LARGE-SCALE LABORATORY SOXHLET EXTRACTION OF WHEAT FLOURS, AND OF INTACT AND CRACKED GRAINS[1]

R. L. CLEMENTS[2]

ABSTRACT

Cereal Chem. 54(4): 865–874

Flours and cracked wheat (5-6 kg) were extracted with hexane on a large-scale Soxhlet extractor, and extracts were sampled at intervals, analyzed for total lipid, and chromatographed by thin-layer chromatography (TLC). About 95% of hexane-extractable lipid was extracted in the first 8 hr. TLC showed no qualitative differences among extracts from different stages of extraction. Hexane-extraction yielded 1.07–1.15, 1.01–1.05, and 0.95–0.97% lipid (dry basis) from soft red (SR), soft white (SW), and hard winter (HW) wheat flours, respectively. Extraction with acetone or chloroform yielded about 20% more lipid than hexane. Hexane-extracted lipid averaged 1.07% (dry basis) from cracked hard wheat and 1.32% from SR and SW wheats. Lipid averaged 0.12, 0.23, and 0.30% from intact hard red (HR), SR, and SW kernels, respectively. Yields from hexane extraction of intact and cracked rye were comparable to yields from wheats, but oat groats gave 3.63% lipid (56% of the lipid from cracked groats). Small amounts of lipids from intact grains appeared to be from the cuticle, but the results suggest little lipid is extracted from unbroken kernels, and lipid yield may be an index of kernel damage.

Flour lipids, as hydrophobic components of complex aqueous systems and as surfactants, may be implicated in many interactions. They are factors in the physical behavior of flour-water systems and in baking behavior (1–3). In the intact grain, lipids may contribute to milling behavior, either directly as textural factors, or indirectly by affecting moisture uptake and distribution. Flow properties of endosperm particles are affected by the lipids, as demonstrated by the change in flow behavior of flours upon removal of free lipids (4,5).

In research on flour, lipid extraction is commonly used for isolation of lipids for qualitative and quantitative determinations, for preparation of 'defatted' flours, and for interaction studies. Usually the free lipids are extracted on a small-scale Soxhlet apparatus of limited capacity (*i.e.*, less than 500 g flour). However, large commercial laboratory extractors are available, and such a unit (accommodating more than 6 kg flour) has been used extensively in this laboratory. Such an apparatus offers several advantages over smaller extractors. It facilitates extraction of flours in quantities sufficient for fractionation, reconstitution and baking studies, and also permits extractions of whole grains in amounts adequate for milling studies on standard test-mills. In addition, the lipid yields facilitate isolation and characterization of individual lipids and studies of their effects in various systems. Finally, the design and capacity of the large-scale apparatus which permits withdrawal of solvent from the extraction chamber during operation provide a convenient means for extraction rate studies.

[1]Cooperative investigation between the North Central Region, Agricultural Research Service, U.S. Department of Agriculture and the Department of Agronomy, Ohio Agricultural Research and Development Center, Wooster, Ohio 44691. Approved as Journal Article No. 132-76 of the Ohio Agricultural Research and Development Center.

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of a product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

[2]Research Chemist, Soft Wheat Quality Laboratory, North Central Region, Agricultural Research Service, U.S. Department of Agriculture, Ohio Agricultural Research and Development Center, Wooster, Ohio 44691.

In this report, data are presented from rate studies made by solvent-sampling during extractions of flours and grain, from quantitative large-scale extractions of flours and of intact and cracked grains with various solvents. The data can provide useful guidelines for routine Soxhlet extractions on both large and small scales.

MATERIALS AND METHODS

Apparatus

Extractions were made on a Quickfit 5-l. multipurpose extractor (Quickfit, Inc., Fairfield, N.J.) in Soxhlet mode, with a stopcock drain on the chamber. The vapor riser was insulated with asbestos, and permitted a maximum reflux rate of about 17 l. hexane per hr (10–12 l. turnover per hr).

Solvents

Hexane (practical grade, or ligroin, bp 63°–75° C), acetone and chloroform (reagent grades) were redistilled before use. Freon-TF (Freon-113; 1,1,2-trichloro-1,2,2-trifluoroethane) was not redistilled.

