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## VARIATIONS IN THE FATTY ACID COMPOSITION OF STORED WHEAT PROTEIN CONCENTRATES PREPARED BY WET AND DRY MILLING

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### ABSTRACT

The relative fatty acid composition of wheat protein concentrates prepared from millrun by dry milling (WPC) and by a wet alkaline process (WAP-WPC) were similar to reported values for bran and shorts. In general, linoleic was  $\geq 50\%$  of total fatty acids, whereas oleic, palmitic, and linolenic were 18.4–25.0, 20–22.5, and 2.5–3.9%, respectively. When stored at 50°C for 12 weeks the fatty acid composition of acid-precipitated WAP-WPC was more stable than that of the heat-precipitated WAP-WPC. GC analyses of the

volatiles of stored samples showed that WPC contained  $\geq 2$  times as much n-hexanal as heat-precipitated WAP-WPC, whereas mixtures of the two protein concentrates contained the least. When samples were tempered to 13% moisture and stored at 35°C for up to 24 weeks, the fatty acids of WPC were more stable than those of heat-precipitated WAP-WPC. Mixtures of these two protein concentrates exhibited greater stability at 35°C than did mixtures stored at their natural moisture at 50°C.

The inadequate supply of protein in the economically developing regions of the world and the maldistribution of protein in the economically developed regions have been well documented (1). The scarcity of protein might be partially alleviated by an increase in the per capita production of conventional sources. In addition, there is a need for more effective utilization of available protein sources which are currently being fed to ruminants. The latter approach has been applied to cereal by-products such as wheat millfeeds.

The annual production of millfeeds in the U.S. is approximately 5 million tons which contain some 800,000 tons of protein. The protein is rich in essential amino acids, especially lysine (4–5g/16g N) (2) and free of antinutritional factors which often accompany plant proteins. Processes have been described whereby millfeed proteins are concentrated by dry milling and sieving (3,4) or wet processing (5,6). Wheat protein concentrates prepared by dry milling (WPC) contain from 23 to 33% protein, whereas those prepared by a wet alkaline process (WAP-WPC) with the starch removed contain from 60 to 70% (3,5). The lysine content of these protein concentrates ranged from 4 to 5g/16g nitrogen (4,7).

The storage stability of WPC as Flour Blend A (70% straight grade hard winter wheat flour, 30% WPC) has been evaluated by criteria such as titratable acidity

and baking properties (8,9). In addition, the lipoxygenase activity of WPC has been reported (10). There are no reports in the literature on the storage stability of WAP-WPC or on the fatty acid composition of either WPC or WAP-WPC.

Since an alkyl resorcinol with antioxidant activity was reported to be present in WAP-WPC at a level of 0.03% (11), it was hypothesized that these protein concentrates might be quite stable during storage. The objectives of this study were to describe and compare the fatty acid composition of WPC, WAP-WPC, and those of other wheat products, and to determine the stability of the fatty acids of these protein concentrates, and combinations thereof, under accelerated storage conditions.

## MATERIALS AND METHODS<sup>1</sup>

### Preparation of Protein Concentrates

Protein concentrates were prepared from the millrun<sup>2</sup> of a hard red spring wheat by a wet alkaline process (5). The millrun was extracted at pH 8.5, the extract was centrifuged to remove starch and cell debris and the protein was then precipitated from the resultant supernatant by either acid (HCl pH 5.0) or heat (steam injection 85°C). Equal portions of both acid- and heat-precipitated fractions were either freeze, spray, or drum dried. Freeze drying was carried out at 25°C in a modified Stokes vacuum oven. Spray drying was done using a Bowen dryer with inlet and outlet temperatures of 232°C and 107–112°C, respectively. A steam-heated double drum dryer with a drum inlet temperature of

<sup>1</sup>Reference to a company or product name does not imply approval or recommendation by the USDA to the exclusion of other products that may be suitable.

<sup>2</sup>Wheat millrun is the fraction of the kernel remaining after removal of the flour during conventional milling of wheat.

