelin of carob germ proteins. All samples were reduced prior to analysis.

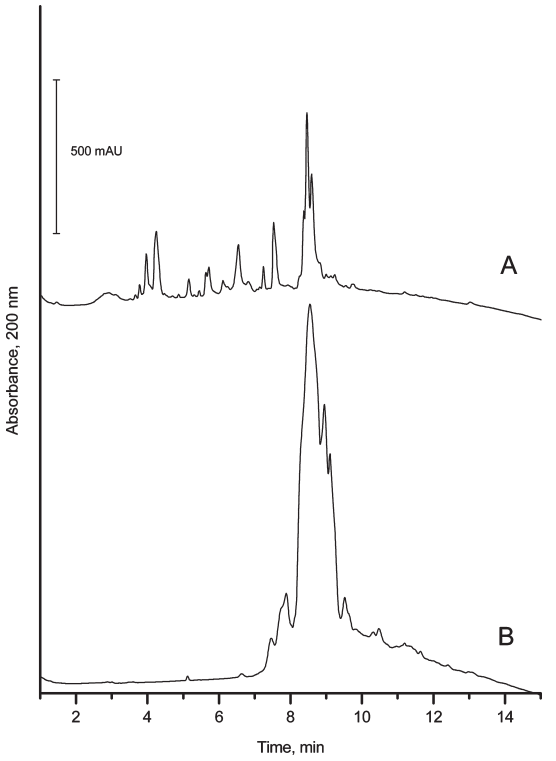

Figure 2. RP-HPLC separations of (**A**) reduced albumin and globulin extract and (**B**) reduced glutelin extract of carob germ protein.

Farinograph. To determine the importance of disulfide bonds on carob germ flour−maize starch dough formation, dough was mixed by a Farinograph-E (Brabender, Duisburg, Germany) at 63 rpm for 20 min. For the control dough, 40 g of a mix containing 30% carob germ flour and 70% corn starch was placed into a farinograph 50 g mixing bowl. One minute of calibration was allowed, and 32 g or 80% water on a flour basis was added and allowed to mix. The reduced dough was prepared as described above, but 2% dithiothreitol (DTT) (w/v) was added to the water prior to mixing.

RESULTS AND DISCUSSION

Protein Characterization. Protein extraction using the Osborne fractionation protocol was efficient, with ∼96% of the total protein being extracted as determined by nitrogen combustion of the residue remaining after all extractions (data not shown). No prolamins were detected by microfluidic analysis (**Figure 1**). The albumin/globulin fraction contained major bands at ∼16 and 46 kDa with minor bands spanning the range from 7 to 96 kDa. Major bands in the glutelins had nominal M_w of ∼16, 46, and 96 kDa with minor bands visible throughout this range (**Figure 1**). In previous work conducted via SDS-PAGE carob proteins were not extracted into different subfractions. However, major and minor protein bands appeared in similar molecular weight ranges (*9*).

Figure 2 shows the RP-HPLC separations of both the albumin/globulin and glutelin fractions. Preliminary experiments showed that no peaks in the prolamin extracts were detected by RP-HPLC (data not shown). The albumin/globulin extract contained peaks with a range of elution times with the major peaks eluting at ∼9 min. The major peaks in the glutelin extract also eluted at the 8−9 min range with only a few additional minor peaks. The albumin/globulin fraction had more early eluting peaks, indicative of lower surface hydrophobicity (i.e., more hydrophilic), than the glutelin fraction. This would be expected from water- and salt-soluble proteins. Quantitative data from the RP-HPLC separations revealed that the glutelins were the most abundant protein class, comprising ∼78% of the total with the albumin/globulin fraction containing the remaining ∼22%. These data confirm the previous results of Plaut et al. (*5*), who found the majority of the proteins extracted were in the glutelin, albumin, and globulin fractions with minimal amounts of prolamin present. However, Plaut et al. (*5*) reported that albumins and globulins accounted for the majority of the protein (∼65% on a total flour protein basis), with the glutelin making up most of the remainder (∼32%). Little information is available on the methodology used by Plaut et al. (*5*), so it is difficult to speculate on the reasons for these differences. In addition to differences in methodology, sample differences and differences in how the carob germ flour was produced could influence the protein composition of the samples.

