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Chemical Characterization and Functionality Assessment of Oat Protein Fractions

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Protein fractions (albumins, globulins, prolamins, and glutelins) were prepared from oat seeds (variety Sentinel). Column chromatography on Sephacryl S-200 revealed that the four solubility fractions had unique polypeptide compositions and there was little cross contamination among the fractions. Isoelectric focusing on polyacrylamide gels resolved the fractions into a large number of bands covering a wide pH range. Differential scanning calorimetric studies showed that albumins and globulins had an endothermic peak at 87 and 110 °C, respectively, while prolamins and glutelins had no thermal response. Some functional properties of the solubility fractions were determined to assess the potential use of oat proteins as a food ingredient. Some fractions had high emulsifying, fat-binding, and water hydration capacities, and the albumins also had excellent foaming properties.

Oats provide a potential source of low-cost proteins with good nutritional value (Hischke et al., 1968) but are not used extensively for human consumption in the form of processed food. This could partly be due to insufficient information on the physicochemical and functional properties of oat proteins. Protein concentrates and isolates have been prepared from oats (Bell et al., 1978; Cluskey et al., 1973, 1978; Wu and Stringfellow, 1973; Youngs, 1974; Ma, 1983a,b) and were found to have some good functionality, suggesting potential use in foods (Ma, 1983a,b). Protein fractions have also been isolated from oats by the Osborne fractionation scheme, and their chemical and amino acid compositions were determined (Wu et al., 1972; Draper, 1973; Pernollet et al., 1982). Oat globulins and prolamins (avenine) have been partially purified and characterized (Peterson, 1978; Kim et al., 1978; Brinegar and Peterson, 1982), while the other two solubility classes, albumins and glutelins, have not been extensively studied. There is a complete lack of information on the functionality of these protein fractions from oats.

In this work, the four solubility fractions from oat groats will be characterized by chromatography, isoelectric focusing, and differential scanning calorimetry to provide information on the polypeptide composition, charge heterogeneity, and conformation of the oat proteins. Such data are needed for comparative studies of cereal proteins that have received recent emphasis (Miflin et al., 1983). Some technological properties of cereals, such as breadmaking properties of wheat and brewing quality of barley, are profoundly influenced by the storage proteins. A

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fundamental understanding of the role of the storage proteins in cereal technology would therefore require detailed physicochemical characterization of the proteins. The functionality of the individual solubility fractions will be determined, and this should provide more detailed information on the characteristics of oat proteins and enable a more accurate assessment of their potential use in foods.

EXPERIMENTAL SECTION

Materials. Oats (variety Sentinel) were grown at the Central Experimental Farm, Ottawa, Ontario. The dehulled groats were ground in a pin mill and defatted by Soxhlet extraction with hexane.

Preparation of Osborne Solubility Fractions. The procedure was similar to that described by Wu et al. (1972) except that 0.5 M $CaCl_2$ was used instead of the more conventional solvent, 1.0 M NaCl. Preliminary experiments showed that 0.5 M $CaCl_2$ gave a higher yield of globulins, and $CaCl_2$ was also recommended to prepare protein isolates from oats (Murray et al., 1978).

Column Chromatography. Gel filtration chromatography of the solubility fractions was performed on Sephacryl S-200 (Pharmacia Fine Chemicals, Uppsala, Sweden) column (2.5 × 90 cm), using an AUC solution (0.1 M acetic acid, 3 M urea, and 0.1 M hexadecyltrimethylammonium bromide) (Meredith and Wren, 1966). The column was calibrated with standard proteins of known molecular weight including human γ-globulin, bovine serum albumin, ovalbumin, trypsin inhibitor, and cytochrome *c*.

Isoelectric Focusing (IEF). IEF was performed on 0.5 mm thick polyacrylamide gel slabs. The gel solution contained 5% acrylamide, 0.15% *N,N'*-methylenebis(acrylamide), 4% Ampholine, pH 3.5–10 (LKB-Produkter AB, Bromma, Sweden), 7% glycerol, and 6 M urea. The sample buffer contained 4% Ampholine, 2% glycine, 6 M urea, and 2% 2-mercaptoethanol. All protein preparations were completely solubilized in this buffer except glutelins, which were about 80% soluble as determined by the micro-Kjeldahl method. DL-Aspartic acid (0.04 M) and NaOH (0.1 M) were used respectively as anolyte and catholyte. Samples (20-μL aliquots) were applied to filter paper strips placed approximately 7 cm from the anode and 3 cm from the cathode. A constant power of 25 W was maintained and electrophoresis was carried out at 10 °C for 1 h. A broad p*I* calibration kit (Pharmacia Fine Chemicals, Uppsala, Sweden) was used for determining the pH gradient. The slabs were stained by the method of Righetti and Drysdale (1974).