TABLE I  
Composition of Wheat Protein Concentrates

Sample	Total solids %	Nitrogen %	Crude fat <sup>a</sup> %	Crude fiber %	Ash %	Starch %	Total sugars %	Reducing sugars %
Wheat Protein Concentrate (WPC)	86.99	2.97	3.35	2.89	2.17	39.9	4.5	0.6
Wet Alkaline Process WPC (WAP-WPC)								
Heat-precipitated								
freeze-dried	95.94	9.03	15.42	0.40	5.18	4.5	8.7	2.1
Spray-dried	99.57	9.13	11.60	0.48	5.27	5.5	7.2	0.6
Drum-dried	95.70	8.59	10.20	0.42	5.03	5.3	8.8	0.6
Acid-precipitated								
freeze-dried	99.18	9.64	12.87	0.47	3.91	6.4	10.8	3.1
Spray-dried	99.60	9.72	7.90	0.51	4.05	8.1	8.2	1.5
Drum-dried	94.12	8.90	7.91	0.42	3.86	7.0	8.8	2.6

<sup>a</sup>Ether extractives.

from 127 to 132°C was used for drum drying.

Dry milled wheat protein concentrate was prepared from the same hard red spring wheat millrun described above according to the procedure of Fellers *et al.* (3). Briefly, the millrun was thrice-milled using the reduction rolls and sifting system of a Brabender Quadrumat Senior flour mill. The fine, flour fractions (WPC) were combined.

Proximate analyses were conducted according to AOAC procedures (12). Starch was determined by the enzymatic procedure of Saunders *et al.* (13), whereas total and reducing sugars were analyzed by the phenol-sulfuric acid (14), and the dinitrosalicylic acid methods (15), respectively.

#### Storage

In Experiment I, dry-milled WPC and each of the protein concentrates prepared by the wet alkaline process as well as mixtures of WPC and WAP-WPC (4:1) were stored at their natural moisture content (Table I) in capped vials at 50°C for 12 weeks. For Experiment II, samples included the heat-precipitated, freeze-dried WAP-WPC, WPC, and various combinations thereof. These

TABLE II  
Lipid Content<sup>a</sup> of Wheat Protein Concentrates  
Stored for 12 Weeks

Sample	Storage Temperature	
	-23°C	50°C
	(% Lipid)	
Wheat Protein Concentrate (WPC)	4.43	3.99
WPC:Wet Alkaline Process WPC (4:1)		
WPC:WAP-WPC heat-precipitated, freeze-dried	8.31	7.26
WPC:WAP-WPC heat-precipitated, spray-dried	8.31	7.29
WPC:WAP-WPC acid-precipitated, freeze-dried	8.34	6.32
WPC:WAP-WPC acid-precipitated, spray-dried	7.45	7.17
Wet Alkaline Process WPC (WAP-WPC)		
Heat-precipitated freeze-dried	21.59	20.00
Spray-dried	22.23	18.79
Drum-dried	21.19	19.36
Acid precipitated freeze-dried	19.47	18.82
Spray-dried	17.69	18.44
Drum-dried	17.97	17.52

<sup>a</sup>Extracted with hot chloroform:methanol (2:1).

samples were tempered to 13% moisture and stored in capped vials. To ensure an adequate oxygen supply, all samples in both experiments were aerated by rotating uncapped vials for 1-2 sec weekly. In both Experiment I and II, duplicate random samples were removed from storage at appropriate time intervals and analyzed. Values expressed are the means of duplicate analyses.

#### Lipid Extraction

Protein concentrates (1g) were extracted with 50 ml of chloroform:methanol (2:1) under reflux for 30 min, cooled, and filtered through a Büchner funnel. The residue was washed with 10 ml chloroform:methanol (2:1); the washings and filtrate were combined and washed with 0.2 vol 0.58% KCl (16). The chloroform phase was collected, evaporated to dryness, and weighed for total lipid determination.

The lipid was then diluted with 25 ml of 0.25*N* ethanolic KOH and heated under reflux for 1 hr. After cooling, the solution was diluted with 50 ml water and twice-extracted with 25 ml of ether. After washing the ether extracts with 10 ml water, the water wash plus aqueous phase were pooled, acidified with 0.5*N* H<sub>2</sub>SO<sub>4</sub> to pH 3, and thrice-extracted with 20 ml ether. The ether extracts were pooled, twice-washed with 15 ml water, and evaporated to dryness under nitrogen to determine the weight of fatty acids.

