A RAPID METHOD FOR THE EXTRACTION OF LIPIDS FROM WHEAT PRODUCTS[1]

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ABSTRACT

A rapid method of extracting lipids from animal tissues by means of phase partition of a ternary mixture of chloroform, methyl alcohol, and water was proposed originally by Folch *et al.* (J. Biol. Chem. **226**: 497–509; 1957). The method has been adapted in this study for the extraction of lipids from wheat products. The modified method is especially useful for studies in which it is necessary to isolate the lipids with minimum changes. The method extracts practically the same amount of lipids from flour and from a corresponding dough or dried dough. For the estimation of lipid content of wheat products, it yields results comparable with those obtained by ethyl or water-saturated n-butyl alcohol extractions or by acid hydrolysis extractions, but gives higher lipid values than petroleum-ether extractions.

In the course of studies on the oxidative changes in lipids during the mixing of doughs (17), it was essential to have a method for extracting lipids that would avoid or minimize any changes in the extracted lipids. None of the methods that have been applied to cereal products was considered adequate for our purpose.

Methods commonly used for quantitative extraction of lipids have a number of limitations. Most require a prolonged extraction; some use high-boiling solvents, such as butyl alcohol (13,14), that are difficult to remove if high temperature is to be avoided; and in one method, acid hydrolysis is used (10). Under such conditions, oxidation, polymerization, and hydrolytic changes may readily take place.

A rapid method for lipid extraction was proposed originally by

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Folch *et al.* (7) for isolation and purification of total lipids from animal tissues by means of phase partition of a ternary mixture of chloroform, methyl alcohol, and water. Later, Bligh and Dyer (3) simplified the method for the extraction of lipids from fish muscle.

The present study was carried out to adapt the rapid method for use with wheat products, to compare it with other methods for the extraction of lipids from various wheat products, and to obtain lipids for studies of oxidative changes.

Materials

The wheat samples were ground by a Wiley mill through a 1-mm. sieve. The flours used in this study were unbleached, improver-free samples commercially milled from a blend of Canadian hard red spring wheat.

Doughs were prepared from 200 g. of flour (14% moisture basis), and sufficient water and salt solution to give an absorption of 59% and a salt content of 1% (flour basis). They were mixed in the GRL mixer (11) for 5 minutes.

Dried doughs were prepared by lyophilization of doughs and reduction of the dried product to fine powders by a micro-Wiley mill with a No. 40 sieve.

Semolina was milled from durum wheat with an Allis-Chalmers experimental mill according to the procedure described by Fisher and Meredith (5).

Macaroni was a commercial product. Bread was baked in this Laboratory. It was sliced and lyophilized. Both the macaroni and dried bread slices were reduced to fine powders by a micro-Wiley mill.

All the chemicals used were of reagent grade. Ethyl alcohol (anhydrous) was purified by distillation in an all-glass apparatus from potassium permanganate and potassium hydroxide (1 and 2 g. respectively, per liter of ethyl alcohol).

Methods

Rapid Method. The solvent system used for the extraction was a mixture of 50 ml. ethyl alcohol, 25 ml. chloroform, and water. The amount of water required was calculated from the proportion of 2:1:0.8; this ratio represents the total volumes of methyl alcohol, chloroform, and water, including the water present in the sample. A 20-g. sample was introduced into the solvent mixture in a micro-Waring Blendor and homogenized for 2 minutes. To the mixture were then added 25 ml. of chloroform and, after 30 seconds' blending, 25 ml. of water were added and blending continued for another 30

seconds. The homogenate was centrifuged at 0°C. with $10,000 \times g$ for 10 minutes. Three layers were then separated in the tube. The upper layer consisted of the methyl alcohol and water extract, the middle layer was a doughlike residue, and the bottom layer the chloroform extract. The upper layer was decanted. While most of the doughlike residue adhered to the tube, the chloroform extract was easily transferred to a separatory funnel. The chloroform extract was then separated. An aliquot of the extract was taken, filtered through Whatman No. 1 paper, and washed thoroughly with additional chloroform. The combined filtrate and washings, after the solvent was evaporated off on a steam bath under a stream of nitrogen, were dried in vacuum oven at 50°C. to constant weight.