Differential Scanning Calorimetry. The thermal behavior of the Osborne fractions was examined with a Perkin-Elmer Model DSC II differential scanning calorimeter equipped with subambient accessory, intercooler II. Polymer-coated Dupont pans were used for sample and reference. An empty pan sealed with a lid was used as the reference. For albumins, a 10% aqueous solution was prepared and 10 μL was added to the sample pan. Due to the low solubility and difficulty in transferring quantitatively the aqueous slurries of the other fractions, the samples (1 mg) were directly weighed onto the pan and 10 μL of distilled water was added. The heating rate was 10 °C/min over the range of 300–400 K. Indium standards were used for temperature and energy calibration. The enthalpy of denaturation (ΔH) was calculated by using the equation

$$\Delta H = RKA/(SW)$$

where R = range control setting, K = calibration constant, S = chart speed, A = area under peak (cm^2), and W = weight of protein (g).

Table I. Kjeldahl Nitrogen Content and Distribution of Protein Fractions in Dehulled, Defatted Sentinel Groats[a]

fraction	N in solids, % (dry basis)	total N extracted, % groat N
albumins	4.8	11.7
globulins	15.3	38.4
prolamins	12.3	13.6
glutelins	10.1	13.6
residues	0.8	13.4

[a] Average of values from three separate experiments.

Functional Properties. Solubility was determined in 1% dispersions in distilled water. The protein dispersions were stirred at room temperature for 20 min, and the pH was adjusted to values between 1.5 and 11.0 with 1 N NaOH and HCl. After centrifugation at 10000*g* for 30 min, the supernatants were analyzed for nitrogen by the micro-Kjeldahl method.

Emulsifying activity index (EAI) and emulsion stability index (ESI) were determined by a turbidimetric method (Pearce and Kinsella, 1978). Water hydration capacity (WHC) was determined according to Quinn and Paton (1979). Fat-binding capacity (FBC) was estimated by the method described by Lin et al. (1974), and foaming properties (foamability and foam stability) were assessed by the procedure of Yatsumatsu et al. (1972).

RESULTS AND DISCUSSION

Distribution of Protein Fractions. Table I shows the Kjeldahl nitrogen content of the protein fractions from Sentinel groats and the portion of nitrogen extracted in each fraction. The globulins had the highest nitrogen content followed by prolamins, glutelins, and albumins. The residue only had 0.8% nitrogen. The salt-soluble globulins were the predominant protein fraction, comprising 38% of the total groat nitrogen. The other fractions were evenly distributed, representing approximately 12–14% of the total groat nitrogen. The yield of all the fractions was nearly 90% of the total groat nitrogen.

Fractionation of plant proteins on the basis of solubility in various solutions is only an approximate estimation of the actual protein composition. Differences in extraction conditions may lead to vastly different values. Hence, globulins were found to be the dominant fraction in oats by some workers (Wu et al., 1972; Peterson and Smith, 1976) while others reported glutelins to be the major proteins (Michael et al., 1961; Volker, 1975). This discrepancy could be attributed to incomplete extraction of globulins that are soluble in weak acids or alkali (Ma, 1983a) and may therefore be included in the glutelins.

Column Chromatography. Figure 1 shows the chromatographic patterns of the four solubility fractions on Sephacryl S-200. The albumins had a major peak with an estimated molecular weight of 15 000 and a minor peak with lower molecular weight (6000). Two poorly resolved peaks with molecular weights of 36 000 and 22 000 were also detected. The globulins showed a small peak near the void volume with an estimated molecular weight of 150 000. There were three poorly resolved peaks with molecular weights of 57 000, 36 000, and 22 000, respectively. A low molecular weight (6000) component was also found in the globulin fraction. The prolamins contained two main peaks with molecular weights of 23 500 and 15 500, respectively. A 36 000 molecular weight component was also detected as a minor peak. The glutelins contained a broad peak at the void volume with a shoulder corresponding to

