INTRODUCTION

INCREASED ATTENTION has been directed in recent years toward study and development of methods for production of protein concentrates and isolates. These processes involve aqueous wet milling fractionation of the cereal flour complex or separation of its components by mechanical means, such as air classification. Research at the Northern Regional Research Center has resulted in methods for producing oat, wheat, corn and triticale concentrates (CLUSKEY et al., 1973; Wu and Sexson, 1975a, 1976a; Wu et al., 1976). The composition and properties of these products have also been reported (Wu et al., 1973, 1976; Wu and Sexson, 1975b, 1976b).

In addition to the above established separations, nonaqueous media have been explored for use in separation of protein from cereal grains. This system, pioneered by Hess (1954) permitted isolation of two protein components from the endosperm of cereal grains and legumes. Rohrlich and Niederauer (1963) investigated the influence of lipids in this type of separation. Later, Barlow et al. (1973) separated wheat starch and storage protein by the solvent suspension method in their starch-protein interface investigations. More recently, Finley (1976) used nonaqueous solvent systems to produce functional proteins, a procedure which if used on a large scale would avoid waste water pollution and eliminate the cost of water removal. He reported the separation of protein and carbohydrates in wheat flour, soybeans, green peas and dried acid whey. The resulting protein fraction from wheat flour contained 58-81% protein while only 0.4-2.9% protein remained in the carbohydrate fraction.

In the present work, the density separation approach was used to separate the protein and carbohydrate fractions of oat flour, a cereal flour previously not investigated with the fluorocarbon solvent system used by Finley (1976). Oat groats were selected for this investigation because they have a high protein content and a nutritionally better amino acid balance than other common cereals (Shukla, 1975). Also, the system offered promise of yielding a protein concentrate valuable for food supplementation. When this investigation began, the fluorocarbons used were considered to be nontoxic. At the present time, their use as propellants in food products is under judgment by the Food & Drug Administration and the Environmental Protection Agency (Anonymous, 1976). Whether the ban will extend to use of fluorocarbons as separation media, which are easily removed and can be recovered, is yet to be determined.

MATERIALS & METHODS

THE OAT FLOUR was a gift from the National Oats Company, Cedar Rapids, IA. This commercial blend flour had been heat processed at the mill and had a protein content (nitrogen x 6.25) of 17.4% (dry basis). In order to reduce particle size, the flour (28% passed through a 100-mesh screen) was remilled twice in a Raymond hammermill until 52% passed through 100-mesh screen.

The fluorocarbons used were Freon 113 (1,1,2 trichlorotrifluoroethane) and Freon 11 (trichlorofluoromethane) by DuPont, Wilmington, DE.

The procedure most frequently used was as follows. Three grams of finely milled oat flour was added to 30 ml of the Freon-hexane mixed solvent contained in a Vitris homogenizing flask. The solvent mixtures were equilibrated to the desired temperature in a thermostated bath, 37°C with Freon 113-hexane and 20°C with Freon 11-hexane. The solvent–flour mixture was blended at high speed (> 9,000 rpm) in a Vitris homogenizer for 45 sec. rapidly transferred to a glass centrifuge tube and returned to the bath until ready for centrifuging. Samples were centrifuged either in an International, Model K, centrifuge fitted with a swinging bucket head (1600 x G) or in a Lourdes, Model LRA, refrigerated centrifuge with a VRA rotor for 14 min at 1600 x G.

The three-phase system which results consist of the floating protein, a solvent layer and the settled carbohydrate layer. The floating protein fraction was rapidly decanted into a tared weighing bottle. Frequently the middle section is decanted simultaneously. Ideally, the interstitial solvent was poured into another weighing bottle, leaving the carbohydrate layer in the centrifuge tube. The solvent was evaporated from the products by passing air over the weighing bottle in a hood.

Protein contents were calculated from duplicate micro-Kjeldahl analysis by multiplying percent nitrogen by 6.25 and are expressed on a dry basis. Nitrogen solubility and hydration capacity of the protein fractions were determined by AACC Approved Methods (1971). Emulsion activity and stability were established by the method of Yasumatsu et al. (1972) for a simple system. This system involved high speed homogenization of the protein with water and soybean oil, subsequent heating, and determination of the ratio of emulsified layer height to total layer height.

