

Defatted and Reconstituted Wheat Flours.

IV. Effects of Flour Lipids on Protein Extractability From Flours That Vary in Bread-Making Quality

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ABSTRACT

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A poor quality flour and two good quality flours were defatted with Skelly B, benzene, acetone, and 2-propanol, listed in the order of increasing ability to remove polar lipids. Protein extractability with 0.01M sodium pyrophosphate (Na-pp) buffer, 0.005N or 0.05N acetic acid (AcOH), or 3M urea in Na-pp buffer decreased curvilinearly with increasing lipid removal. Decreases were greater for gluten (urea or AcOH extracts) than for albumin and globulin (Na-pp extracts) proteins. The decreased protein solubilities of defatted flours were, to a large extent, restored when the defatted flours were reconstituted with their respective extracted nonpolar or total (nonpolar plus polar) lipids, but not with polar lipids alone. Protein solubil-

ity, like bread-making quality, was almost completely restored when lipids extracted with Skelly B were returned to the defatted flour, but not when 2-propanol extracted lipids were recombined with their flour residue. The effects of lipids on protein extractability in flour depended on the quantities and types of lipids and their association with proteins. Urea extraction differentiated best between good and poor quality flours; 0.05N AcOH extraction appeared to be a more sensitive indicator of the relation between protein extractability and bread-making characteristics (especially mixing time) of flours reconstituted with lipids.

Wheat flour lipids and their role in bread making have been comprehensively reviewed (Chung and Pomeranz 1977, Mecham 1971, Morrison 1976, Pomeranz 1971, Pomeranz and Chung 1978), and the importance of wheat flour proteins in bread making is well recognized (Goforth et al 1977, Hosney et al 1969 a,b, Kasarda et al 1971, Pomeranz 1965). Investigators have also studied, using several extractants, protein extractability from flours that vary in bread-making quality (Huebner 1970, Huebner and Wall 1976, Orth and Bushuk 1972, Tanaka and Bushuk 1972) or from a single flour (Bietz and Wall 1975, Jankiewicz and Pomeranz 1965). Sodium pyrophosphate (Na-pp) buffer generally extracts albumin and globulin proteins; much gluten protein can be extracted with a dilute acid, such as acetic acid (AcOH), or urea solution. Extraction of high-molecular-weight glutenin proteins requires rather harsh solvents, such as dilute HgCl₂ solution (Mecham et al 1972), which nevertheless does not dissolve all glutenin proteins. Use of denaturing or reducing agents, detergents, or alkali with HgCl₂ solution may still leave some proteins insoluble (Bietz and Wall 1975).

In studying the lipids associated with gliadin and glutenin, Olcott and Mecham (1947) found that more than 80% of the gluten lipids were associated with glutenin proteins precipitated from gluten by acid. Chung and Tsen (1975) fractionated acid-soluble proteins into glutenin and gliadin by gel filtration chromatography. In flour, 61% of acid-soluble gluten lipids (48.4% of nonpolar and 100% of polar) were associated with glutenin; but in the optimally mixed dough, 77% (66.3% of nonpolar and 94.3% of polar) were associated with glutenin. Ponte et al (1967), however, found that most lipids were associated with gliadin isolated from gluten by 70% alcohol. Later, Hosney et al (1970) discovered that association of lipids with gliadin and glutenin depended on the conditions used to

isolate glutenin and gliadin.

Simmonds and Wrigley (1972) found that much less protein was extracted with 6M urea from gluten than from storage proteins defatted by chloroform-benzene mixtures. Reconstitution experiments, involving wetting of storage proteins in the presence of the added flour lipids, suggested that the reduced gluten solubility resulted from lipid-protein association during dough formation. Charbonnier (1973), however, showed that protein solubility in 55% ethanol decreased when flour was defatted with ethanol-ether-water (2:2:1, by vol). Fractionation of proteins on Sephadex G-100 and by electrophoresis and characterization by amino acid analyses showed that the decrease in protein extractability was mainly due to a decreased solubility of glutenin. Recently, Kobrehel and Bushuk (1977) demonstrated that AcOH-soluble glutenin isolated by a pH precipitation method (Orth and Bushuk 1973) was completely solubilized in aqueous sodium palmitate or sodium stearate solutions. Of fatty acids tested, only hexanoic acid solubilized some glutenin, suggesting that glutenin becomes insoluble largely because of hydrophobic interactions among proteins, perhaps involving flour lipids.