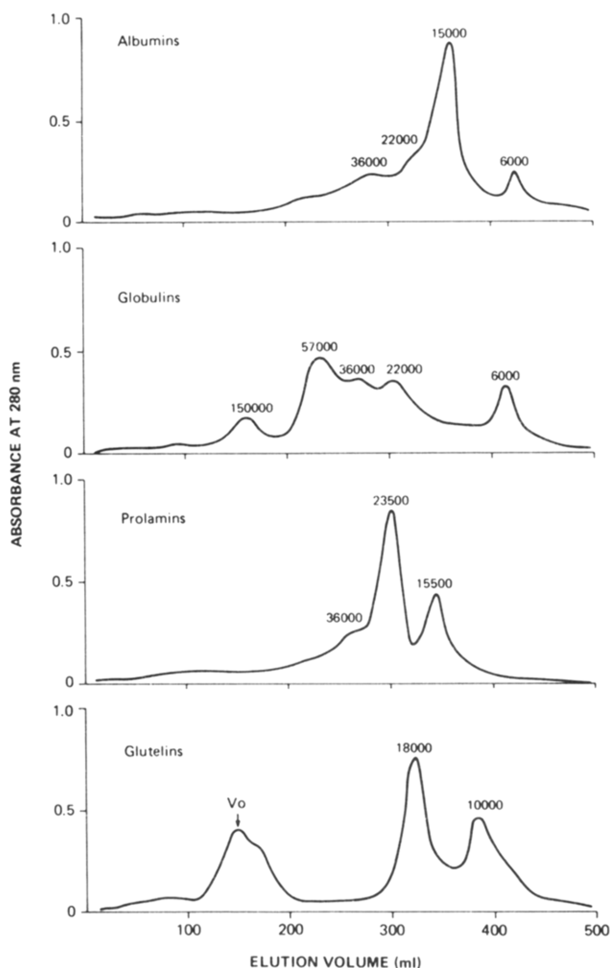

Figure 1. Column chromatography of oat protein fractions on Sephacryl S-200.

the 150 000 component in globulins. The major glutelin component had an estimated molecular weight of 18 000 and there was also a minor 10 000 molecular weight peak.

The chromatography data show that the four solubility fractions from oats had unique polypeptide composition. There was little cross contamination among the fractions except the presence of some globulin components in other classes. This is consistent with the finding of Pernollet et al. (1982), who showed that Osborne fractions from oat meal were clearly individualized. The estimated molecular weight (23 500) of the major prolamin component was close to the reported value (22 500) of purified avenins (Kim et al., 1978). The molecular weights of two of the major globulin components were also close to those reported for the α and β subunits in oat globulins (Peterson, 1978; Brinegar and Peterson, 1982). Brinegar and Peterson (1982) observed that the α and β subunits associate through a disulfide bond to form a dimer with a molecular weight of 58 000.

Isoelectric Focusing. Figure 2 shows the isoelectric focusing (IEF) patterns of the four solubility fractions. The loading position was found to significantly affect the resolution. Optimal resolution was obtained when samples were loaded near the cathode at a position corresponding to approximately pH 8. The albumins had isoelectric components covering a wide pH range. The majority of the albumin bands had an isoelectric point (p*I*) between 4 and 7.5. Some alkaline components were also observed as diffused bands (Figure 2a). Most of the globulin components were found above pH 5.5 and were grouped essentially into an acidic (pH 5.5–6.5) and alkaline (pH 8–10) region (Figure 2b). According to Brinegar and Peterson (1982), these correspond respectively to the α and β sub-

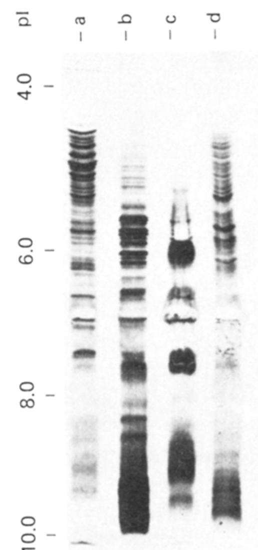

Figure 2. Isoelectric focusing patterns of oat protein fractions. (a) Albumins; (b) globulins; (c) prolamins; (d) glutelins.

