Density Fractionation of Wheat Flours in Nonaqueous Solvents. I. Effect of Flour Moisture Level on Distribution of Solids, Protein, and Ash Among Fractions[1]

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ABSTRACT

Moisture in pin-milled hexane-extracted soft and hard wheat flours was adjusted to low, intermediate, and high levels. The flours at each moisture level were then fractionated by sequential flotations at 0°C in a series of Freon TF-hexane mixtures, sp gr 1.350–1.450 at 25°C in 0.010 increments, to give 10–12 floating fractions plus residues. Protein was highest (68–75% db) in the first floating fraction from soft wheat flour and maximum (54–64%) in the first or second fraction from hard wheat flour. About 70% of the total solids from soft wheat flour were distributed in two high-density fractions containing 1–4% protein. About 75% of the solids from hard wheat flour were distributed in three to four high-density fractions containing 1–14% protein. Protein was high (up to 23%) in residues. Ash was uniform among floating fractions from both flours, but high (6–23%) in residues. Increasing flour moisture caused high-density components to float off earlier in the solvent sequence, but had little overall effect on fractionation.

Sedimentation in aqueous media is an invaluable technique for fractionating wheat flour, usually yielding four fractions: starch, gluten, starch tailings, and water-soluble constituents (Fellers 1973, Finney 1943, Sollars 1958, Yamazaki et al 1977). Water, which is a highly polar and reactive solvent, disrupts aggregates and extracts water-soluble material; gluten proteins are hydrated and agglutinated to form gluten, and starch is released. The properties of water that make it such an effective fractionation medium, however, can cause irreversible chemical and physical changes, and isolated fractions may not represent components as they exist in the original flour or endosperm (Gallus and Jennings 1971, Kasarda et al 1971). Such effects may not be disadvantageous in studies of processes that normally involve flour-water systems (eg, most baking processes). Relocations, reactions, and interactions caused by exposure to water, however, can be detrimental to studies in which the objectives are location, isolation, or characterization of components in their native states or a combination of these. They can also be detrimental when it is desirable to maintain specific associations (eg, starch-protein), particle size distributions, or other physical characteristics of the unfractionated flour.

To avoid solvent effects, density separations in nonaqueous media have been used for isolation and purification of small amounts of specific wheat fractions and for morphologic studies (Barlow et al 1973, Hess 1954, Hess and Mahl 1954, Kent 1966, Rohrlich et al 1972, St. Clair 1970, Simmonds 1972, Stevens 1973, Stevens et al 1963). Nonaqueous density methods also have been used to detect or remove mineral additives (Gustafson 1931, Kent-Jones and Amos 1947). Finley (1976) and Finley and Hautala (1973) made pilot plant-scale separations in nonaqueous media to determine the feasibility of such methods on a commercial scale for protein enrichment of various cereal flours.

The purpose of the present study was to develop a procedure for laboratory-scale separations of wheat flour into chemically or physically discrete fractions or both by sedimentation in nonaqueous solvents, and to characterize the fractions. This initial report describes the procedure, and provides data regarding distribution of solids, protein, and ash among fractions obtained from soft and hard wheat flours fractionated at different moisture levels.

MATERIALS AND METHODS

Flours

The soft wheat flour was a blend of straight-grade flours from soft red and soft white varieties (10.92% protein and 0.386% ash, 14% mb). The hard winter wheat flour was a blend of flours from Purkof and Comanche varieties (11.44% protein and 0.428% ash, 14% mb). Flours were milled in the Wooster Laboratory, pin milled at 18,000 rpm on an Alpine Kolloplex Type 160Z mill, and extracted with hexane in a Soxhlet extractor for 24 hr. For high moisture levels, flours were hydrated in a humidity cabinet. For low moisture levels, flours were dried under vacuum for five to six days. Each flour was also adjusted to an intermediate moisture level.

Solvents

Hexane (practical grade, or ligroin, bp 63–75°C) was redistilled before use. Freon TF (Freon 113, 1,1,2-trichloro-1,2,2-trifluoroethane) was used without purification.