Grains and Flours

The Kansas hard red winter (HRW) wheat flour was obtained from the Hard Red Wheat Quality Laboratory, U.S. Grain Marketing Research Center, at Manhattan, Kans. All other grains and flours were from stocks on hand at the Soft Wheat Quality Laboratory at Wooster. Flour mixtures were composites of straight-grade flours from several varieties of the same class. Grains were passed through a dockage tester several times to remove small and broken kernels and, for cracking, were tempered to 12.5% moisture basis and passed through the rolls of a Tag-Heppenstall moisture meter with a combination of shims to crack without shattering. The cracked grain was air-dried to about 10% moisture basis before extraction.

Extraction Procedure

The sample was placed in the thimble (about 3 kg flour) or in several cotton bags (350 g per bag, up to 6.3 kg total) and transferred to the chamber. Solvent (10–15 l.) was added to the still pot, and reflux was usually carried out for three 8-hr periods (*i.e.*, 3 days); the sample was submerged in solvent in the extraction chamber for 16 hr between reflux periods. Reflux rate for hexane (*i.e.*, rate of condensation from boiling solvent at equilibrium) was 15–17 l. per hr, but because of cycling, actual solvent turnover was less (10–11 l. per hr, or 240–260 l. during 24-hr extraction). Cycle frequency (*i.e.*, syphons per hr) depended on the sample, but generally ranged from 9 to 12 for flours and 4 to 6 for grain. After the final reflux period, the chamber was allowed to syphon, and the sample was removed and air-dried in a hood stream at room temperature. The extract was concentrated to 0.5–1.5 l. by distillation, then was filtered and made up to 2 l. Total lipids in hexane and Freon-TF extracts were determined by evaporating aliquots and weighing after 1 hr at 100° C. Lipids in other solvents were determined by concentrating aliquots, taking them on a Celite 545 and air drying at room temperature, and extracting the Celite with hexane on a Goldfisch

extractor for 6 hr. Residues were weighed after 1 hr at 100° C.

For studies of extraction rates, the sample (5–6 kg) in cotton bags was placed in the chamber. Reflux was started, and during each of the specified cycles 350–400 ml of solvent was withdrawn from the chamber by opening the drain cock at the beginning of a particular syphoning; solvent was collected until the chamber had drained, and then the cock was closed. Duplicate subsamples (100 or 150 ml) were filtered and evaporated in weighed beakers, dried 1 hr at 100° C and weighed for quantitative determination of lipid. Additional subsamples (100 ml) were evaporated and taken up in chloroform:methanol (2:1, v/v) for thin-layer chromatography (TLC). Solvent was added to the still as necessary between reflux periods to compensate for sample removal. When extraction was completed, the chamber was allowed to fill from reflux until just short of the syphon level, the solvent was drained from the chamber, and the volume measured to determine solvent turnover per cycle.

Germ Lipids

Germ (about 1 g) was excised from non-extracted kernels and from hexane-extracted kernels of intact wheat (Genesee). The germ was ground in a mortar with Celite 545 and extracted with hexane for 6 hr in a Goldfisch extractor. Extracts were dried 1 hr at 100° C and weighed to determine lipid.

Thin-Layer Chromatography

Extracts were chromatographed on precoated silicic acid plates (Quantum Types Q1 and Q5, 250 μ thick, from Quantum Industries, Fairfield, N.J.).

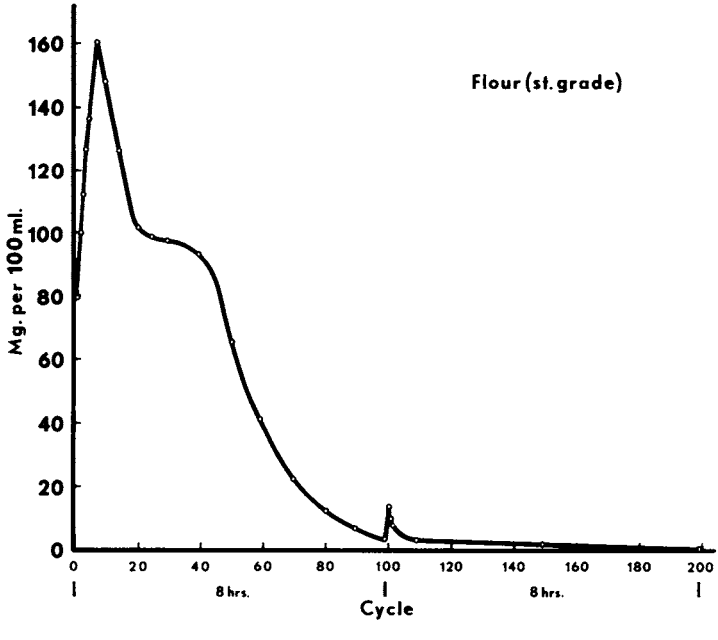

Fig. 1. Hexane Soxhlet extraction of a soft wheat flour (red and white blend, straight grade). Sample: 6.3 kg (9.0% moisture basis). Solvent turnover: 850 ml per cycle.