Thus, much evidence indicates that lipids can suppress (Simmonds and Wrigley 1972) or enhance (Charbonnier 1973) the solubility of isolated or flour proteins. We have extended previous studies by further investigating: (a) effects of lipid removal on flour protein extractability; (b) effects of lipid and protein extractants on protein solubility; (c) extent to which polar, nonpolar, and total (unfractionated lipids, ie, the sum of polar and nonpolar) lipids restore protein solubility in defatted flours; (d) effects of lipid extractants, especially 2-propanol, on flour components other than lipids, likely proteins; and (e) differences in protein-lipid interactions among flours that vary in bread-making quality. These investigations may give a better understanding of flour lipid-protein interactions and of their effects on bread making.

MATERIALS AND METHODS

Materials

Untreated straight-grade flours were experimentally milled (Allis) from two hard red winter wheats harvested in 1973 (Chiefkan/Tenmarq [Cfk/Tm, KS501097] and Shawnee [C.I. 14157]) and from a composite grist of many wheat varieties harvested throughout the Great Plains in 1973 (Regional Baking Standard [RBS-74]). The untreated and reconstituted flours (the extracted lipids were added back to the defatted flour) had the characteristics listed in Table I (Chung et al 1977). Shawnee and RBS-74 were good bread-making quality flours and Cfk/Tm was

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extremely poor.

All reagents and solvents were analytical reagent grade and deionized distilled water was used throughout.

Analytical Methods

Protein, ash, and moisture were determined by AACC methods. Nitrogen contents of protein extracts were determined by the AOAC micro-Kjeldahl method; protein content was calculated as $N \times 5.7$.

Lipid Extraction and Fractionation

Lipids were extracted from flours with Skelly B, benzene, acetone, and 2-propanol by a regular or a modified vacuum Soxhlet, as previously described (Chung et al 1977a). The extracted flour lipids were fractionated by silicic acid column chromatography into nonpolar and polar fractions with chloroform and methanol, respectively (Chung et al 1977a). Total lipids are defined as unfractionated lipids, ie, the sum of nonpolar and polar lipids, which were extracted with the solvents given in the text. Nonpolar lipids and polar lipids are defined as fractions eluted by chloroform and methanol, respectively. Each extraction was replicated twice, and lipid fractionation was duplicated for each replicate. Total recovery from silicic acid column fractionation ranged from 90.1 to 99.2%; average recovery was 95.1%

Reconstituting Defatted Flours with Flour Lipids

The defatted flour (75 g, dry basis [db]) was blended with total, nonpolar, or polar lipids in a Stein mill for 1 min, so that the reconstituted flour would contain the same amount of lipids as did the extracted. Reconstituting defatted flour with polar lipids required blending (by hand) in a mortar for several minutes before blending in a Stein mill, because the polar lipids were semisolid and sticky, and made homogeneous blending difficult. As examples of the blending mixture, 75-g samples (db) of Shawnee flour defatted with Skelly B were reconstituted with (a) 787.5 mg (1.05% of flour weight) of unfractionated total lipids extracted with Skelly B, (b) 532.5 mg (0.71% of flour weight) of Skelly B-extracted nonpolar lipids, and (c) 255.0 mg (0.34% of flour weight) of Skelly B-extracted polar lipids (Table II).

Protein Extraction

Flour samples (2.000 g, db) were dispersed in 30 ml 0.01M Na-pp buffer (pH 7.0), 0.005N AcOH, or 0.05N AcOH and shaken for 30 min on a wrist-action shaker (Burrell Corporation, Pittsburgh, PA) at room temperature. Urea-dispersible proteins were extracted from 0.5000 g (db) flour with 40 ml 3M urea in 0.01M Na-pp buffer (pH 7.0) as described above. Extractants were prepared

TABLE I
Characteristics of Flours (Untreated or Reconstituted)^a

Characteristics	Flours		
	Cfk/Tm	Shawnee	RBS-74
Protein (%; 14% mb) ($N \times 5.7$)	13.4	12.7	12.4
Ash (%; 14% mb)	0.43	0.40	0.41
Bake mixing time (min)			
Untreated	7%	5.0	4.0
Defatted and reconstituted			
Skelly B	7%	6½	4%
Benzene	7%	6.0	6%
Acetone	1½	12%	8%
2-Propanol	3½	2(∞) ^b	2(∞) ^b
Loaf volume (cc)			
Untreated	53.6	78.3	77.7
Defatted and reconstituted			
Skelly B	53.6	78.9	76.5
Benzene	52.6	79.2	74.0
Acetone	58.1	72.7	74.0
2-Propanol	65.7	28.3	25.8

^aFrom Chung et al 1977b.