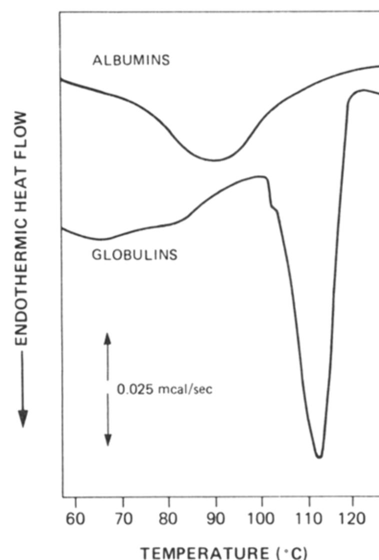

Figure 3. Differential scanning calorimetric thermograms of albumins and globulins from oats.

units of oat globulins. Three main groups of prolamins were observed at pH around 6, 7.6, and 9 (Figure 2c). The glutelins were also grouped into an acidic (pH 4.5–6.5) and alkaline (pH 9–10) region. Some highly acidic glutelin components had low affinity for protein dye and were detected as white bands (trichloroacetic acid fixed) on the gels (Figure 2d).

Differential Scanning Calorimetry (DSC). DSC has been established as a technique for studying thermal denaturation and conformation transition of proteins and has been used in the study of various food systems (Donovan et al., 1975; Donovan, 1977; Quinn et al., 1980). Since DSC does not require dissolving the materials, the technique is paticularly useful to characterize plant proteins such as oat storage proteins, which are not readily soluble in aqueous buffers.

The DSC thermograms of albumins and globulins from oats are presented in Figure 3. The albumin fraction showed a broad endothermic peak with a T_d (denaturation temperature or peak maximum temperature) of approximately 87 °C. The globulin fraction showed a sharp symmetrical endothermic peak with a T_d of about 110 °C and a half-peak width of 9.6 °C. The ΔH of the globulin fraction was 5.39 cal/g, and the ΔH for albumins was not calculated because of the difficulty in establishing an accurate base line. The prolamins and glutelins did not show

Table II. Functional Properties of Protein Fractions from Sentinel Groats[a]

fraction	EAI,[b] m²/g	ESI,[c] min	FBC,[d] mL/g	WHC,[e] mL/g	foam-ability, %	foam stability, %	
						30 min	60 min
albumins	31.2	3.2	2.8	2.4	240	70	47
globulins	27.6	3.0	1.6	0.8	100	73	60
prolamins	36.8	9.5	1.7	0.9	50	67	63
glutelins	45.0	2.0	2.1	1.9	45	37	27

[a]Average of duplicate determinations. [b]Emulsifying activity index. [c]Emulsion stability index. [d]Fat-binding capacity. [e]Water hydration capacity.

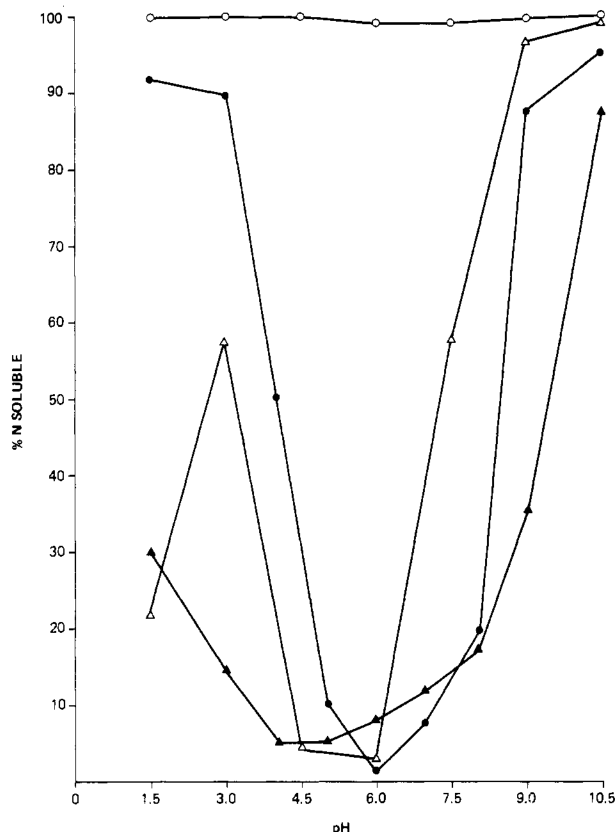

Figure 4. pH–solubility curves of oat protein fractions. (○) Albumins; (●) globulins; (△) prolamins; (▲) glutelins.