The fatty acids were then dissolved in 30 ml methylating agent (2 ml H<sub>2</sub>SO<sub>4</sub> per 230 ml methanol) and refluxed for 1 hr under nitrogen. After cooling, the methylated fatty acids were diluted with 50 ml water and thrice-extracted with 20 ml hexane. Pooled hexane extracts were twice-washed with 15 ml water, dried over anhydrous sodium sulfate, filtered and concentrated to ~ 5 ml under nitrogen.

#### Gas-Liquid Chromatography of Methyl Esters

Methyl esters of fatty acids were analyzed on a Micro-Tech gas chromatograph Model 2000 R using a flame ionization detector. The 6 ft × 1/8

TABLE III  
Lipid Content<sup>a</sup> of Wheat Protein Concentrates  
Stored at 35°C

Sample	Storage Time in Weeks		
	0	12	24
Wheat Protein Concentrate (WPC)	4.49	4.24	4.15
WPC:Wet Alkaline Process WPC <sup>b</sup>			(% Lipid)
9:1	6.35	5.60	5.60
4:1	8.38	6.92	7.59
3:2	13.41	11.21	10.80
Wet Alkaline Process WPC <sup>b</sup>	21.66	21.45	18.48

<sup>a</sup>Extracted with hot chloroform:methanol (2:1).

<sup>b</sup>Wet alkaline process WPC was heat-precipitated and freeze-dried.

in. i.d. stainless-steel column contained 10% FFAP<sup>R</sup> on Chromosorb W, acid-washed DMCS. The gas chromatograph was operated isothermally at 170°C with detector and inlet temperatures of 250°C. The flow rate of the carrier gas, helium, was 30 cc/min at the detector. Depending on the concentration of the sample, 0.25  $\mu$ l. of the final methylated fatty acid solution was applied to the column.

The retention times of standard methyl esters were used to identify the major methyl esters of the protein concentrates. Quantitation was done by peak area and the use of correction factors obtained by gas chromatography of quantitative standards.

#### Gas-Liquid Chromatography of Volatile Constituents

Volatiles were collected from WPC, WPC:WAP-WPC (4:1), and WAP-WPC (heat-precipitated, freeze-dried), of Experiment I stored at -23° and 50°C for 12 weeks. The apparatus used was similar to that previously described (17). The samples (0.3g) were held at 40°C and stirred while helium (30 cc/min) swept the volatiles through a Carle switching valve and into a double-helix sample trap immersed in liquid nitrogen. After collection of the volatiles, the sample trap was

TABLE IV  
Fatty Acid Concentration of Wheat Protein Concentrates  
Stored for 12 Weeks (moisture-free basis)

Sample	Storage Temperature °C	Palmitic Oleic Linoleic Linolenic			
		(mg fatty acid/g of material)			
Wheat Protein	-23	5.2	4.8	15.1	1.0
Concentrate (WPC)	50	6.7	6.0	11.8	0.6
WPC:Wet Alkaline Process WPC (4:1)					
WPC:WAP-WPC, heat- precipitated, freeze-dried	-23	13.1	13.1	34.8	2.2
	50	10.5	11.8	29.2	2.0
WPC:WAP-WPC, heat- precipitated, spray-dried	-23	12.1	13.0	34.5	2.5
	50	10.8	10.8	28.0	2.0
WPC:WAP-WPC, acid- precipitated, freeze-dried	-23	11.4	11.6	32.9	2.1
	50	7.9	8.4	21.5	1.4
WPC:WAP-WPC, acid- precipitated, spray-dried	-23	11.2	10.3	28.1	1.9
	50	10.1	8.2	23.3	1.6
Wet Alkaline Process WPC (WAP-WPC)					
Heat-precipitated	-23	33.6	41.1	86.1	5.5
freeze-dried	50	28.9	35.6	73.5	6.7
Heat-precipitated	-23	36.5	40.7	80.1	5.4
spray-dried	50	36.5	31.4	64.1	5.1
Heat-precipitated	-23	32.8	33.4	86.7	4.7
drum-dried	50	24.2	27.4	68.7	4.0
Acid-precipitated	-23	33.1	37.5	75.3	5.1
freeze-dried	50	29.9	31.0	71.4	5.8
Acid-precipitated	-23	28.1	32.0	71.4	4.9
spray-dried	50	32.1	29.6	67.3	4.4
Acid-precipitated	-23	25.1	28.4	69.0	3.1
drum-dried	50	24.3	26.5	68.7	4.2

heated to 200°C for 2.5 min and the trapped volatiles were carried by water-saturated helium (20–25 cc/sec) through the Carle valve and into the column.