^bMixed for 2 min to incorporate the ingredients.

daily. Suspensions were centrifuged for 30 min at 4°C at 43,542 × g in a Sorvall RC-5 refrigerated centrifuge (DuPont Instruments, Newton, CT). Extraction was duplicated on two replicates of each defatted or reconstituted flour. Supernatants from Na-pp extracts were diluted to 100 ml, from AcOH extracts to 250 ml, and from urea extracts to 50 ml with the respective extractant. After 1 hr at room temperature, absorbances of diluted extracts were measured at 280 nm with a Beckman DU spectrophotometer. Average standard deviations of absorbance were 0.015, 0.013, 0.013, and 0.012 for Na-pp, 0.005N and 0.05N AcOH, and 3M urea extracts, respectively.

Calibration Curves

Protein concentrations could be determined from absorbance at 280 nm (A_{280}) using calibration curves that were developed. For Na-pp and AcOH extracts, A_{280} was plotted vs. nitrogen content, determined by micro-Kjeldahl analysis (Jankiewicz and Pomeranz 1965, Pomeranz 1965). For the 3M urea extracts, A_{280} is proportional to biuret transmission at 550 nm (Greenaway and Johnson 1974), and N content and biuret transmission are also linearly related (Pomeranz 1965), so A_{280} is linearly related to protein content.

RESULTS AND DISCUSSION

Flour Lipids

Increasing the solubility parameter of the solvent (Skelly B, benzene, acetone, and 2-propanol—in increasing order) increased flour lipid extractability (Table II). Solubility parameter is defined as the square root of cohesive energy density; Hoy (1970) calculated solubility parameter by using the vapor pressure for numerous solvents. Each solvent extracted from RBS-74 more lipids by regular Soxhlet extraction than by the modified vacuum Soxhlet, as reported previously (Chung et al 1977a). Increases in the amounts of total (unfractionated) lipids extracted by solvents of increasing solvent parameters were mainly due to polar lipids for all three flours.

Among lipid extractants, water-saturated butanol (WSB) is considered the most efficient for flour lipid extraction at room temperature (Mecham 1971, Morrison 1976). Our experience has shown that WSB extracts 1.60–1.64% total lipids (0.78–0.81% nonpolar and 0.79–0.86% polar) and petroleum ether (PE) extracts

TABLE II
Flour Lipids Extracted with Four Solvents

Flour	Lipid Extractant	Lipids (%; db) ^a Extracted		
		Total	Nonpolar	Polar
Chiefkan/Tenmarq	Skelly B (7.27) ^b	0.88	0.71	0.17
	Benzene (9.16)	1.04	0.74	0.30
	Acetone (9.62)	1.24	0.74	0.50
	2-Propanol (11.44)	1.41	0.78	0.63
Shawnee	Skelly B	1.05	0.71	0.34
	Benzene	1.18	0.71	0.47
	Acetone	1.23	0.71	0.52
	2-Propanol	1.47	0.75	0.72
RBS-74	Skelly B V ^c	0.91	0.64	0.27
	R	1.03	0.68	0.35
	Benzene V	1.07	0.69	0.38
	R	1.12	0.67	0.45
	Acetone V	1.14	0.66	0.48
	R	1.17	0.65	0.52
	2-Propanol V	1.34	0.70	0.64
	R	1.43	0.72	0.71

^aAverages of two extractions for total lipids (overall standard deviation: 0.01) and of four fractionations for nonpolar and polar lipids (overall standard deviation: 0.019).

^bSolvent solubility parameter at 25°C, according to Hoy (1970).

^cV=vacuum Soxhlet; R=regular Soxhlet. For Chiefkan/Tenmarq and Shawnee flours, a regular Soxhlet was used for lipid extraction.

0.83–1.09% total lipids (0.65–0.84% nonpolar and 0.11–0.27% polar) from many hard red winter wheat flours. Differences in solubility provide a convenient and useful means of separating wheat flour lipids into the major categories of free and bound. Lipids extracted by PE are arbitrarily defined as free and those by WSB, following PE extraction, as bound. We assume that WSB extracts all free and practically all bound lipids from flour. We further assume that Skelly B extracts all free lipids and that 2-propanol extracts free lipids plus most bound lipids (94% of nonpolar and 83% of polar lipids extracted with WSB).

Nonpolar lipids consist of glycerides (tri, di, and mono), steryl esters, free fatty acids, and free sterols; triglycerides are major components. Polar lipids are a mixture of glycolipids and phospholipids; digalactosyldiglyceride is a major glycolipid and (lyso)phosphatidylcholines and acylated (lyso)phosphatidylethanolamines are major phospholipids (MacMurray and Morrison 1970). The four solvents extracted similar types and amounts of nonpolar, but not polar, lipids (Chung et al 1977a).