Preparation of Solvent Mixtures

Freon TF (sp gr 1.565 at 25°C) and hexane (sp gr 0.6559 at 25°C) were mixed to give the desired specific gravity (±0.002) measured at 25°C. Measurements were made with hydrometers calibrated at 60°F (15.56°C) against water at 60°F, and the values reported are direct readings, without correction for deviations from calibration temperatures. Solvent mixtures (filtrates) were reused after readjustment of specific gravities.

Fractionation Procedure

Duplicate samples of flour, each equivalent to 500 g of flour at intermediate moisture level, were weighed into 1-l polypropylene screw-cap centrifuge bottles. The bottles were filled to the shoulder with Freon TF-hexane, sp gr 1.350 at 25°C (precooled to 0°C), capped, and placed in a refrigerated centrifuge at 0°C (Sorvall Model RC-3 with swinging bucket rotor HG-4L). After equilibration for at least 1 hr with occasional vigorous shaking, the samples were centrifuged for 5 min at 250 × *g*, for 5 min at 1,000 × *g*, and for 20 min at 6,500 × *g*. The floating layer and liquid phase were removed and filtered by vacuum on a Büchner funnel, and the resulting solids (Fraction I) were air dried at room temperature for 30–60 min until solvent odor could no longer be detected.

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Precooled medium, sp gr 1.360 at 25°C, was added to the sediment remaining in each bottle, and the bottles were returned to the centrifuge, equilibrated with shaking, and centrifuged as before. The floating layer and liquid phase were filtered and dried to give Fraction II. Flotation in media of increasing specific gravity (in increments of 0.010) was continued in this way until the sediment ("residue") was less than 10 g (estimated).

Analytic Procedure

Ash was determined gravimetrically after incineration of samples at 555°C for 15 hr; protein was calculated from Kjeldahl nitrogen (N × 5.7).

RESULTS AND DISCUSSION

Finley and Hautala (1973) have pointed out the advantages of fluorocarbons as media for density separations. Freon TF-hexane mixtures provide appropriate densities for flour fractionations, are nontoxic, and are easily removed from products by evaporation. The authors also noted the effect of temperature on solvent densities, and emphasized that temperature must be controlled precisely for protein-starch separations. Low temperatures facilitate control. Also, evaporation is minimized at 0°C and centrifugation can be performed in polypropylene centrifuge bottles, which swell on prolonged contact with Freon TF-hexane at higher temperatures. Over the density range used (sp gr 1.350–1.470 at 25°C), the following linear effect was established for the range from −20°C to 25°C:

$$\text{sp gr } t = \text{sp gr at } 25^\circ\text{C} + \frac{25^\circ\text{C} - t}{536^\circ\text{C}}$$

Therefore, sp gr at 0°C = sp gr at 25°C + 0.0466.

Data from fractionation of soft wheat flour of high moisture content (13.8%) are presented in Table I as an example of typical specific gravities, solids yields, protein and ash recoveries, and moisture distribution. "Actual" specific gravities were from measurements on filtrates at 25°C corrected to 0°C, and reflect decreases in media densities due to residual solvent in the sediments and to evaporation of Freon during filtration. Generally, solids yields duplicated within 5%, but variation was greater between duplicates of the high-yield, high-density fractions. Protein and ash contents of duplicates agreed within 6%. For the fractionation illustrated in Table I, total recoveries of protein and ash were 98.0 and 103.6% of flour protein and ash, respectively. Overall, recoveries of protein and ash ranged from 95.1 to 105.6% and 96.4 to 105.9%, respectively, for the six fractionations.

Protein/ash ratios were calculated for all fractions (three moisture levels) and used as indexes of protein-ash separation. Fractions I or II always gave highest values (133–175), and the residues gave the lowest value (1 in every case).