RESULTS & DISCUSSION

THE FRACTIONATION of cereal flours on the basis of density depends greatly on the effective density of the protein and the starch components. The work of Hess (1954) established densities of starch and protein of wheat at 1.500 ± 0.005 g/ml and 1.345 ± 0.002 g/ml respectively on a moisture-free basis. More recently Finley (1976) has extrapolated these data to moisture contents up to 16% and calculated the difference in density between the starch and the protein. He found that for effective separation of protein and carbohydrate fractions from wheat the moisture levels in the components should be between 10-12%, and optimum solvent density should be between 1.303-1.440. Oat groat flour has a moisture content of approximately half the protein available in the flour remaining in this fraction. Functional properties of the protein fractions such as hydration capacity, emulsifying activity, emulsion stability and nitrogen solubilities were determined.

ABSTRACT

Protein and carbohydrate fractions in oat flour were separated on the basis of density using nonaqueous solvent systems. Optimum solvent densities and solvent:flour ratios for maximum yield of protein fraction were determined. Fractions with over 70% protein content can be separated; however, they represent only about 40% of the protein available in the flour. The carbohydrate fraction contains about 10% protein, with approximately half the protein available in the flour remaining in this fraction. Functional properties of the protein fractions such as hydration capacity, emulsifying activity, emulsion stability and nitrogen solubilities were determined.

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Table 1—Protein composition of separated oat flour fractions in Freon 113-hexane at 30°C

<table>
<thead>
<tr>
<th>Solvent density</th>
<th>Solvent:flour ratio</th>
<th>Protein content (%)</th>
<th>Recovery solids (wt %)</th>
<th>Total flour protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Float</td>
<td>Sink</td>
<td>Float</td>
<td>Sink</td>
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<td></td>
<td></td>
<td></td>
</tr>
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<td>30:1</td>
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<td>8</td>
<td>9</td>
<td>76</td>
</tr>
<tr>
<td>10:1</td>
<td>57</td>
<td>9</td>
<td>12</td>
<td>77</td>
</tr>
<tr>
<td>5:1</td>
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<td>11</td>
<td>14</td>
<td>80</td>
</tr>
<tr>
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<td>12</td>
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<td>82</td>
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<td>61</td>
<td>14</td>
<td>10</td>
<td>84</td>
</tr>
</tbody>
</table>

a Defatted flour used

The relationship between density and solvent composition for mixtures of Freon 11 and hexane and Freon 113 and hexane is shown in Figure 1. Measurements of density variation were made with the Freon 113-hexane mixtures at 20 and 30°C. These plots illustrate the importance of temperature control relative to the change in solvent density.

Solvent to flour ratio

Complete solvent removal is important in the preparation of products which may be used for human or animal food. Ease of removal may also be an energy consideration; a lower boiling liquid requires less energy than a higher boiling one if the heat of vaporization and the heat capacity of the solvent mixture are similar. Along with ease of removal and recovery, the volume of solvent to be removed is a prime economic factor as well as an energy conservation consideration. Therefore, optimum solvent volume giving maximum separation with least volume was sought.

The effect of solvent:oat flour ratio on the separation of groat protein at two solvent densities is shown in Table 1. The highest ratio used in this work was 30:1, the lowest 1:1. Both defatted and nondefatted oat flour were tested.

With nondefatted flour, the optimum protein content and yields in the float portions were exhibited at the 5:1 ratio with both density solvents. As the ratio of solvent to flour decreased, the protein content of the float and the sink portions increased, as did the percentage of total flour protein separated into the two fractions. In the higher density solvent mixture the protein content of the float is generally lower than in the lower density solvent, whereas the amount of the total protein in the higher density solvent is greater. Most of the total flour protein usually remains in the sink portion in both solvents.

The protein content and recovery of the float fraction from the defatted flour were relatively constant in the 1.391 density solvent at all ratios; however, the percent total flour protein in the float varied. As the ratio is decreased in the 1.421 density solvent, the optimum total defatted flour protein in the float occurred in the 3:1 and the 2:1 solvent ratios; the highest protein content of 64% is shown at 2:1 ratio. As shown in Table 1, a decrease in the solvent:flour ratio results in an increased recovery of total flour protein both with defatted and nondefatted flours (excluding 1:1 ratio).

Solvent density vs protein separation

The protein content and amount of total flour protein contained in the float fraction is dependent on the solvent and the
density of the solvent. The relationship between solvent density and the protein content of the float fraction is shown in Figure 2. Results of protein extractions in two solvent systems, Freon 11-hexane (20°C) and Freon 113-hexane (30°C) are given.

The twice-milled oat flour separated in Freon 11-hexane at 20°C exhibits an increase in protein content of the float fraction with increase in solvent density to 1.38. The protein content of the float is 61% at this maximum in the curve. As the density is increased from 1.38, a decrease in protein in the float fraction is observed.