Effect of Lipid Removal on Protein Extractability

Defatting slightly decreased the protein contents of the flours; 2-propanol caused the largest decrease. The poor quality Cfk/Tm flour exhibited a greater decrease in protein content (from 13.4 to 12.9%, 14% moisture basis [mb]) than the good quality Shawnee (from 12.7 to 12.6%) or RBS-74 (from 12.4 to 12.2%) upon defatting with 2-propanol. Lipid reconstitution either did not affect protein content or slightly increased it for the 2-propanol treatment. Part of the nitrogenous material in the lipid extracts was lost during purification and fractionation.

We compared the protein extractants 0.01M Na-pp, 0.005N and 0.05N AcOH, and 3M urea using the untreated composite RBS-74 flour and that flour defatted to different extents by the four solvents

listed in Table II. Figure 1 shows that proteins were extracted most by 3M urea, next by 0.05N AcOH, and least by Na-pp from the untreated and the defatted flours. Protein extractability decreased upon lipid removal. The decrease was minor for Na-pp-soluble proteins, but substantial for 0.005N AcOH, 0.05N AcOH, and 3M urea-soluble proteins. Irrespective of type of Soxhlet extraction, protein extractability depended on the amount of lipids removed; the relationship was, however, not linear (Fig. 1). Decrease in protein extractability was greatest, by far, for the 2-propanol defatted flour due to removal of bound lipids or to possible alteration of proteins by the solvent (2-propanol) itself, or both. These results indicate that flour lipids affect extractability of gluten proteins substantially more than extractability of albumin and globulin proteins, as noted by Charbonnier (1973).

We compared the extractabilities of proteins from flours, both untreated and defatted, varying in bread-making quality. The extractants were 0.05N AcOH and 3M urea (Figs. 2 and 3). More protein was extracted by 0.05N AcOH from the poor quality flour than from the good quality flours at any lipid level (Fig. 2). Others have shown also that more acid-soluble proteins can be obtained from poor than from good quality flours (Heubner and Wall 1976, Orth and Bushuk 1972). For the three flours used in this study, protein extractability decreased curvilinearly as amounts of extracted lipids increased.

A similar relation was established between the A_{280} of the 3M urea extracts and the amount of total lipids extracted (Fig. 3). Figures 2 and 3 are similar but suggest that poor and good quality flours are differentiated better with 3M urea than with 0.05N AcOH. Although both Shawnee and RBS-74 were good flours, Shawnee was the slightly better flour. A previous study (Pomeranz 1965) also showed that protein dispersibility in 3M urea differentiates between good and poor quality flours.

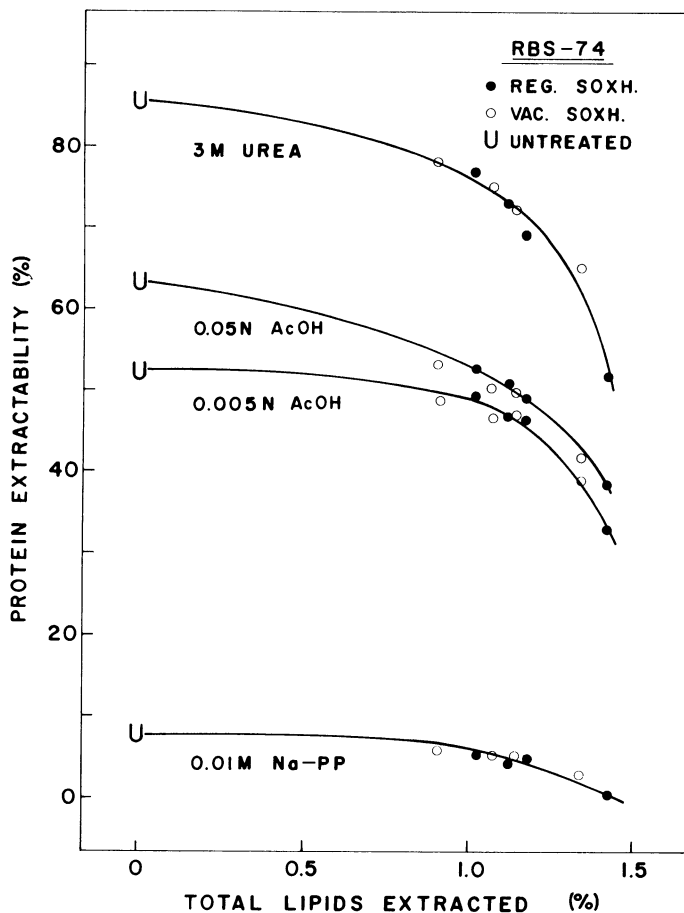


Fig. 1. Effects of unfractionated total (nonpolar plus polar) lipid removal on protein extractability in 0.01M Na-pp, 0.005N or 0.05N AcOH, and 3M urea from RBS-74 flour.