SEC-MALS was used to characterize the M_w of the polymeric protein complexes found in carob germ flour. MALS provides an "absolute" M_w measurement for proteins, is not reliant on standard protein molecular weight curves, and removes bias in M_w estimates by SEC due to factors such as differences in hydrodynamic radius and protein structure (*19*). SEC-MALS analysis of the nonreduced albumin/globulin and glutelin fractions showed major differences between the two protein classes in their molecular weight distribution (**Figure 3**). The albumin/globulin fraction had proteins that eluted across a wide time frame, indicating a wide M_w distribution. Relatively low amounts of the early eluting high M_w material was seen in the albumin/globulin fraction. Little change was seen in the chromatograms for the reduced samples, indicating low levels of disulfide-bonded polymers present in these proteins (**Figure 3A**). The glutelin fraction exhibited high levels of early eluting peaks, indicating polymers of high M_w. Upon reduction, the majority of the early eluting peaks showed a large decrease in absorbance with subsequent appearance of new peaks eluting later in the chromatogram, suggesting that the early eluting peaks were large polymers linked through disulfide bonds (**Figure 3B**).

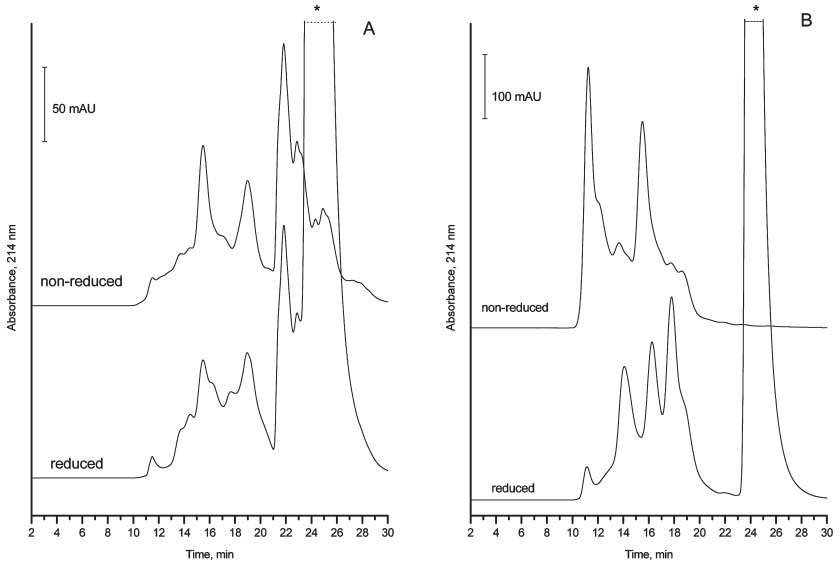

Figure 3. Size exclusion chromatograms of reduced and nonreduced (**A**) albumin and globulins and (**B**) glutelins of carob germ proteins. The asterisk marks the location of the β-ME peak, which has been artificially truncated for scale.

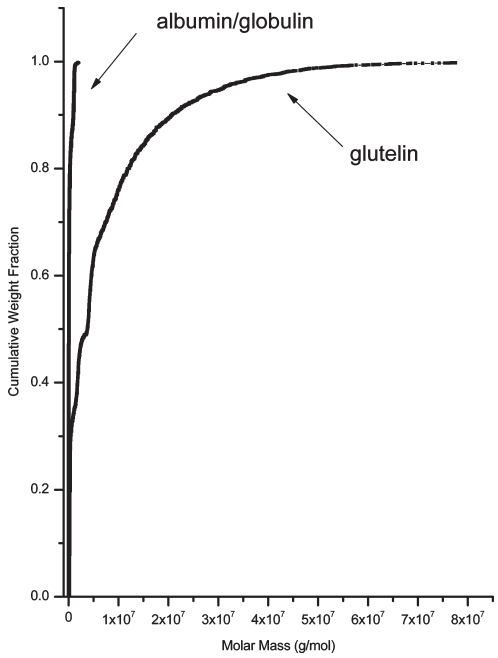

Figure 4. Cumulative molecular weight curves for the nonreduced polymeric peaks of albumin/globulin and glutelins of carob germ proteins.