The column is one of a pair mounted in a modified Beckman Thermotrac oven with dual hydrogen-flame ionization detectors which were maintained at 200°C. The 920 ft × 0.03 in. i.d. open-tubular stainless-steel column was coated with methyl silicone SF96(50) (General Electric, Waterford, N.Y.) containing 5% Igepal CO-880 (General Aniline and Film Corporation, New York, N.Y.). The column was programmed from 60° to 180°C with  $\cong 2^\circ\text{C}$  rise per min. The volatiles of each sample were analyzed by two separate runs. Hexanal, the aldehyde of interest, was identified by the retention time of standard n-hexanal (J. T. Baker Chemical Co.).

## RESULTS AND DISCUSSION

### Composition

The WPC and WAP-WPC used in this study varied in composition as a result of preparation method (Table I). The dry milled WPC was higher in moisture, crude fiber, and starch; whereas, WAP-WPC contained 3 to 4 times as much nitrogen, more crude fat and ash than WPC. The general composition of WAP-WPC prepared and dried by different methods was similar for most analyses with the exception of crude fat. The AOAC (12) method with ether as the extractant was used for these determinations. The apparent differences in lipid content of the various WAP-WPC preparations were not evident when chloroform:methanol (2:1) was used as the extractant (Table II). Saunders and Betschart (18) have reported the difficulties of obtaining accurate lipid extraction with ether from protein concentrates which have been heated either during preparation or drying. Thus, WAP-WPC more accurately contains from 4 to 5 times as much lipid as WPC, and the lipid content of WAP-WPC prepared by various methods is not as variable as it may appear in Table I.

The influence of storage upon total lipid content is apparent in Tables II and III. In general, the quantity of lipid extracted by chloroform:methanol (2:1) diminished as a result of storage. The accelerated storage conditions rendered the lipids either less extractable or caused destruction of portions of the lipid fraction by oxidation and degradation.

### Fatty Acid Composition of Control Samples

Information on the fatty acid composition of WPC and WAP-WPC can be gleaned from the control samples of Experiment I (stored at  $-23^\circ\text{C}$ ). Fatty acids comprised 59% of the total lipid of WPC, whereas 77 and 78% of the lipid present in freeze-dried, heat- and acid-precipitated WAP-WPC, respectively, were fatty acids. These values were slightly lower than similar data for wheat bran (82%) and shorts (80%) (19). On a moisture-free basis, WPC contained 2.7% fatty acids, whereas freeze-dried, heat- and acid-precipitated WAP-WPC contained 16.6 and 15.5%, respectively. In general, WAP-WPC contained  $\geq 5$  times the quantity of each of the major fatty acids found in WPC (Table IV). Thus, the relative proportions of fatty acids of WPC and WAP-WPC were similar (Table V) and the fatty acid component of the protein concentrates differed mainly in quantity rather than quality or composition.

In agreement with earlier reports, the fatty acid composition of wheat lipids were relatively simple with 50% or more of the fatty acids of protein concentrates from wheat being linoleic (20,21). This has nutritional significance especially in WAP-WPC which contains 69.0 to 86.7 mg linoleic acid per g of material (Table IV). From 78 to 80% of the total fatty acids of protein concentrates prepared from wheat were unsaturated with 61.5% of the WPC and 51.7–58.6% of the WAP-WPC fatty acids being polyunsaturated.

The relative proportions of fatty acids of protein concentrates prepared from wheat, wheat shorts, and bran were quite similar (Table V) considering the difficulties of comparing fatty acid data from several laboratories in which different extraction procedures were used. Palmitic and oleic acids were slightly higher in the protein concentrates than in the shorts or bran, whereas some of the linoleic values for the protein concentrates were slightly lower.

#### Influence of Storage on Fatty Acid Composition

Deleterious changes in stored wheat products and protein concentrates from several sources have been attributed to alterations of the lipid fractions (8,22). If protein concentrates contain substantial quantities of unsaturated fatty acids, hydroperoxides are often produced. Breakdown products of these hydroperoxides are responsible for a variety of undesirable odors and flavors (23). Since linoleic acid comprises  $\geq 50\%$  of the fatty acids composition of WPC and WAP-WPC, the changes in concentration of this fatty acid as well as other major fatty acids were used as a criterion of deterioration during storage in this study.