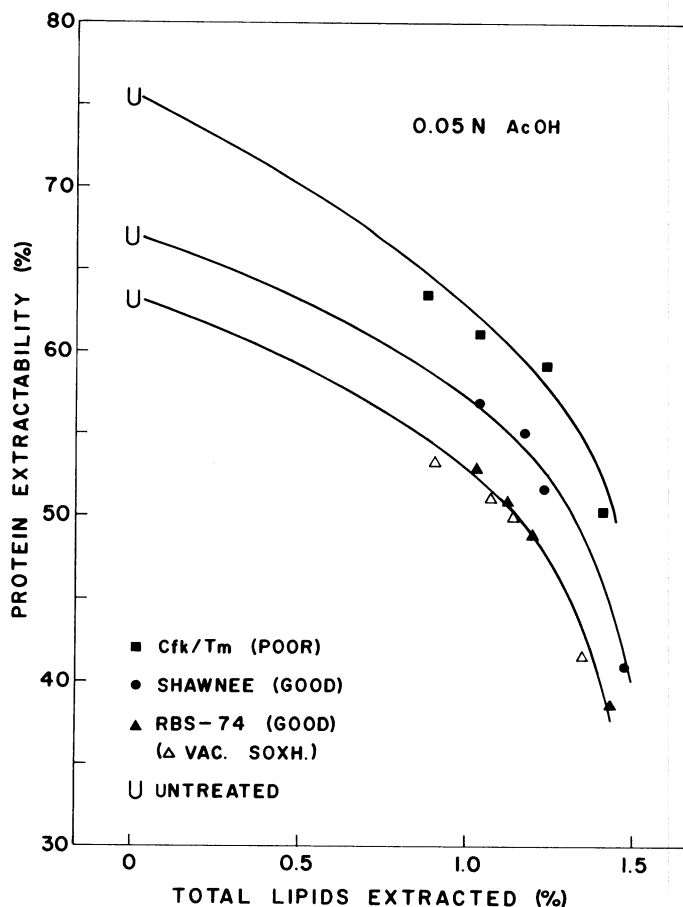


Fig. 2. Effects of total (nonpolar plus polar) lipid removal on protein extractability in 0.05N AcOH from poor (Chiefkan/Tenmarq) and good quality (Shawnee and RBS-74) flours.

Effects of Lipid Reconstitution on Protein Extractability

We next determined whether the decreases in protein extractability resulting from lipid removal can be reversed by reconstitution of the defatted flours with their extracted polar, nonpolar, or total lipids.

Reconstituting defatted flours with their polar lipids did not restore AcOH solubility of proteins in the poor quality flour (Fig. 4a), and slightly depressed solubility of proteins in the good quality flours (Fig. 4b and c). These effects on protein solubility contrast with the improving effects that polar lipids have on bread making when added to defatted flour (Chung and Pomeranz 1977, Mecham 1971, Morrison 1976, Pomeranz 1971, Pomeranz and Chung 1978). Addition of nonpolar or total lipids largely restored protein solubility of both poor and good quality defatted flours, but the extent of the improvement depended on the amount and type of lipids (free or bound) that had been extracted. For flours defatted with Skelly B which presumably extracts only free lipids, reconstitution with their extracted nonpolar or total lipids (extracted with Skelly B) completely or almost completely restored protein extractability (Fig. 4). However, as more bound lipids were extracted by solvents of increasing solubility parameter, protein extractability could only partially be restored by addition of the nonpolar or total lipids extracted by those solvents. Thus, reconstituting defatted flour with its extracted lipids (free plus bound) does not restore the original binding state between lipids and proteins.

The same conclusion could be made on the basis of baking studies of defatted flours reconstituted with total lipids (Chung et al 1977b) in the same manner as in the present study (Table I). For flours extracted with Skelly B or benzene and reconstituted with the extracted lipids, loaf volume was equal to that for the untreated flours, and mixing time was somewhat extended. Extraction with 2-propanol impaired the functionality of good flours, however, and substantially improved that of the poor quality flour. These results could be interpreted to mean that 2-propanol alters lipid-protein interactions to such an extent that adding the lipids (extracted with 2-propanol) back to the defatted flours does not completely restore

the original binding states and protein extractability.