The cumulative molecular weight distribution curves showed that the albumin/globulin fraction contained polymeric proteins with an upper M_w range of up to $\sim 5 \times 10^6$ Da (**Figure 4**). Note, this number represents the largest polymeric proteins found in the albumin and globulin fractions. Approximately 75% of the albumin and globulins had M_w of $< 1.24 \times 10^5$ Da. Again, this is for unreduced proteins only. This agrees well with the results from the microfluidics analysis, which showed that in the reduced form, the albumin and globulins ranged in M_w from 16 to 96 kDa (**Figure 1**).

In contrast to the albumin and globulin fraction, the glutelin fraction contained large M_w proteins ranging up to $\sim 8 \times 10^7$ Da. Approximately 25% of the proteins in the glutelins fraction had M_w of $> 5.49 \times 10^6$ (**Figure 4**). Again, these M_w numbers are for unreduced polymeric protein complexes.

Wheat typically contains $\sim 10 - 15\%$ albumins/globulins, 67−76% prolamins (gliadins + glutenins), and 14−18% glutelins (*13*, *20*, *21*), whereas carob germ flour contained no extractable prolamins. Prolamins in wheat are rich in proline and glutamine, and this fraction is known to contribute significantly to wheat gluten functionality. More specifically, the large polymeric glutenins are directly correlated to dough strength in wheat (*17*). In the classical Osborne fractionation scheme, the glutenins of wheat are sometimes classified as glutelins; however, more modern work places the glutenins in the prolamin subclass (*22*). Regardless of their nomenclature, the glutenins of wheat have significantly different solubility than the carob glutelins fraction (e.g., solubility in aqueous alcohols). Differences in solubility do not necessarily represent differences in functionality between proteins, and caution should be used in the comparison of Osborne fractions across different types of materials. Given that amino acid differences between carob and gluten have already been reported (*7*), the differences in solubility reported here support previous research that whereas carob and gluten proteins both contain large M_w protein complexes, other factors may be involved in their functionality.

Another significant difference is the M_w between the gluten polymeric proteins and those found in the glutelin fraction of carob. When compared to previous measurements of the M_w of wheat polymeric proteins, the polymeric proteins of the carob glutelins were found to be slightly lower in terms of the upper range of M_w. Wheat has been reported to contain polymeric protein complexes that range up to $1 \times 10^7 - 1 \times 10^8$ Da (*18*, *23*, *24*). Note that these values represent the upper ranges of the M_w, not the average M_w of the wheat polymeric protein complexes (which have been reported in the $\sim 3 \times 10^6$ Da (*23*) range). Although the data presented here represent only one sample of carob and therefore should be regarded as preliminary, the data do point to an important functional difference between the polymeric proteins of wheat and carob germ flour.

In addition to characterizing wheat proteins using Osborne fractionation, researchers have focused on more straightforward procedures to extract wheat flour proteins into two broad classes, soluble and insoluble or unextractable (*17*). This is done without reducing agent, and the resulting protein fractions are typically analyzed by SEC to determine their overall molecular weight distribution. To better compare carob proteins to wheat, this type