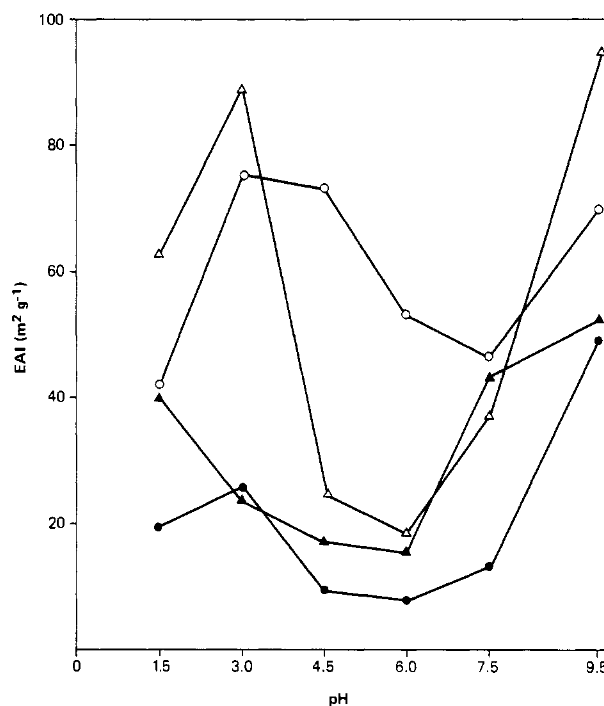

Figure 5. pH–EAI curves of oat protein fractions. (○) Albumins; (●) globulins; (△) prolamins; (▲) glutelins.

any detectable thermal reaction.

The estimated T_d and ΔH for oat globulins were close to those reported for the protein isolates prepared by a procedure similar to the Osborne fractionation of globulins (Arntfield and Murray, 1981) and were considerably higher than the T_d values of other plant proteins. The sharp peak observed for globulins with a relatively low half-peak width value suggests that oat globulins belong to a protein system with high cooperative thermal transition. the broad endotherm of albumins suggests a multistep thermal transition related to a heterogeneous mixture of proteins. The absence of an endothermic peak in oat glutelins may be due to extensive denaturation of proteins during extraction. Arntfield and Murray (1981) showed the disappearance of a typical endothermal peak in faba bean proteins extracted at pH above 11.5. The extraction procedure for prolamins is not likely to cause extensive protein denaturation. The absence of a thermal response in prolamins, as suggested in the case of wheat gluten (Arntfield and Murray, 1981), could be due to the cancellation of the endothermicity of the polar interaction breakup by the exothermic disruption of a sufficient number of hydrophobic bonds.

Functional Properties. *Solubility.* Figure 4 shows the pH–solubility curves of the four protein fractions. The albumins were almost completely soluble over a wide pH range. The globulin fraction showed a bell-shaped solubility curve with minimum solubility at pH 6–7 and high solubility (over 90%) at the acidic and alkaline ends of the curve. The prolamins also exhibited a bell-shaped curve, with minimum solubility at a pH between 5 and 6. The solubility of prolamins was higher at alkaline pH than at acidic pH. The glutelin fraction had low solubility (below 20%) at a pH between 3 and 8. The solubility was much higher at the alkaline end (about 90% at pH 10.5) than at acidic end (30% at pH 1.5). The relatively high solubility of all the oat fractions at alkaline pH suggests that alkali can extract most of the proteins from oats. High extractability of protein from oat groats at relatively low alkali concentration had been demonstrated (Cluskey et al., 1973; Ma, 1983a). Oat protein concentrates prepared by alkaline extraction were also shown to contain substantial quantities of all four protein fractions (Ma, 1983a).

Emulsifying Properties. Table II summarizes the functional properties of the solubility fractions from oats. The emulsifying activity index (EAI), which is related to the interfacial area of the emulsion, varied between 27.6 m^2/g for globulins and 45 m^2/g for glutelins. The emulsion stability indices (ESI) were found to be low in the oat fractions with the exception of prolamins.