Moisture was determined in all fractions exceeding 10 g of solids, and the values in Table I typify moisture distribution. In general, moisture in low-density, high-protein fractions was lower than the original flour moisture, and moisture in high-density low-protein fractions was equal to or higher than flour moisture. These levels prevailed, however, after exposure of fractions to the atmosphere for 30–60 min under variable humidity. Total moisture in fractions usually approximated moisture content of the original flour, but total moisture in fractions from the low-moisture soft wheat flour averaged higher (5–6% moisture) than moisture in the original flour (2.9%), presumably due to moisture uptake during solvent removal.

Data from fractionations of the soft and hard wheat flours at intermediate moisture levels are given in Table II; Fig. 1 and 2 show patterns of solid yields at three moisture levels. Fraction I from fractionations of soft wheat flour constituted 7–8% of total solids regardless of flour moisture content, and contained about 70% protein (db). Fraction I from the hard wheat flour contained about 60% protein and contributed only 2–4% of total solids. Protein concentration was highest in Fraction I in every fractionation except that of the hard wheat flour of intermediate moisture (with maximum protein in Fraction II). Protein concentration generally declined with increasing density, but small secondary peaks appeared in the concentration curves (Fig. 3 and 4). In every fractionation, protein was substantially higher in the residue than in the final floating fraction, and exceeded 20% in residues from both high-moisture flours. Because of low solids yields, however, residues never contributed more than 1% of total protein. Protein concentrations were lowest (about 1%) in the final high-yield floating fractions, which were not necessarily the final floating fractions.

TABLE I
Nonaqueous Density Fractionation of Soft Wheat Flour (13.8% Moisture) by Flotation at 0°C in Freon TF-Hexane Mixtures: Specific Gravities, Solid Yields, and Protein and Ash Recoveries From a Typical Fractionation[a]

Fraction	Specific Gravity			Weight			Protein (Av)		Ash (Av)			
	Original Medium[b] (25°C)	Filtrate[b] (25°C)	Actual[c] (0°C)	Dupl. (1) (g)	Dupl. (2) (g)	Av (g)	Concn. (%)	Wt./100 g flour (g)	Concn. (%)	Wt./100 g flour (g)	Protein (%) / Ash (%)	Moist. (Av) (%)
I	1.349	1.333	1.380	37.80	38.08	37.94	74.48	6.14	0.448	0.037	166	10.7
II	1.359	1.342	1.389	4.42	4.82	4.62	70.38	0.71	0.429	0.004	164	–[d]
III	1.369	1.357	1.404	7.45	7.18	7.32	40.89	0.65	0.598	0.010	68	–[d]
IV	1.380	1.367	1.414	8.69	8.89	8.79	37.14	0.72	0.688	0.013	54	12.1
V	1.391	1.379	1.426	5.81	6.21	6.02	29.98	0.40	0.590	0.008	51	–[d]
VI	1.398	1.389	1.436	11.97	12.39	12.18	23.36	0.62	0.582	0.016	40	11.5
VII	1.411	1.398	1.445	27.08	25.47	26.28	17.92	1.04	0.565	0.034	32	12.7
VIII	1.419	1.408	1.455	27.71	27.55	27.63	11.39	0.70	0.457	0.028	25	13.2
IX	1.429	1.420	1.467	74.66	70.47	72.57	4.39	0.72	0.390	0.064	11	13.9
X	1.440	1.431	1.478	231.10	237.79	234.35	1.23	0.65	0.357	0.188	3	13.5
XI	1.451	1.446	1.493	12.08	8.48	10.29	3.34	0.08	2.114	0.049	2	13.2
XII		(Residue)		0.27	0.25	0.26	22.94	0.01	22.811	0.014	1	–[d]
Total						448.25		12.44		0.465		
Flour (db)						455.00		12.70		0.449	28	
Recovery (%)						98.5		98.0		103.6		

[a] All data on dry basis.

[b] Measured with hydrometers calibrated at 15.56°C against water at 15.56°C.

[c] Calculated from specific gravity of filtrate at 25°C.

[d] Not determined.