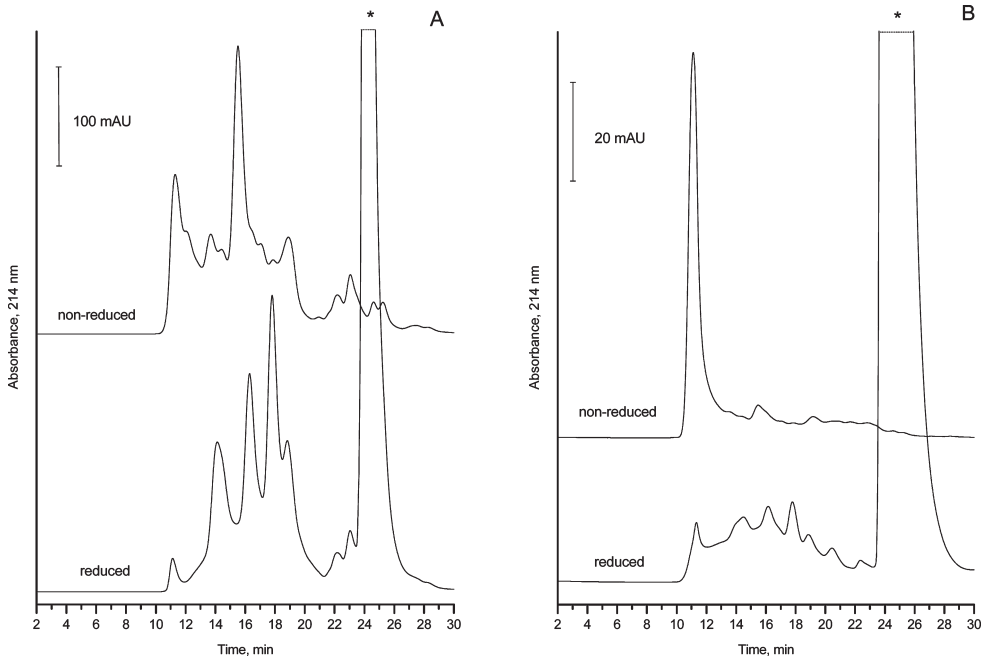

Figure 5. Size exclusion chromatograms of (**A**) nonreduced and reduced soluble proteins (SP) and (**B**) nonreduced and reduced insoluble proteins (IP) of carob germ proteins. The asterisk marks the location of the β-ME peak, which has been artificially truncated for scale.

of extraction was carried out on carob germ flour. **Figure 5** shows the SEC chromatograms of the SP and IP fractions of carob germ flour, both reduced and nonreduced. The SP fraction was found to comprise ~93% of the total protein, whereas IP was ~5% and residual protein (RP) was ~2%. This is much different from the distribution typically found in wheat, where IP typically accounts for 30−50% of the protein depending on the type of wheat and the extraction methodology used (*16*, *25*, *26*). This again points to important differences between wheat polymeric proteins and those of carob germ flour.

Reduction of the SP and IP samples was carried out to identify disulfide-containing peaks in the SEC chromatograms of each fraction. In the SP fraction, peaks eluting from 10 to 16 min substantially decreased or their elution times shifted to longer times when the samples were reduced, demonstrating that these were polymeric proteins linked via disulfide bonds. Furthermore, along with the decrease in early eluting peaks, an increase in the peak at ~19 min was observed (**Figure 5**). Other regions of the chromatogram showed only minor changes (**Figure 5**), indicating that the SP extract most likely contained a mixture of polymeric, oligomeric, and monomeric proteins.

The SEC chromatogram of the IP extract showed that this fraction was composed of mainly large polymers (**Figure 5**). This was evident when the IP sample was reduced and analyzed via SEC. Reduced chromatograms of both the SP and IP were overall similar with some slight differences in the 12−14 min range. This possibly suggests that the polymeric proteins in the SP and IP were composed of the same set of monomers and thus differed only in their degree of polymerization (i.e., M_w). The quantitative differences in the reduced SP and IP extracts, for example, the proteins eluting at 16−18 min, were present in much greater proportion to the other proteins than in the reduced IP sample. Comparing the results (**Figure 5**) to the chromatograms (**Figure 3**), one may gain some insight into the composition of the SP and IP. **Figure 3** shows that the albumin and globulins contained only low levels of large disulfide-bonded polymeric proteins. Conversely, the glutelins showed a large peak in the unreduced samples at 10−12 min that almost completely disappeared when reduced. Because both the SP and IP fractions