Concentrations and application rates of extracts were adjusted to give comparable loads (about 500 μg lipid) insofar as possible. Solvent systems were hexane:diethyl ether:glacial acetic acid, 70:30:2 and 70:40:3 (v/v/v), and chloroform:methanol:water, 65:25:4 (v/v/v) (6,7). For detection of lipids plates were exposed to iodine vapor (6,7), or dipped in 3% cupric acetate in 8% phosphoric acid and heated at 130°C for 30 min (8).

RESULTS AND DISCUSSION

Extraction Rate Studies

The extraction curve from sampling of a single hexane extraction of a straight-grade soft wheat flour is shown in Fig. 1. Sampling was terminated at the end of the second 8-hr extraction period when residues had declined to less than 1 mg per 100 ml. The peak at cycle-101 is residue from the sample taken from the chamber after the 16-hr submersion between extraction periods, and is typical of the overnight soakings. This extraction was essentially complete after two 8-hr extractions and one 16-hr soaking, and indicated that three 8-hr periods are sufficient for flour extraction. Lipid yield from the first 8-hr period (from integration) accounted for 96.5% of total yield from the two 8-hr periods. Extracted lipid rose rapidly to a maximum at cycle-8 (*i.e.*, about 30 min extraction), and fell sharply during the following 10–12 cycles. Extraction rate remained almost constant during the next 20 cycles, then fell steadily to cycle-100

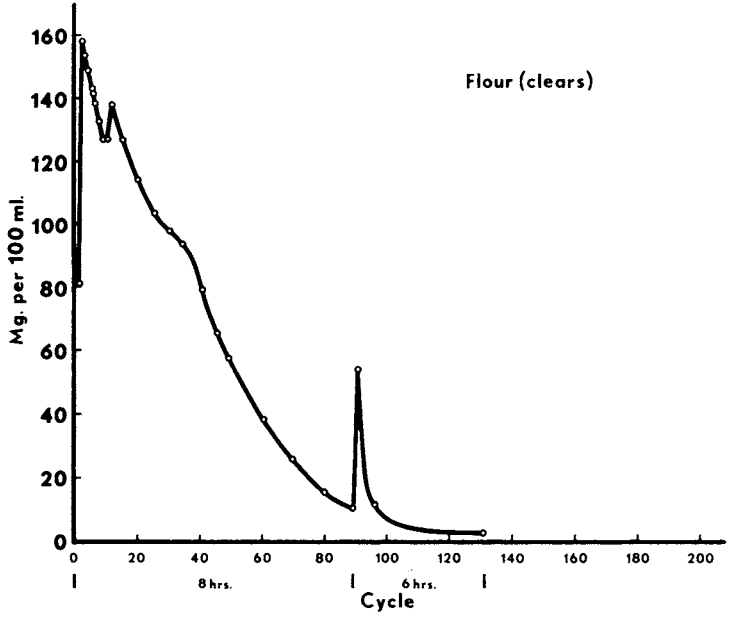

Fig. 2. Hexane Soxhlet extraction of a soft red winter wheat flour (clears). Sample: 5.6 kg (12.0% moisture). Solvent turnover: 875 ml per cycle.