In contrast with protein, ash was distributed rather uniformly throughout the floating fractions (Fig. 5 and 6). It was much higher in the residues than in the floating fractions, however, and like protein, exceeded 20% in the residues from the high-moisture flours. Ash was generally slightly lower in the high-density fractions than in low-density fractions (ie, 0.4 versus 0.5%), but because of high solids yields, most of the total ash was contributed by the high-density fractions.

The effect of flour moisture on densities of flour components is evident from the solids patterns (Fig. 1 and 2). Hydration lowered densities, and consequently accelerated flotation in the solvent sequence. Therefore, with increasing moisture, solids peaks were displaced toward the low-density fractions. The protein curves (Fig. 3 and 4) also show the effects of moisture. Curves from both soft and hard wheat flours were shifted to lower densities, without significant changes in profiles. Ash peaks were likewise displaced (Fig. 5 and 6). Aside from these displacements, however, differences in flour moisture levels had little influence on protein and ash patterns.

The preceding data are condensed in Table III to allow comparison of the six fractionations. For each sample, data from Fractions I–VI were combined, as were data from Fractions VII–Residue. The table thus represents data for the hypothetical fractionation of each flour by a single flotation at 0° C in a medium of sp gr 1.400 at 25°C. Fractions I–VI would be combined as a single floating fraction, leaving the remaining fractions as a sediment. For each floating fraction, an index of protein enrichment was calculated as the ratio:

$$\frac{\text{Protein concentration in fraction}}{\text{Protein concentration in flour}}$$

which is also equal to the ratio:

$$\frac{\text{Percent of total protein in fraction}}{\text{Percent of total solids in fraction}}$$

A value greater than 1 indicates protein enrichment; less than 1, depletion. Analogous values were also calculated for ash.

TABLE II
Nonaqueous Density Fractionations of Soft and Hard Wheat Flour at Intermediate Moisture Levels by Flotation at 0°C in Freon TF-Hexane Mixtures[a]

Fraction	Sp gr[b] of Medium (25°C)	Solids (% of Total)	Protein Concn.[c] (%)	Protein % of Total	Ash Concn.[c] (%)	Ash % of Total
			Soft wheat flour (9.0% moisture)			
I	1.350	7.05	73.55	41.42	0.448	6.90
II	1.360	1.07	52.47	4.42	0.483	1.19
III	1.370	1.23	54.80	5.29	0.417	1.19
IV	1.380	1.32	35.82	3.73	0.483	1.43
V	1.390	1.65	36.22	4.68	0.593	2.14
VI	1.400	1.73	27.93	3.81	0.549	2.14
VII	1.410	1.66	24.05	3.12	0.488	1.67
VIII	1.420	6.30	23.89	11.70	0.406	5.48
IX	1.430	3.23	14.76	3.73	0.359	2.38
X	1.440	6.51	9.42	5.29	0.341	5.24
XI	1.450	40.89	3.17	10.14	0.361	31.67
XII	1.460	25.88	0.95	1.91	0.374	20.71
XIII	(Residue)	1.48	6.76	0.78	5.649	17.86
			Hard wheat flour (11.1% moisture)			
I	1.350	1.49	55.76	6.56	0.447	1.33
II	1.360	2.26	64.64	11.55	0.370	1.78
III	1.370	1.07	40.96	3.41	0.422	0.89
IV	1.380	1.55	36.32	4.46	0.483	1.56
V	1.390	3.01	35.81	8.40	0.517	3.11
VI	1.400	3.53	24.54	6.65	0.511	3.56
VII	1.410	7.30	19.85	11.29	0.524	7.56
VIII	1.420	23.32	14.00	25.28	0.413	18.89
IX	1.430	15.92	9.34	11.55	0.382	12.00
X	1.440	29.02	4.15	9.36	0.385	22.00
XI	1.450	11.04	1.15	0.96	0.701	15.11
XII	(Residue)	0.49	14.63	0.52	12.633	12.22

[a] Sp gr 1.350–1.460 at 25°C. Averages from 500-g duplicates.
[b] ± 0.002.
[c] Dry basis.