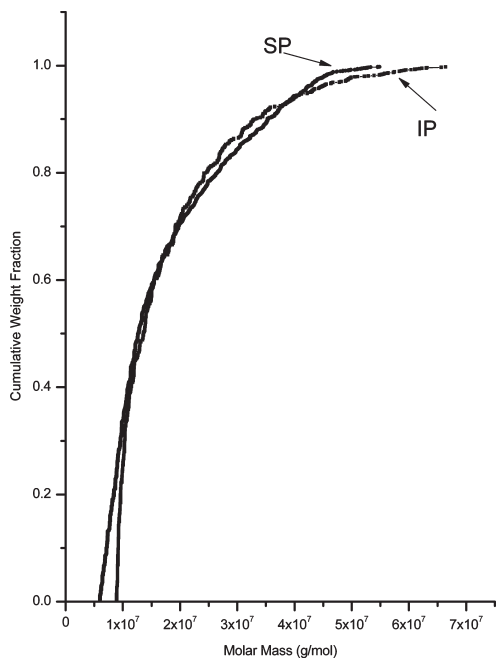

Figure 6. Cumulative molecular weight curves for the nonreduced polymeric peaks of soluble and insoluble proteins of carob germ proteins.

contained large polymeric protein peaks at 10−12 min, the data suggest that the large polymeric proteins found in IP fractions of carob are composed mainly of glutelin. As discussed previously, this may have implications for the functionality of carob germ proteins with respect to viscoelastic dough formation.

The cumulative molecular weight distribution curves as determined by SEC-MALS for the SP and IP fractions for both SP and IP were similar (**Figure 6**). However, as found in wheat (*18*), the IP fraction contained proteins of higher molecular weight than the SP fraction. These higher molecular weight proteins have been shown to play a major role in wheat gluten functionality (*18*, *23*, *24*). Carob germ proteins were previously shown to have functional properties similar to those of wheat gluten, which may

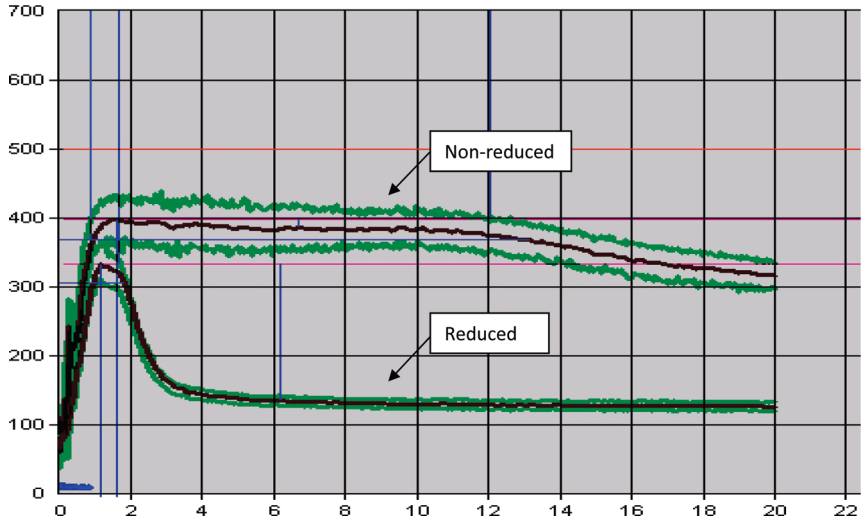

Figure 7. Farinograms of carob germ flour protein−maize starch dough under nonreducing conditions and reducing conditions.

provide high-quality gluten-free food products for the celiac market. Understanding how proteins other than wheat gluten form viscoelastic dough will allow for a better understanding of wheat gluten functionality (*7*). The above results show that carob germ proteins contained mostly (~95%) "soluble" proteins with maximum M_w up to ~5 × 10^7 Da with only ~5% IP proteins, whereas wheat has been reported to contain 30−50% IP depending on the type of wheat analyzed (*16*, *27*, *28*). The carob germ protein contains polymeric proteins with M_w close to that of wheat; the levels of these largest proteins are very low compared to wheat. The M_w distribution was skewed to monomeric and smaller M_w polymers, and this may be one reason for differences between the functionality of wheat gluten and carob germ protein. Relating the functionality of SP and IP in carob to that of wheat should be approached with caution until more understanding of carob germ proteins can be gained.