EAI were also measured at pH ranging from 1.5 to 9.5 and Figure 5 shows the pH–EAI curves of the four protein fractions. Albumins were found to have higher EAI at acidic (3.0–4.5) and alkaline pH (9.5) than at neutral pH (6.0–7.5). At highly acidic pH (1.5), however, albumins had a lower EAI value. The pH–EAI curves of the other three

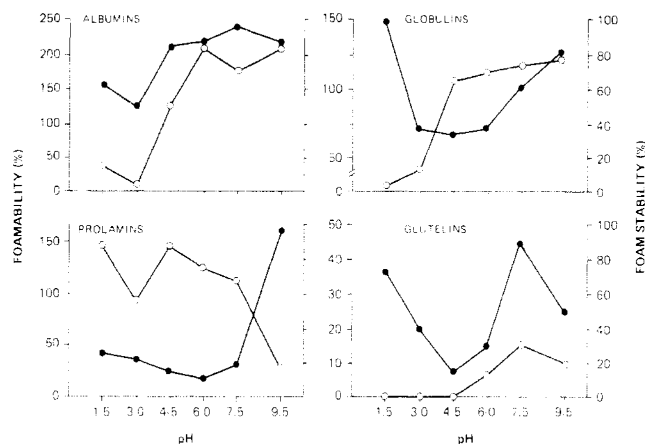

Figure 6. pH–foamability and pH–foam stability curves of oat protein fractions. (●) Foamability; (○) foam stability.

fractions followed the pH–solubility curves with lowest EAI values at the pH corresponding to lowest solubility.

Fat-Binding Capacity. Fat absorption is an important functionality for applications such as meat replacers and extenders. Oat albumins had fat-binding capacity (FBC) considerably higher than the other solubility fractions (Table II). The albumins also had the lowest bulk density, which is consistent with the view that fat absorption is attributable to the physical entrapment of oil by the material (Kinsella, 1976).

Water Hydration Capacity. Albumins and glutelins were found to have water hydration capacity (WHC) much higher than globulins and prolamins (Table II). An inverse relation between solubility and water-binding ability was observed in many food proteins (Hermansson and Akesson, 1975; Lin et al., 1974). The high WHC value for oat glutelins could therefore be due to the low solubility of this fraction. Non-protein components in the albumin fraction, such as carbohydrates, may contribute to higher WHC in this fraction, despite its high solubility.

Foaming Properties. Albumins had much greater foamability than the other three fractions and it was comparable to that (250%) of liquid egg white (Lin et al., 1974). This could be attributed to high solubility of this fraction and the presence of non-protein constituents. Lawhon et al. (1972) reported that many water solubles in oilseeds had high whipping potential. The foam stability was high in all the fractions except glutelin (Table II).

Figure 6 shows the changes in foamability and foam stability at pHs ranging from 1.5 to 9.5. Albumins had low foamability and foam stability at acidic pH with a minimum at pH 3.0. The foamability and foam stability increased gradually with an increase in pH and leveled off above pH 6.5. The pH–foamability curves of the other three fractions were similar to the pH–solubility curves, with lowest foamability at slightly acidic pH. The foam stability in globulins and glutelins increased with increase in pH, while in prolamins, the foam stability was lower at alkaline pH than at acidic or neutral pH.

The functionality of vital gluten and soy protein isolates, two widely used plant proteins, had been determined and compared to that of oat protein concentrates (Ma, 1983a) and isolates (Ma, 1983b). The present data show that the oat solubility fractions had emulsifying activity and fat-binding capacity comparable to those of gluten and soy isolate and the albumin fraction had much better foaming properties. The comparatively higher foamability of oat protein concentrates than of isolates (Ma, 1983a,b) could also be due to the presence of the albumin fraction in the concentrates.

Protein denaturation is generally regarded as detrimental to functionality (Kinsella, 1976). The present data, however, show that oat glutelins still had functional properties comparable to those of the other oat fractions. The results suggest that glutelins were not completely denatured, and a partial denatured conformation is considered desirable in proteins for certain functionality (Kinsella, 1976).

The data from this study show that oat protein fractions, particularly globulins, have properties unique among other cereals. Oat globulins are shown to have striking similarity with the legumin-like (11S) storage globulins of some legume seeds in terms of amino acid composition and sequence, molecular weight of the subunits, and the association of the subunits through disulfide linkages (Peterson, 1978; Brinegar and Peterson, 1982; Walburg and Larkins, 1983). Wright and Boulter (1980) studied the thermal transitions of extracted proteins from some legume meals and found that legumin has a T_d above 100 °C, considerably higher than that of the other globulin fraction, vicilin. The unusually high T_d in both legumin and oat globulins further substantiates the similarity between the two types of proteins in structural organization, since the thermal stability (and denaturation) of macromolecules is dependent on the molecular structure and nature of the bondings. As suggested by other workers (Brinegar and Peterson, 1982; Walburg and Larkins, 1983), such a homology may indicate a common ancestral gene for the two types of polypeptides.