Because the amounts and types of AcOH-soluble proteins are related to flour quality (Orth and Bushuk 1972), significant differences in protein aggregation may influence flour quality. If there is a "critical point" in protein aggregation, excessive aggregation beyond the critical point would be markedly detrimental to breadmaking quality. For the poor quality flour, Cfk/Tm, extraction with 2-propanol followed by reconstitution with total lipids decreased the level of acid-soluble proteins to nearly that in the untreated good flours (Fig. 4). The decrease might have been the cause of the improved mixing time and loaf volume of the reconstituted Cfk/Tm. In good quality flours, however, 2-propanol extraction might aggregate some proteins (glutenins, according to Charbonnier 1973) beyond the critical point such that protein solubility and good bread-making quality cannot be restored by lipid addition to the defatted flours. The irreversibly impaired bread-making quality of propanol-treated good quality flours could be due to excessive aggregation of glutenin proteins beyond the critical point. This possibility would agree with a previous report that glutenin governs mixing time, while gliadin controls loaf volume potential of wheat flour (Hoseney et al 1969a).

Increased solubility of proteins caused by addition of lipids to the defatted flour was detectable not only with AcOH but also with 3M urea extracts (Fig. 5). A₂₈₀ of urea extracts was higher than that of AcOH extracts, and was substantially higher for poor than for good quality flours at each lipid level. Adding unfractionated total lipids to defatted flours increased protein solubility to a greater extent than adding nonpolar or polar lipids separately. This suggests that both nonpolar and polar lipids may interact each other with proteins. Adding back the nonpolar or total lipids extracted by Skelly B from Cfk/Tm or Shawnee flour actually increased protein solubility over that in the untreated flour. We do not know the mechanism of this increase in solubility.

When 2-propanol defatted flours were reconstituted with their extracted lipids, more proteins could be extracted by 3M urea than by 0.05N AcOH. Of the two protein extractants, 3M urea seems to better differentiate among flours that vary in bread-making quality and 0.05N AcOH might more sensitively indicate changes in lipid-protein interactions. Possibly, 3M urea is too harsh to reveal the small decreases, shown by 0.05N AcOH, in protein solubility of good quality flours reconstituted with polar lipids.

Certain mechanisms of aggregation are consistent with the observed effects of lipids on solubility of proteins in flour. Proteins (probably glutenins) from defatted good quality flours might aggregate directly in the absence of lipids, but may aggregate to a slightly greater extent upon reconstitution with extracted polar lipids through protein-polar lipid-protein interactions. Protein aggregation could be largely reversed by addition of nonpolar or total lipids if aggregation had not exceeded the critical point. If these concepts are correct, the aggregation effects of polar lipids (in the absence of nonpolar lipids), as reflected by the protein concentrations in the AcOH extracts, should be more marked in an optimally mixed dough than in flour. Lipid binding is accelerated during dough-mixing and even by simply raising flour moisture content from 20 to 40% (Davies et al 1969); over that moisture range, binding of polar lipids was far greater than that of nonpolar lipids (Daniels 1974). Because water is important in hydrophobic bonding between proteins and lipids (Burley 1971), lipid-protein interaction would markedly differ in flour and dough. Our results of decreased protein extractability due to lipid removal agree with those of Charbonnier (1973) but not with Simmonds and Wrigley (1972), probably because Charbonnier and we studied protein extractabilities from flour. Simmonds and Wrigley (1972), on the other hand, studied solubility of freeze-dried purified proteins, which were mixed with water to make a dough-like mass after defatted and purified storage proteins had been reconstituted with lipids.

The effect of lipids on protein extractability from flour depended on the quantity and type of lipids involved as well as on their association with proteins. Our study was confined to wheat flours. Yet, our findings on lipid-protein interactions and their effects on protein solubility may have practical implications in bread making.

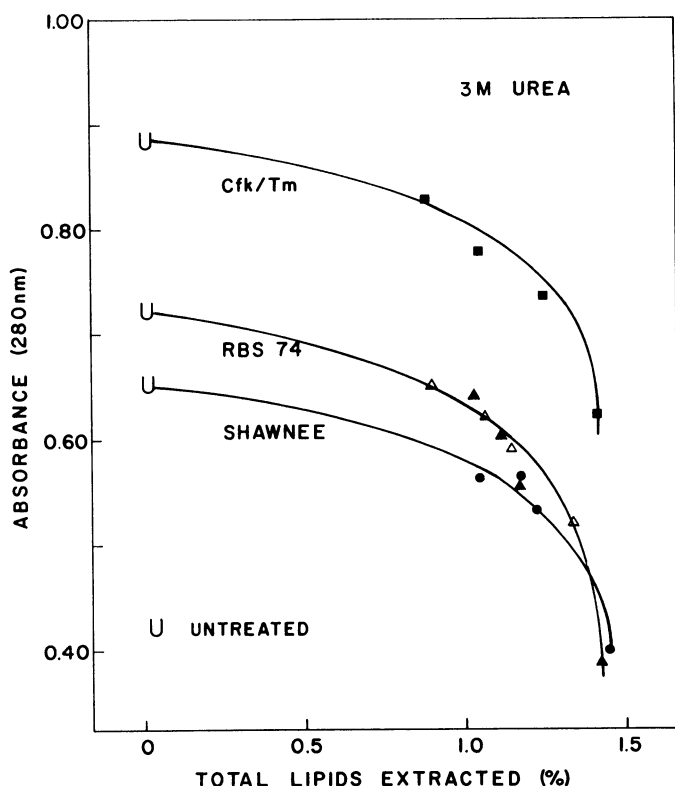


Fig. 3. Effects of total (nonpolar plus polar) lipid removal on absorbance (280 nm) of 3M urea extracts from poor (Chiefkan/Tenmarq) and good (Shawnee and RBS-74) flours.