Because the polymeric proteins investigated during this research were apparently formed through disulfide bonds, we decided to perform a simple experiment to determine if the polymeric proteins in carob were important at a functional level. A carob germ flour−maize starch dough was mixed in a farinograph, both unreduced and reduced (achieved by adding the reducing agent DTT to the dough during mixing). It is clear that when the dough was reduced, the mixing curve was drastically altered (**Figure 7**), demonstrating the importance of the disulfide-bonded large polymeric proteins found in carob germ flour to its ability to form viscoelastic dough. On a side note, these experiments were also attempted with a mixograph, which is known to have much higher shear than a farinograph during mixing. However, no mixing curve could be produced, indicating that the proteins of carob germ flour were not able to form as strong of a dough as those of wheat. This follows the data found in this paper that carob germ flour proteins have a substantially different MWD than that of gluten, which may result in a much weaker dough.

There are few known proteins capable of dough formation. For this reason carob germ proteins' ability to form protein networks is significant in helping to better understand the properties of viscoelastic proteins. This functional property attribute in carob may open new avenues for future gluten-free foods. Whereas the gluten-like properties of carob germ protein have been reported, the biochemical analysis proved caroubin to be quite different from gluten. The M_w distribution of carob germ proteins was shifted to lower M_w protein and was present in relatively smaller quantities than that of wheat gluten. Furthermore, in the Osborne extractions caroubin was found to contain no measurable amounts of prolamin, a protein fraction that is attributed to gluten functionality. These major biochemical differences may be the causative factor in the rheological differences reported by Feillet and Roulland (*7*). More research is needed to gain a further understanding of these chemical differences and the chemical interactions that take place during dough formation so that carob may be better utilized.

LITERATURE CITED

(1) Fasano, A.; Catassi, C. Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum. *Gastroenterology* **2001**, *120*, 636–651.
(2) Weiser, H.; Koehler, P. The biochemical basis of celiac disease. *Cereal Chem.* **2008**, *85*, 1–13.
(3) Batlle, I.; Tous, J. *Carob Tree: Ceratonia silique L. Promoting the Conservation and Use of Underutilized and Neglected Crops 17*; Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute: Rome, Italy, 1997.
(4) Bienenstock, M.; Csaski, L.; Pless, J.; Sagi, A.; Sagi, E. U.S. Patent 2,025,705, 1935.
(5) Plaut, M.; Zelcbuch, B.; Guggenhem, K. Nutritive and baking properties of carob germ flour. *Bull. Res. Council Israel* **1953**, *3*, 129–131.
(6) Rice, A.; Ramstad, P. Amino acid composition of wheat and carob glutens. *Cereal Chem.* **1950**, *27*, 238–243.
(7) Feillet, P.; Roulland, T. Caroubin: a gluten-like protein isolate from carob bean germ. *Cereal Chem.* **1998**, *75*, 488–492.
(8) Wang, Y.; Belton, S.; Bridon, H.; Garanger, E.; Wellner, N.; Parker, M.; Grant, A.; Feillet, P.; Noel, T. Physicochemical studies of caroubin: a gluten like protein. *J. Agric. Food Chem.* **2001**, *49*, 3414–3419.
(9) Bengoechea, C.; Romero, A.; Villanueva, A.; Moreno, G.; Alaiz, M.; Millan, F.; Guerro, A.; Puppo, M. Composition and structure of carob (*Ceratonia siliqua* L.) germ proteins. *Food Chem.* **2008**, *107*, 675–683.
(10) Osborne, T. B. *The Proteins of the Wheat Kernel*; Carnegie Institute: Washington, DC, 1907.
(11) Shewry, P.; Hill, J.; Pratt, H.; Leggatt, M.; Miflin, B. An evaluation of techniques for the extraction of hordein and glutelins from barley seed and a comparison of the protein composition of Bomi and Riso 1508. *J. Exp. Bot.* **1978**, *29*, 677–692.
(12) Taylor, J.; Schussler, L.; Van der Walt, W. Fractionation of proteins from low-tannin sorghum grain. *J. Agric. Food Chem.* **1984**, *32*, 149–154.
(13) Sapirstein, H.; Fu, B. Intercultivar variation in the quantity of monomeric proteins, soluble and insoluble glutenin, and residue