The exceptional high T_d for oat globulins may also have some practical significance in foods, indicating that this protein fraction can be used in food formulations requiring high thermal stability. However, such stability could limit the use of oat globulins in food applications that require a change of state at conventional processing temperature, e.g., heat-induced gelation or coagulation in baking and meat emulsion systems.

Oat protein concentrates (Ma, 1983a) and isolates (Ma, 1983b) were found to have good emulsifying and binding properties. The present data show that the oat protein fractions also possess such functionality, suggesting a potential use of the oat proteins as a meat binder or extender. Specific fractions such as albumins may find use in foods requiring high foamability, e.g., cake and whipped topping. A full assessment of the potential of oat proteins as a food ingredient would require evaluation of the proteins in various food formulations. With a gradual decline in worldwide oat production, identification of the unique functionality in oat components through research may help to rekindle the interest in this nutritionally superior crop and promote its use for human consumption.

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Fractionation of Proteins from Low-Tannin Sorghum Grain

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The effects of various parameters on the extraction of the Osborne protein fractions from low-tannin sorghum grain were investigated with the objective of obtaining a protein fractionation procedure specifically optimized for sorghum. Methods were obtained for the quantitiative extraction of low molecular weight nitrogen (amino acids and peptides), albumins plus globulins, and prolamins. The protein fractionation procedure was applied to 16 locally (South African) grown sorghums and an International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) collection of 25 economically important sorghums. It was found that nearly 50% of the protein is in the form of prolamins and that the albumins plus globulins plus low molecular weight nitrogen make up nearly 25%. The figure of less than 30% for the residual glutelin fraction is much less than that found in previous investigations.

The fractionation of cereal seed proteins by the use of different solvents, based on the classical work of Osborne (1924), is still a most useful technique.

In sorghum it has been used for many purposes: Jones and Beckworth (1970) fractionated the proteins to determine their chemical composition. More recently this type of work has been extended to compare the composition of the proteins from different varieties of sorghum (Guiragossian et al., 1978; Neucere and Sumrell, 1979; Paulis and Wall, 1979). Fractionation has been used to explain the different responses of rats fed high- and low-tannin sorghum (Jambunathan and Mertz, 1973) and to determine which proteins are increased in high-lysine varieties (Jambunathan et al., 1975). It has shown which proteins are affected when sorghum grain is dehulled (Chibber et al., 1978) and micronized (Shiau and Yang, 1982) and which were rendered insoluble in high-tannin cultivars (Daiber and Taylor, 1982). The technique has also been used to investigate the changes in sorghum protein composition during seed development (Johari et al., 1981) and germination (Wu and Wall, 1980; Taylor, 1983).

Despite the importance of protein fractionation to sorghum research there has been no recent systematic investigation into the efficiency of the procedures used. This is in contrast to the situation with other cereals such as barley (Shewry et al., 1978) and maize (Landry and Moureaux, 1981). Workers have in general used adaptations of the method of Landry and Moureaux (1970) developed for maize. These authors introduce two important changes compared to previous methods of grain protein fractionation: the use of aqueous alcohol plus reducing agent after the aqueous alcohol extraction and a final extraction with basic buffer containing sodium dodecyl sulfate ($NaDodSO_4$) plus reducing agent. These changes resulted in much improved protein extraction. For example, Skotch et al., (1970), using a traditional-type procedure, only extracted an average of 31% of the protein in sorghum grain, whereas Jambunathan and Mertz (1973), using a Landry and Moureaux procedure, extracted an average of 89%.

However, the fact that procedures for fractionating sorghum proteins have not been more thoroughly investigated is surprising especially as it is known that kafirin (sorghum prolamin) is less soluble than zein, maize prolamin (Wall and Paulis, 1978). The difference is probably due to the more hydrophobic nature of kafirin.

In this study the effect of different parameters on the extraction of each protein group was investigated with the objective of obtaining a protein fractionation procedure specifically optimized for sorghum grain. The resulting procedure was then applied to a number of locally (South African) grown sorghums and to some from other parts of the world so that a general picture of sorghum seed protein

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