Petroleum-Ether and Ethyl-Alcohol Extractions. Two-gram samples were extracted overnight (16–18 hours) with petroleum ether (Skellysolve F 95) or ethyl alcohol in a Goldfisch extraction apparatus by the direct method for grain and starch feeds (2). The alcohol extract, after the solvent was evaporated off as in the rapid method, was further purified by dissolving it in chloroform and filtering through Whatman No. 1 paper. The combined filtrate and washings were then dried and weighed.

Water-Saturated n-Butyl-Alcohol Extraction. The method used was the one described by Mecham and Mohammad (13). The extraction times were 30 minutes and 17 hours.

Acid Hydrolysis Method. The procedure followed was the one described in *Cereal Laboratory Methods* (1), with two modifications: First, separatory funnels were used in place of Röhrig or Mojonnier fat-extraction apparatus; second, the ether extract was filtered through Whatman No. 1 paper instead of through a pledget of cotton. The results obtained were reported as percent lipids by the acid hydrolysis method. In addition, the determination was modified in the following manner. After the solvents were evaporated off the extract was dissolved in chloroform and filtered. The filtrate and washings were dried and weighed. The results were reported as percent chloroform-purified lipids by the acid hydrolysis method.

Total nitrogen was determined by the Kjeldahl procedure. Total phosphorus by the method of Harris and Popat (8).

All results were reported as percent, dry basis, together with standard deviations. The figure in parentheses indicates the number of determinations for each test.

Results and Discussion

Lipids Extracted by the Ternary Mixture. To find out the op-

timum ratio of methyl alcohol, chloroform, and water in the mixture for the extraction of lipids in flour, the following experiment was made. Twenty grams of straight-grade flour were extracted by various mixtures of the solvents as shown in Table I. The results, in Table I, show that water is required for the extraction in order to obtain more lipids. As far as the optimum ratios are concerned, our results agree well with the findings of Bligh and Dyer (3). Therefore, the proportions of methyl alcohol, chloroform, and water before and after dilution of 2:1:0.8 and 2:2:1.8 used by Bligh and Dyer (3) were adapted in this study. While the ratios were kept constant, it was found that variation in the volume of the solvents did not significantly change the amount of lipids extracted. However, the smaller the volume of the mixture used, the more difficult it is to separate the extract.

TABLE I

LIPIDS EXTRACTED BY TERNARY MIXTURE OF CHLOROFORM-METHYL ALCOHOL-WATER

Initial Extraction Mixture			Dilution Solvents		Lipids Extracted
Chloroform	Methyl Alcohol	Water	Chloroform	Water	
ml	*ml*	*ml*	*ml*	*ml*	%
25.0	50.0	...	25.0	25.0	1.32
25.0	50.0	15.0	25.0	25.0	1.63
25.0	50.0	2.0	25.0	25.0	1.43
12.5	25.0	8.6	12.5	12.5	...[a]
25.0	50.0	18.0	25.0	25.0	1.63
37.5	75.0	25.8	37.5	37.5	1.58
50.0	100.0	34.4	50.0	50.0	1.59

[a] The extract could not be separated.

Effect of the Blending Time on Lipid Extraction. Twenty grams of straight-grade flour were used for the extraction according to the rapid method with variation in blending times. The results are presented in Fig. 1. They show that the amount of lipids extracted is slightly increased when the blending time is extended from 2 to 3 minutes and that, if the time is further prolonged from 3 to 8 minutes, the level of lipids extracted is rather constant.

Relation of the Volume of Extract and the Amount of Lipids Extracted. Unlike animal tissues, once flour or dough was blended with the solvent mixture, a considerable amount of chloroform was left in the doughlike residue and could not be separated even with high-speed centrifugation. It was therefore difficult to calculate the lipid content of a sample based on the total volume of chloroform extract, as Folch *et al.* (7) and Bligh and Dyer (3) were able to do. In