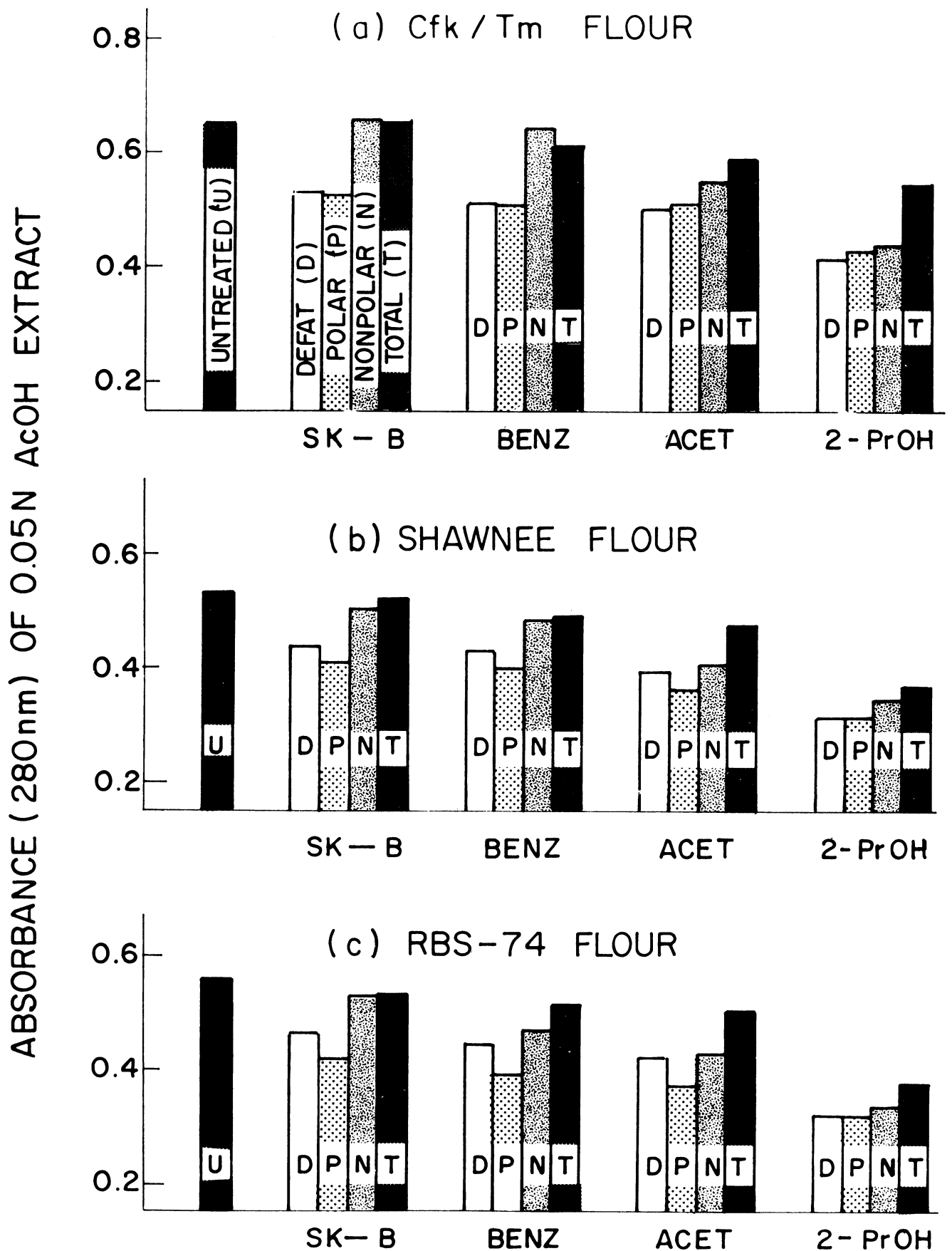


Fig. 4. Absorbance (280 nm) of 0.05N AcOH extracts of (a) Chiefkan/Tenmarq, (b) Shawnee, and (c) RBS-74 flours. The flours were untreated (U); defatted (D) with Skelly B (SK-B), benzene (BENZ), acetone (ACET), or 2-propanol (PrOH); or reconstituted with the extracted polar (P), nonpolar (N), or total (T) lipids.