Table III clearly shows that when the separations are reduced to the above two fractions, increases in flour moisture greatly increased recovery of solids, protein, and ash in the combined low-density fractions (Fractions I–VI). Protein recovery in this fraction was considerably higher for the soft wheat flour than for the hard wheat flour at all moisture levels. Protein enrichment index was about 4.5 for the low-density solids from the soft wheat flour, but only about 3 for the hard wheat solids. Ash enrichment index, on the other hand, was approximately 1 for every fraction, indicating little change in ash level from the original flour ash level.

Overall, the high-protein, low-density fractions and the low-protein, high-density fractions fall in the density ranges of gluten protein and starch, respectively (Finley and Hautala 1973); preliminary examinations substantiate these identities. Presumably, intermediate fractions consist mainly of starch-protein aggregates. Studies are in progress to characterize fractions further.

The separation of protein from minerals is significant, since usually protein and ash in flours and millstreams is positively correlated (Ziegler and Greer 1971). The relatively uniform level of ash among fractions indicates a general distribution, not a pronounced association with high-protein portions of the endosperm. Since 80% or more of flour mineral matter can be extracted with water, most of it appears in the water-soluble fraction from aqueous fractionation (Clements 1977). Microscopic examinations of high-ash, high-density residues show high concentrations of spherical bodies less than 2 μ in diameter and assumed to be derived from aleurone (Stevens 1973). Protein/ash ratios approached 1 in the high-density residues, suggesting a direct

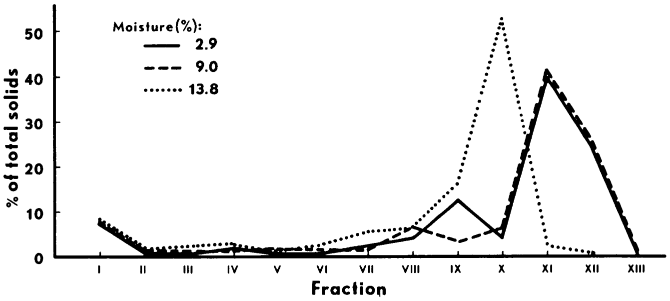

Fig. 1. Distribution of solids in floating fractions and final residue from nonaqueous density fractionations of soft wheat flour at three moisture levels. (Flotations at 0°C in Freon TF-hexane mixtures, sp gr 1.350–1.460 at 25°C in increments of 0.010.)

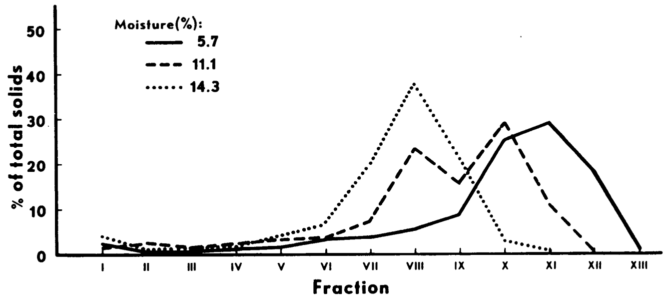

Fig. 2. Distribution of solids in floating fractions and final residue from nonaqueous density fractionations of a hard wheat flour at three moisture levels. (Flotations at 0°C in Freon TF-hexane mixtures, sp gr 1.350–1.460 at 25°C in increments of 0.010.)

association between protein and minerals in this fraction. Minerals appearing so associated usually were distributed between the residue and the last floating fraction, and comprised more than 10% of total flour ash (Table II).

The effects of moisture level agree with calculations that predict maximum differences between densities of flour proteins and starch at low moisture levels (Finley and Hautala 1973). These calculations, however, assume equal distribution of moisture between protein and starch in hydrated flour, and this seems unlikely. Data from this study suggest that at a particular flour moisture, moisture concentration is higher in starch than in nonstarch components of the flour. Such distributions, however, must be confirmed by appropriate moisture equilibrium studies.