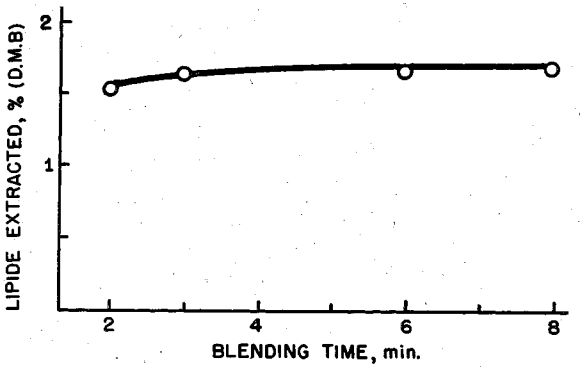

Fig. 1. Effect of blending time on lipid extraction.

order to determine the lipid content by this rapid method, two assumptions were made: that there was little or no chloroform lost during the operation, and that the lipids were evenly distributed in the chloroform phase. The calculation was simply as follows:

$$\text{Lipids, } \% = \frac{\text{wt. of lipids in aliquot} \times \text{total vol. of chloroform used} \times 100}{\text{volume of aliquot} \times \text{sample weight}}$$

To verify these assumptions, the following study was made. Twenty-five grams of dough prepared from Bakers' strong flour were extracted according to the rapid method. After blending, the homogenate was subjected to various centrifugal forces. The relation of the volume of the extract and the percent of lipids is presented in Fig. 2. The results show that regardless of the variations in volume of the

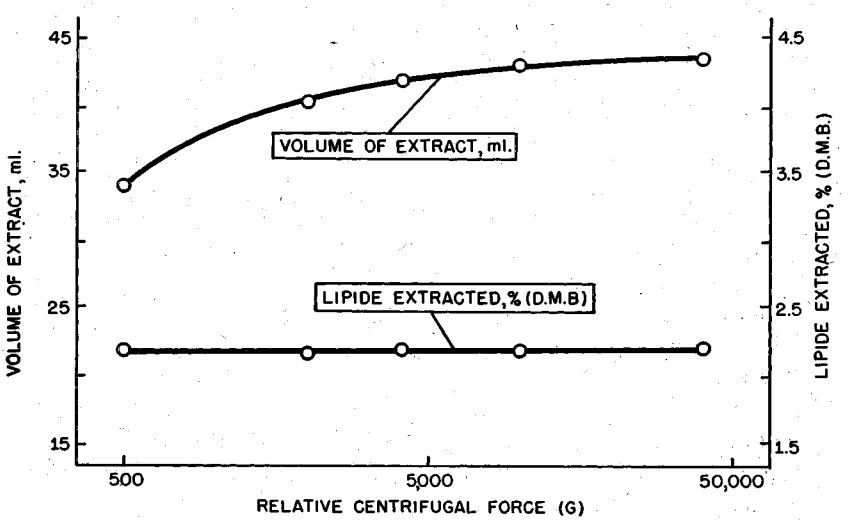

Fig. 2. Relation of the volume of extract and the amount of lipids extracted.

extract, the percent of lipids calculated by the formula remains practically the same. It is evident that these results support our assumptions. However, we have to admit that in spite of the short extraction period and cold centrifugation, there must be some chloroform volatilized during the operation, introducing some error in the determination.

Extraction of Lipids from Flour, Dough, and Dried Dough. The results in Table II present several interesting features. Firstly, by the rapid method, the percent lipids obtained is almost identical for flour, dough, and dried dough prepared from the same flour. It is well known that flour lipids become increasingly unextractable after dough is formed. McCaig and McCalla have suggested that the protein-lipid complex may be formed during the formation of dough (12). Olcott and Mecham have demonstrated that mere wetting of flour can cause binding of a considerable part of the lipids (16). The solvent mixture used in this study is quite effective for the extraction of lipids from dough. The effectiveness may be explained as follows: (a) Water and methyl alcohol may intensify the dispersion system of dough in solvents to render the lipids more accessible to the extracting solvent. (b) Water and methyl alcohol increase the polarity of the solvent system and make it easier to dissociate bonds, mainly hydrogen bonds, between lipids and proteins.

Secondly, by the rapid method, the lipids can be extracted directly from dough without lyophilization and pulverization (Table II). This simplifies the procedure and obviates some deleterious manipulations.