WPC, WAP-WPC prepared in a variety of ways, and mixtures of these protein concentrates were evaluated at 13% moisture as well as at their natural moisture content. WPC was mixed with WAP-WPC to increase the relatively-low protein content of WPC, and to evaluate the potential antioxidant activity of WAP-WPC.

In Experiment I, in which the samples were stored at their natural moisture, the most dramatic changes were observed in oleic and linoleic acids. The heat-precipitated WAP-WPC showed a marked reduction in the concentrations of

TABLE V  
Fatty Acid Composition of the Lipids of Wheat Protein Concentrates  
Shorts and Bran

	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Others
	(% Total fatty acids)					
Wheat Protein						
Concentrate (WPC)	20.0	...	18.4	57.6	3.9	...
Wet Alkaline Process	20.0–	...	21.2–	49.2–	2.5–	...
WPC (WAP-WPC) <sup>a</sup>	22.4		25.0	55.0	3.1	
Shorts <sup>b</sup>	17.7	1.3	15.7	58.2	5.9	1.3
Bran <sup>b</sup>	17.9	1.0	16.8	57.3	5.6	1.3
Bran <sup>c</sup>	18.3	1.1	20.9	57.7	1.3	0.9

<sup>a</sup>Range of values for control samples prepared by acid and heat precipitation from the millrun of hard red spring wheat.

<sup>b</sup>Burkwall, M. P., and Glass, R. L. (1965). Milled from hard red spring wheat.

<sup>c</sup>Nelson, J. H., Glass, R. L., and Geddes, W. F. (1963). Milled from hard red spring wheat.

oleic and linoleic acids (Table IV). In contrast, the fatty acid composition of acid precipitated WAP-WPC, especially the drum-dried preparation, was quite stable after 12 weeks storage at 50°C. WPC exhibited losses of linoleic acid with concomitant increases in oleic and palmitic acids. Proportionally, the loss of linoleic acid in WPC was analogous to that of heat-precipitated WAP-WPC. However, since the total lipid content of WPC was  $\approx 20\%$  that of WAP-WPC, the absolute loss of linoleic acid was less.

In the WPC:WAP-WPC mixtures (4:1) the relative proportions of oleic and linoleic acids were similar in samples stored at  $-23^\circ\text{C}$  and  $50^\circ\text{C}$ . However, since less total lipid was extracted after  $50^\circ\text{C}$  storage there were similar decreases in all the fatty acids. In general, the fatty acids of mixtures of WPC:heat-precipitated WAP-WPC were more stable than those of the individual WAP-WPC. Since the lipids of heat-precipitated WAP-WPC were partially stabilized by mixing with WPC, the freeze-dried, heat-precipitated WAP-WPC was further investigated in a variety of combinations with WPC.

In Experiment II the moisture level was standardized to 13% for all samples and the temperature lowered to  $35^\circ\text{C}$  to simulate warm storage and shipping conditions. Under these conditions, as anticipated, WPC showed less deterioration than in Experiment I, especially with regard to linoleic acid concentration (Table VI). The heat-precipitated, freeze-dried WAP-WPC showed larger losses of linoleic acid after 12 weeks than did the samples stored at natural moisture for 12 weeks at  $50^\circ\text{C}$ . In addition to the linoleic acid losses,

TABLE VI  
Fatty Acid Concentration of Wheat Protein Concentrates  
Stored at  $35^\circ\text{C}$  (moisture-free basis)

Sample	Weeks Stored	Palmitic	Oleic	Linoleic	Linolenic	
		(mg/g fatty acids/g of material)				
Wheat Protein Concentrate (WPC)	0	5.5	4.9	15.0	1.1	
	12	5.1	5.2	15.1	0.9	
	24	4.9	4.9	13.4	0.7	
WPC:Wet Alkaline Process WPC <sup>a</sup>						
	9:1	0	6.4	7.0	18.8	1.2
		12	6.2	5.9	18.1	1.2
		24	6.1	6.4	18.8	1.2
	4:1	0	8.6	10.0	25.6	1.5
		12	6.8	7.3	20.1	1.2
		24	8.3	9.3	25.2	1.7
	3:2	0	15.1	16.8	41.3	2.8
		12	13.1	14.6	39.9	2.5
24		13.4	14.1	37.9	2.2	
Wet Alkaline Process WPC <sup>a</sup>	0	29.2	34.5	81.6	5.4	
	12	23.9	24.9	65.3	5.3	
	24	20.0	23.1	62.2	3.7	