The results of this study provide guidelines for a technique that is not new but that has been applied only to a limited extent for fractionation of whole flours. Since the solvent does not interact with hexane-extracted flour components, the separation process should not affect their properties. Because of this lack of interaction, however, aggregated material is not broken down, and separation into homogeneous fractions requires prior disruption of aggregates. Soft wheat flours are therefore more amenable than hard wheat flours to fractionation. Preliminary baking studies of flours reconstituted from fractions obtained by the procedures described in this study produced cookies that were identical to those produced from the original flours. This preservation of functionality agrees with results reported by Finley and Hautala (1973), who found that nonaqueous processing did not affect bread quality adversely.

Although the sedimentation process in itself should not affect functionality, pretreatments such as pin milling or other means of comminution designed to reduce aggregation may affect baking behavior. Therefore, for application to reconstitution and baking studies, the fractionation technique should be most useful when particle-size reduction is a normal flour treatment (eg, cake flours). For applications in which such a pretreatment can affect baking behavior adversely (eg, reduce cookie spread), avoidance of mechanical reduction, with a sacrifice in fractionation efficiency, may be desirable. Studies are now in progress to determine the usefulness of nonaqueous fractionation for reconstitution and baking studies of soft wheat flours.

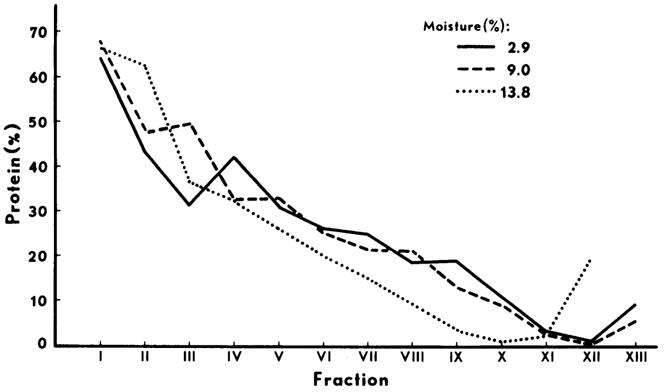

Fig. 3. Protein concentration (as-is basis) in floating fractions and final residue from nonaqueous density fractionations of soft wheat flour at three moisture levels. (Flotations at 0°C in Freon TF-hexane mixtures, sp gr 1.350–1.460 at 25°C in increments of 0.010.)

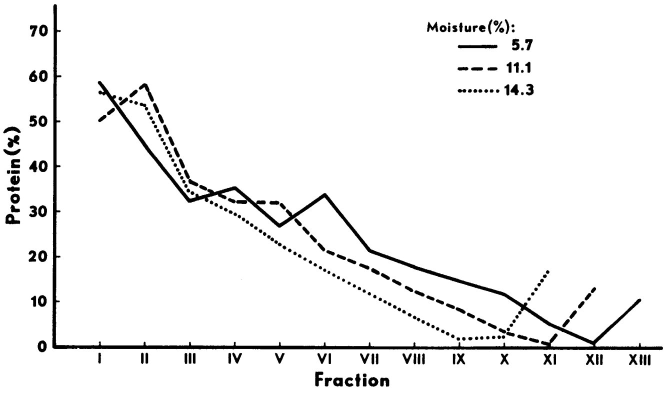

Fig. 4. Protein concentration (as-is basis) in floating fractions and final residue from nonaqueous density fractionations of hard wheat flour at three moisture levels. (Flotations at 0°C in Freon TF-hexane mixtures, sp gr 1.350–1.460 at 25°C in increments of 0.010.)