Thirdly, a higher amount of lipids can be extracted by ethyl alcohol and water-saturated n-butyl alcohol than by the rapid method (Table II). However, evaporation of the alcohols from the extracts presents a difficulty for lipid studies. This is especially so if water-saturated n-butyl alcohol is used. Changes of the lipids could take place as a result of overheating.

Lastly, when compared with other methods, the highest percent lipids was obtained by the acid hydrolysis method. The results are in agreement with the observation of Herd and Amos (9). However, by this method, hydrolysis of a part of the lipids cannot be avoided, as noted in the next section. Also, the method is quite laborious.

Nitrogen and Phosphorus Contents of the Lipids. As the amount of lipids extracted varied with different solvents, the nitrogen and phosphorus contents of the extracts were determined. The results (Table III) show that there are only small differences in the nitrogen and phosphorus contents of the lipids extracted by the rapid method

TABLE II

LIPIDS EXTRACTED FROM FLOUR, DOUGH, AND DRIED DOUGH BY VARIOUS METHODS

EXTRACTION METHOD	FLOUR	DOUGH	DRIED DOUGH
	%	%	%
	Straight Grade		
Rapid method	1.63±0.02(8)	1.60±0.01(4)	1.59±0.01(6)
Petroleum ether	1.01±0.08(8)		0.45±0.06(8)
Ethyl alcohol	1.88±0.06(8)		1.70±0.07(8)
n-Butyl alcohol			
(30-minute extraction)	1.81 (2)		
(17-hour extraction)	2.09 (2)		
Acid hydrolysis	2.14±0.07(4)		1.32±0.07(4)
Chloroform-purified	2.11 (2)		1.25±0.10(4)
	Bakers' Strong		
Rapid method	2.12±0.02(8)	2.10±0.05(4)	2.10±0.05(11)
Petroleum ether	1.48±0.07(8)		0.72±0.02(8)
Ethyl alcohol	2.26±0.04(8)		2.13±0.06(8)
n-Butyl alcohol			
(30-minute extraction)	2.42 (2)		
(17-hour extraction)	2.73 (2)		
	Bakers' Special		
Rapid method	1.28±0.02(8)	1.29±0.03(4)	1.28±0.04(8)
Petroleum ether	0.75±0.03(8)		0.32±0.04(8)
Ethyl alcohol	1.42±0.04(8)		
n-Butyl alcohol			
(30-minute extraction)	1.65 (2)		
(17-hour extraction)	1.87 (2)		

TABLE III

NITROGEN AND PHOSPHORUS CONTENTS OF EXTRACTED LIPIDS BY VARIOUS METHODS

EXTRACTION METHOD	NITROGEN	PHOSPHORUS
	%	%
	Flour	
Rapid method	0.74(2)	0.68±0.02(4)
Petroleum ether	0.71(2)	0.24±0.06(4)
Ethyl alcohol	0.94(2)	0.74±0.04(4)
Acid hydrolysis	0.07(2)	0.06(3)
Chloroform-purified	0.02(2)	0.06(3)
	Dried Dough	
Rapid method	0.63(2)	0.78±0.07(4)
Petroleum ether	0.15(2)	0.03±0.01(4)
Ethyl alcohol	0.76(2)	0.50±0.06(4)
Acid hydrolysis	0.03(2)	0.06(3)
Chloroform-purified	0.01(2)	0.05(3)
	Dough	
Rapid method	0.65(2)	0.62±0.06(4)

from flour, dough, and dried dough.

With petroleum ether, the nitrogen and phosphorus contents of the lipids extracted from dough are lower than those of flour lipids. This result is in accord with the findings of Olcott and Mecham (16) that phospholipids are bound preferentially after the dough is made. As a result, both the phosphorus and nitrogen contents are lowered.

With the exception of the phosphorus content of the lipids extracted from dried dough, both the nitrogen and phosphorus contents of the lipids extracted with ethyl alcohol are the highest among the methods tested. This suggests that ethyl alcohol extracts more phospholipids than the other solvents.