<sup>a</sup>Wet alkaline process WPC was heat-precipitated and freeze-dried.



decreases of all fatty acids of this WAP-WPC sample stored at 35°C for 12 weeks were proportional as a result of diminished extractability of total lipid. In general, as expected, WAP-WPC was more stable when stored at the natural moisture level of 5%.

The fatty acids of various mixtures of WPC and WAP-WPC exhibited good stability at 13% moisture (Table VI). All of these mixtures stored 24 weeks at 35°C showed less loss of linoleic acid than did the mixtures stored at natural moisture and 50°C for 12 weeks (Table IV). Although the moisture level was less in samples stored at 50°C, the higher temperature was apparently sufficient to significantly accelerate deteriorative changes.

#### **n-Hexanal Content of Volatiles**

Cereal products which are stored at high temperatures in the presence of oxygen undergo deterioration with the resultant production of off flavors and odors. The lipid fraction, especially unsaturated fatty acids, are the main source of these undesirable organoleptic qualities (24). The initial reaction products of the oxidation of an unsaturated fatty acid are 13-hydroperoxy-9, 11-octadecadienoic acid (13-LAHPO) and 9-hydroperoxy-10,12 - octadecadienoic acid (9-LAHPO) (25,26). Hydroperoxides are associated with undesirable flavor notes such as bitter, musty, and stale (23). Enzymatic and nonenzymatic decomposition of hydroperoxides result in a variety of compounds. When linoleic acid was exposed to several lipoxygenases the following homologous aldehydes were detected in the headspace in order of decreasing concentration: n-hexanal; n-pentanal; n-butanal; n-propanal; and ethanal (27). Others have also identified n-hexanal as an important degradation product of linoleic acid (25,28,29). Since the concentration of n-hexanal has been correlated with flavor deterioration of other stored food products (30) it was used as an index of oxidative deterioration in this study.

The n-hexanal content of WPC, heat-precipitated, freeze-dried WAP-WPC and a 4:1 mixture stored under conditions of Experiment I was determined. No attempt was made to quantitate the hexanal. On the basis of visual comparison and attenuations used, WPC stored at -23°C for 12 weeks contained  $\cong$ 2 times as much n-hexanal as WAP-WPC (Fig. 1). The 4:1 mixture contained less than either of the constituent protein concentrate preparations. The chromatograms also illustrate the more complex array of carbonyl compounds present in WPC as compared to WAP-WPC (Fig. 1).

The samples stored at 50°C for 12 weeks contained  $\geq$ 2 times the quantity of n-hexanal as their respective controls at -23°C. Also, the relative n-hexanal content of WPC, WAP-WPC and the mixture were similar to the controls, *i.e.*, WPC contained  $\geq$ 2 times as much as WAP-WPC with the mixture containing the least. After 50°C storage more high-molecular-weight carbonyl compounds were present in the WAP-WPC. It is apparent that the 4:1 mixtures stored under both conditions contain the least amount of n-hexanal and show the least amount of unsaturated fatty acid degradation. These data confirm the fatty acid data in that the mixture of WPC:WAP-WPC appears to be quite stable. However, these results are somewhat in conflict with the fatty acid data upon examining individual WPC and WAP-WPC (Table IV). It may be of significance that the fatty acid composition of WPC stored at 50°C showed a definite decrease in linoleic with concomitant increases in palmitic and oleic acids. The

results for WAP-WPC, in contrast, indicate a general decrease in all fatty acids rather than just linoleic (Table IV), as a result of less total lipid being extracted.