TABLE III
Nonaqueous Density Fractionations of Soft and Hard Wheat Flours at Three Moisture Levels[a]

Flour Moisture (%)	Fractions I–VI (Combined) % of Total Solids	% of Total Protein	% of Total Ash	Protein[b] / Solids	Ash[c] / Solids	Fractions VII–Residue (Combined) % of Total Solids	% of Total Protein	% of Total Ash	Protein[b] / Solids	Ash[c] / Solids
					Soft wheat flour					
2.9	10.80	49.88	10.87	4.62	1.01	89.20	50.12	89.13	0.56	1.00
9.0	14.05	63.35	14.99	4.51	1.07	85.95	36.65	85.01	0.43	0.99
13.8	16.78	74.73	19.41	4.45	1.16	83.22	25.27	80.59	0.30	0.97
					Hard wheat flour					
5.7	10.46	34.62	9.88	3.31	0.94	89.54	65.38	90.12	0.73	1.01
11.1	12.91	41.03	12.23	3.18	0.95	87.09	58.97	87.77	0.68	1.01
14.3	18.96	52.17	19.48	2.75	1.03	81.04	47.83	80.52	0.59	0.99

[a] Data from sequential flotations combined to show fractionation predicted from single flotation at 0° in medium of sp gr 1.440 at 25°C.

[b] $\frac{\text{\% of Total protein}}{\text{\% of Total solids}} = \frac{\text{Protein concn. in fraction}}{\text{Protein concn. in flour}} = \text{Protein enrichment index.}$

[c] $\frac{\text{\% of Total Ash}}{\text{\% of Total solids}} = \frac{\text{Ash content of fraction}}{\text{Ash content of flour}} = \text{Ash enrichment index.}$

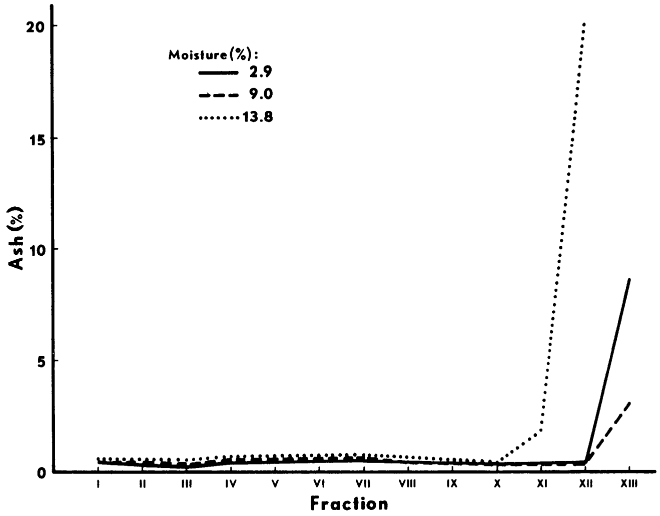

Fig. 5. Ash content (as-is basis) of floating fractions and final residue from nonaqueous density fractionations of soft wheat flour at three moisture levels. (Flotations at 0°C in Freon TF-hexane mixtures, sp gr 1.350—1.460 at 25°C in increments of 0.010.)

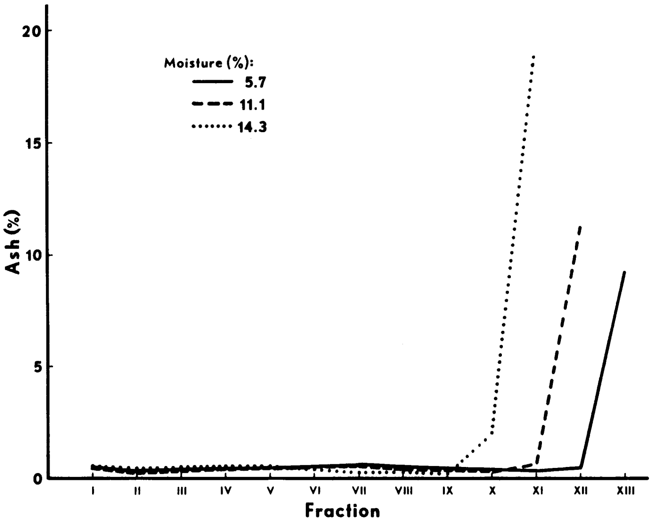

Fig. 6. Ash content (as-is basis) of floating fractions and final residue from nonaqueous density fractionations of hard wheat flour at three moisture levels. (Flotations at 0°C in Freon TF-hexane mixtures, sp gr 1.350—1.460 at 25°C in increments of 0.010.)

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