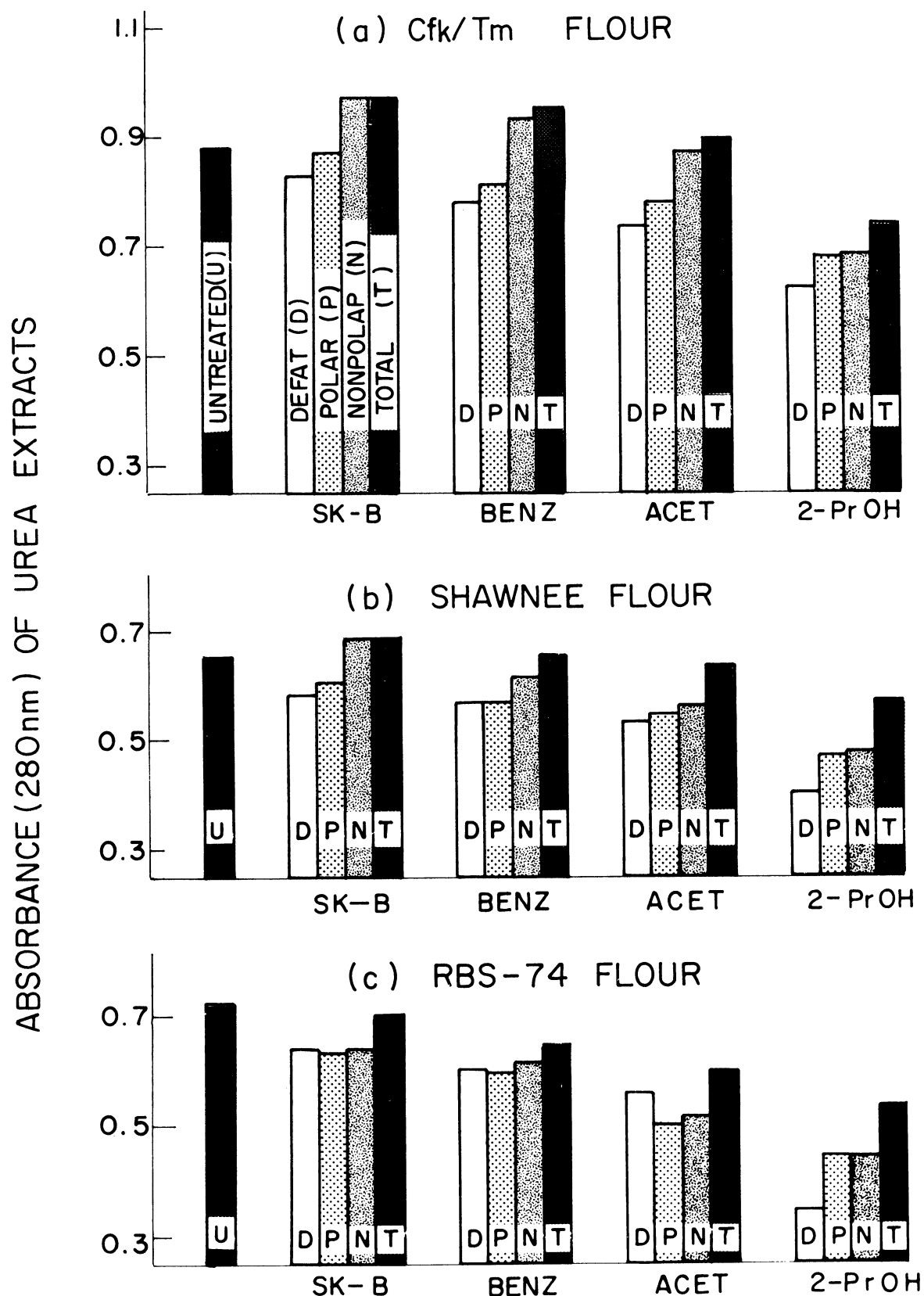


Fig. 5. Absorbance (280 nm) of 3M urea extracts of (a) Chiefkan/Tenmarq, (b) Shawnee, and (c) RBS-74 flours. The flours were untreated (U); defatted (D) with Skelly B (SK-B), benzene (BENZ), acetone (ACET), or 2-propanol (PrOH); or reconstituted with the extracted polar (P), nonpolar (N), or total (T) lipids.

Thus, additional investigations on the interactions between lipids and proteins in gluten during dough development, processing, and baking appear to be warranted.

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