protein in wheat flour and relationships to bread making quality. *Cereal Chem*. **1998**, *75*, 500–5007.
(14) Marion, D.; Nicolas, Y.; Popineau, Y.; Branlard, G.; Landry, J. A new and improved sequential extraction procedure of wheat proteins. In *Wheat Kernel Proteins*; Univerit Degli Studi Della Tuscia: Viterbo, Italy, 1994; pp 197−199.
(15) Weegels, P.; Hamer, R.; Schofield, J. Functional properties of wheat glutenin. *J. Cereal Sci*. **1996**, *23*, 1−18.
(16) Bean, S.; Lyne, R.; Tilley, K.; Chung, O.; Lookhart, G. A rapid method for quantization of insoluble polymeric proteins in flour. *Cereal Chem*. **1998**, *75*, 374–379.
(17) Southan, M.; MacRitchie, F. Molecular weight distribution of wheat proteins. *Cereal Chem*. **1999**, *75*, 827–836.
(18) Bean, S.; Lookhart, G. Factors influencing the characterization of gluten proteins by size-exclusion chromatography and multiangle laser light scattering (SEC-MALLS). *Cereal Chem*. **2001**, *78*, 608–618.
(19) Wyatt, P. Light scattering and the absolute characterization of macromolecules. *Anal. Chim. Acta* **1993**, *272*, 1–40.
(20) Fu, B.; Sapirstein, H. Procedure for isolating monomeric proteins and polymeric glutenin of wheat. *Cereal Chem*. **1996**, *73*, 143–152.
(21) Lookhart, G.; Bean, S. Cereal composition of their major fractions and methods for identification. In *Handbook of Cereal Science and Technology*, 2nd ed.; Kulp, K., Lorenz, K., Eds.; Dekker: New York, 2000; pp 363−383.
(22) Shewry, P.; Tatham, A.; Forde, J.; Kreis, M.; Miflin, B. The classification and nomenclature of wheat gluten proteins: a reassessment. *J. Cereal Sci*. **1986**, *4*, 97–106.
(23) Carceller, J.; Aussenac, T. Size characterization of glutenin polymers by HPSEC-MALLS. *J. Cereal Sci*. **1999**, *33*, 131–142.
(24) Stevenson, S.; You, S.; Izydorczyk, M.; Preston, K. Characterization of polymeric wheat proteins by flow-flow fractionation/MALLS. *J. Liq. Chromatogr. Relat. Technol*. **2003**, *26*, 2771–2781.
(25) Gupta, R.; Khan, K.; MacRithie, F. Biochemical basis of flour properties in wheats. I. Effects of variations in the quantity and size distribution of polymeric proteins. *J. Cereal Sci*. **1993**, *18*, 23–41.
(26) Ciaffi, M.; Tozzi, L.; Lafiandra, D. Relationships between flour protein composition determined by size-exclusion chromatography and dough rheological parameters. *Cereal Chem*. **1996**, *73*, 346–351.
(27) Gupta, R.; Popineau, Y.; Lefebvre, J.; Cornec, M.; Lawrence, G.; MacRitchie, F. Biochemical basis of flour properties in bread wheats. II. Changes in polymeric protein formation and dough/gluten properties associated with the loss of low Mr or high Mr glutenin subunits. *J. Cereal Sci*. **1998**, *21*, 103–116.
(28) Schober, T.; Bean, S.; Khun, M. Gluten proteins from spelt (*Triticum aestivum* ssp. *spelta*) cultivars: a rheological and size-exclusion high-performance liquid chromatography study. *J. Cereal Sci*. **2005**, *44*, 161–173.

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