In summary, these experiments have shown that the fatty acid composition of acid-precipitated WAP-WPC is rather stable at its natural moisture level for up to 12 weeks at 50°C. Heat-precipitated WAP-WPC was less stable. The stability of the lipids of mixtures of the two protein concentrates was good with the WPC:heat-precipitated WAP-WPC being as stable as the WPC: acid-precipitated WAP-WPC combinations. When stored at -23° and 50°C for 12 weeks, the n-hexanal content of WPC was  $\geq 2$  times that of heat-precipitated,

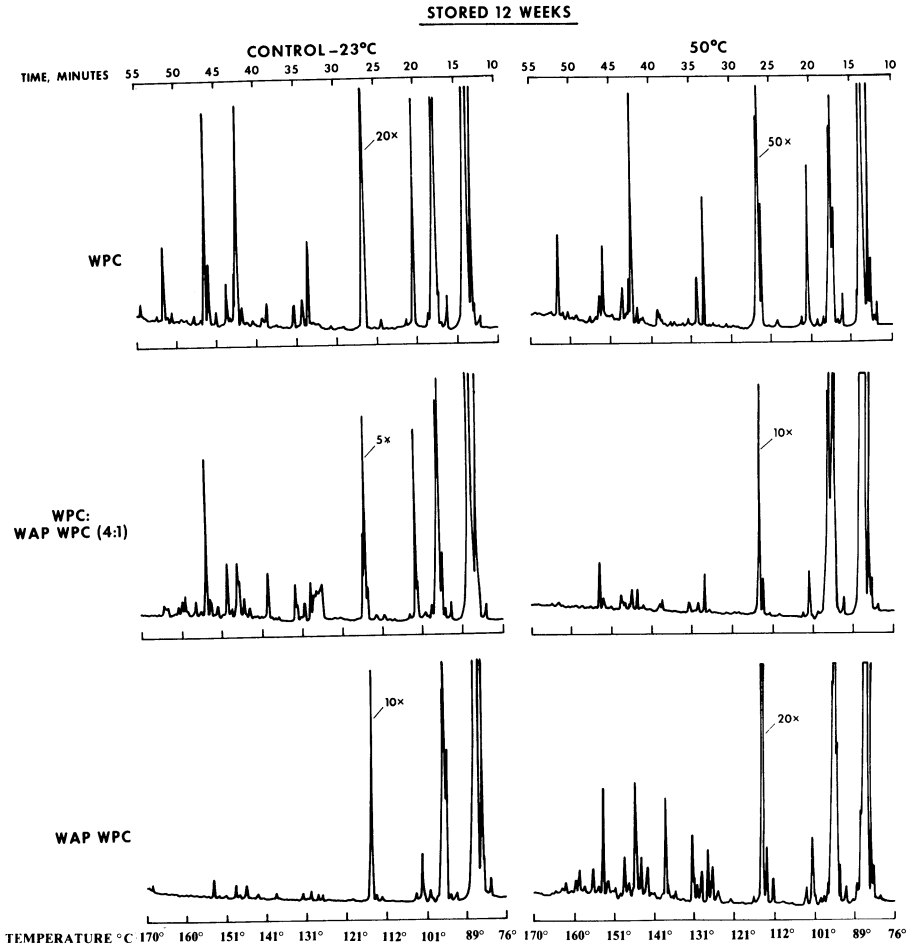


Fig. 1. Gas-liquid chromatography of the volatiles of select samples of stored wheat protein concentrates. WPC = dry milled wheat protein concentrate; WAP-WPC = wet alkaline process WPC isolated by heat precipitation and freeze dried; WPC:WAP-WPC = 4:1 (w/w) mixture. The attenuation for n-hexanal indicates the relative concentration in samples. See text for chromatographic conditions.

freeze-dried WAP-WPC. In the 4:1 mixtures of WPC:WAP-WPC, the n-hexanal concentration was less than in the individual protein concentrate components. This would suggest some synergistic effect between the two protein concentrates.

At 13% moisture the fatty acids of heat-precipitated, freeze-dried WAP-WPC were less stable than those of WPC. The antioxidant potential of the alkyl resorcinol identified in WAP-WPC appears to be minimal under these conditions. In general, WAP-WPC should be stored at its natural moisture level (1-6%) until use, especially when it is to be combined with higher-moisture ingredients such as wheat flour. Mixtures of the two protein concentrates were quite stable at 13% moisture. Since combinations of these protein concentrates exhibited good storage stability, the protein content of WPC is enhanced by WAP-WPC, and WAP-WPC has been reported to perform well in baking studies (7), these mixtures may offer advantages which neither protein concentrate does alone.

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