Although the highest amount of the "crude" lipids can be obtained by the acid hydrolysis method, the nitrogen and phosphorus contents of these lipids are the lowest for all the methods tested. It is known that phospholipids can be hydrolyzed by hydrochloric acid (15), and choline has been separated from animal tissue and from salad cream and eggs (4,6) by hydrochloric acid hydrolysis rather than by solvent extraction. Thus, the low nitrogen and phosphorus contents reflect clearly that the phospholipids are hydrolyzed by the acid used in the hydrolysis method. These results confirm the assumption of Herd and Amos (9).

Extraction of Lipids from Various Wheat Products. In addition to flours and doughs, the rapid method was also applied to various wheat products and the results were compared with those obtained by the petroleum-ether extraction. The results are presented in Table IV and show that the rapid method extracts more lipids than does the petroleum ether, and results obtained by the rapid method are consistent and reproducible. Although the rapid method is investigated in this study primarily for the isolation of lipids with minimum

TABLE IV

LIPIDS EXTRACTED FROM WHEAT PRODUCTS BY VARIOUS METHODS

WHEAT PRODUCT	LIPIDS EXTRACTED	
	Rapid Method	Petroleum Ether
	%	%
Whole grain		
No. 2 Manitoba Northern	2.32±0.04(8)	1.64±0.02(8)
No. 4 Manitoba Northern	2.24±0.04(8)	1.69±0.07(8)
Extra 4 Amber durum	2.26±0.03(6)	1.49±0.02(8)
3 C.W. Amber durum	2.57±0.06(8)	1.90±0.01(8)
Semolina	1.53±0.04(8)	0.92±0.04(8)
Macaroni	1.18±0.06(8)	0.16±0.01(8)
Bread	1.83±0.02(8)	0.37±0.03(8)

changes, it is also found useful for the estimation of lipids in wheat products.

Literature Cited

1. American Association of Cereal Chemists. Cereal laboratory methods (6th ed.). The Association: St. Paul, Minn. (1957).
2. Association of Official Agricultural Chemists. Official methods of analysis (8th ed.). The Association: Washington, D.C. (1955).
3. Bligh, E. G., and Dyer, W. J. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37:** 911–917 (1959).
4. Daubney, C. G., and Sexton, G. E. W. The determination of egg in salad cream. Analyst **75:** 305–309 (1950).
5. Fisher, M. H., and Meredith, W. O. S. Note on a semimicro milling procedure for durum wheat. Cereal Chem. **23:** 565–568 (1946).
6. Fletcher, J. P., Best, C. H., and Solandt, O. M. The distribution of choline. Biochem. J. **29:** 2278–2284 (1935).
7. Folch, J., Lees, M., and Stanley, G. H. S. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. **226:** 497–509 (1957).
8. Harris, W. D., and Popat, P. Determination of the phosphorus content of lipids. J. Am. Oil Chemists' Soc. **31:** 124–127 (1954).
9. Herd, C. W., and Amos, A. J. Fat: Its estimation in wheaten products. Cereal Chem. **7:** 251–269 (1930).
10. Hertwig, Raymond. Summary of the activities of the AOAC for 1924 on studies of methods for the analysis of cereal foods. Cereal Chem. **2:** 46–52 (1925).
11. Hlynka, I., and Anderson, J. A. Laboratory dough mixer with an air-tight bowl. Cereal Chem. **32:** 83–87 (1955).
12. McCaig, J. D., and McCalla, A. G. Changes in the physical properties of gluten with the aging of flour. Can. J. Research **C19:** 163–176 (1941).
13. Mecham, D. K., and Mohammad, A. Extraction of lipids from wheat products. Cereal Chem. **32:** 405–415 (1955).
14. Morton, R. K. Separation and purification of enzymes associated with insoluble particles. Nature **166:** 1092–1095 (1950).
15. Munsey, V. E. Report on choline in egg noodles. J. Assoc. Offic. Agr. Chemists **36:** 766–769 (1953).
16. Olcott, H. S., and Mecham, D. K. Characterization of wheat gluten. I. Protein-lipid complex formation during doughing of flours. Lipoprotein nature of the glutenin fraction. Cereal Chem. **24:** 407–414 (1947).
17. Tsen, C. C., and Hlynka, I. The role of lipids in oxidation of doughs. Cereal Chem. **39:** 209–219 (1962).