The protein content of the float fractions separated in Freon 113-hexane mixtures at 30°C from hexane-defatted and nondefatted oat flour is also shown in Figure 2. A solvent density of 1.38 is again the density where the protein content of the float showed a maximum value of 78% for nondefatted flour. As with Freon 11-hexane, increase of solvent density above 1.38 gives a decrease in protein content of the float fraction. Solvent densities lower than 1.38 tend to separate a float fraction containing less protein. In general, little difference in protein content of the float was observed when defatted instead of nondefatted oat flour was separated in Freon 113-hexane systems in the density range of 1.38–1.43. This observation is compatible with the conclusions of Rohrlich and Niederauer (1963) in their study of the influence of lipids on protein-density separation by organic systems. They showed that hexane separates only the neutral fats from the groat flour, and if the flour is further extracted with alcohol-ether, lipoproteins will be removed and will hinder the oat proteins from being separated from the flour.

Furthermore, the amount of the total oat flour protein contained in the float fraction varies with solvent and solvent density. Figure 3 shows the total flour protein separation behavior between densities of 1.31 and 1.45. In Freon 11-hexane the percent of the total protein separated in the float increased gradually as the density increased. In Freon 113-hexane no great difference was noted between defatted and nondefatted flour separations; however, a more rapid increase in amount of protein occurred between densities 1.36 and 1.42. Continued measurement of the defatted flour up to density 1.45 showed a constant increase to 65% total flour protein contained in the float fraction.

Functional properties of float protein

Isolated groat protein (45.2% protein) from a 2:1 ratio (1.419 Freon-hexane:flour) separation was refractionated in a similar manner to obtain a purer protein fraction for use in functional properties investigations. The refractionated float protein contained 60% protein.

The hydration capacity for the purified float protein was measured at neutrality and had a value of 4.28 (weight of hydrated sediment per weight of dry sample) (Table 2). The hydration capacity of an oat protein concentrate containing no water solubles from the similar commercial groat flour was 3.68.

Emulsifying activity and emulsion stability of the refractionated float protein were determined at neutral pH. Percentage values of 5 and 3, respectively, compared well with those of the concentrate used for comparison, namely, 4 and 2% Table 2. Promine D (soybean protein isolate) was concurrently run as a control and gave an emulsifying activity value of 33% and an emulsion stability of 43% under similar conditions. The low values given by the float fraction are no doubt caused by the pH range in which the tests were run. Oat protein concentrate prepared from the oat cultivar, Garland, has an emulsion stability value of 55% at pH 2.2 compared to 56% for Promine D, and at pH 8.6 both proteins have emulsion stability values of 47% (Wu et al., 1973).

Nitrogen solubility

The solubility of oat float protein at various pH values was determined by mixing 0.1g of the fraction with 10 ml of water and adjusting the pH from 2.0 to 9.8 by HCl or NaOH. After stirring and centrifuging (800 X G), the supernatant was analyzed for nitrogen by the micro-Kjeldahl method. The percentage of nitrogen soluble at various pH values is plotted in Figure 4. Maximum solubility of the float fraction is observed at pH 2 where 54% of the nitrogen is soluble. The fraction exhibits similar high solubility in the alkaline region near pH 10, where 48% of the nitrogen is soluble. Minimum solubility occurs between pH 4 and 7. The nitrogen solubility curve for an oat protein concentrate (67% protein) prepared from the same...
flour by a wet-milling procedure (Cluskey et al., 1976) at 1% solids concentration is also presented in Figure 4. The similarity of conformation of the curves indicated no striking difference in the two protein samples, although a difference in amount of protein concentrate solubilized is evident. The protein separated in a wet-milling process is considerably more soluble at the acidic (pH 2–4) and basic (pH 7–10) ends of the curve.

Now, in addition to wet-milling and air-classification procedures, oat protein concentrate has been prepared by density separation of oat flour, using nonaqueous solvent systems. Density-separated and wet-milled protein concentrates prepared with similar solvent-to-solid ratios both have protein contents of 59–60%. Separation of oat flour by freon-hexane mixtures, especially Freon 113-hexane, is comparatively simple to carry out and could possibly become a practical system for future oat concentrate preparation.

REFERENCES

Wu, Y.V. and Sexson, K.R. 1976b. Protein concentrate from normal and high-lysine corns by alkaline extraction: Composition and properties. J. Food Sci. 41: 512.

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