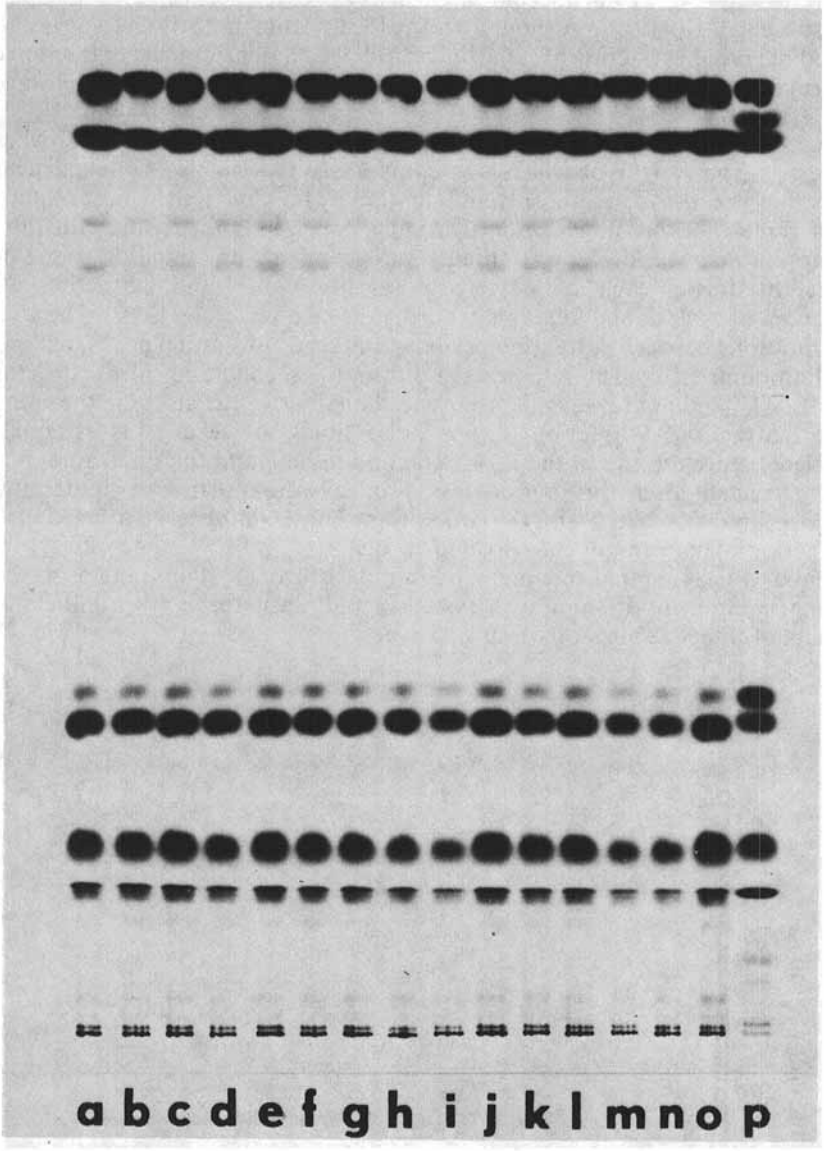

Fig. 3. Thin-layer chromatograms of hexane extracts of a soft red wheat flour (clears) collected at intervals during Soxhlet extraction. Silicic acid plates (Quantum Q5) developed in hexane:diethyl ether:acetic acid, 70:40:3, v/v/v (bottom), and chloroform:methanol:water, 65:25:4, v/v/v (top). Lipids detected with 3% cupric acetate in 8% phosphoric acid. Key: a–j, cycles 1–10; k, cycle 12; l, cycle 15; m, cycle 30; n, cycle 60; o, cycle 91 (first 16-hr soaking); p, reference mixture (cholesteryl oleate, triolein, oleic acid, cholesterol, and lecithin).

(the end of the first 8-hr period). A similar curve resulted from extraction of a soft red clear flour (Fig. 2), but a second sharp peak appeared between the initial peak and subsequent plateau. Although the patterns from both flours suggest an immediate rapid extraction of some lipids, followed by a protracted extraction of the remainder, TLC did not indicate qualitative differences between the stages (Fig. 3).

Figure 4 is a curve from hexane extraction of a cracked soft white wheat (Genesee). The weight of wheat was comparable to weights of the extracted flours, but because of greater solvent retention by the flours, the wheat required about three times as much reflux for each cycle. In comparison with flour, therefore, time per cycle was three times as great, and equilibration and extraction during each cycle were more thorough. Lipid extraction was maximum at cycle-1, and fell rapidly during the next ten cycles (3 hr). The 16-hr equilibrations between extraction periods produced substantial peaks, but only small amounts of lipid were extracted during the second and third extraction periods. TLC did not reveal any pronounced qualitative variations in the course of the extraction. Extraction of free polar lipids, however, was essentially complete before the end of the first extraction period, and the small amount of lipid extracted after this period was primarily nonpolar. An unidentified nonpolar lipid associated with the cuticle was almost completely extracted in the first period. This component (isolated in quantity and now under study) was evident as a heavy, rapid-moving band in polar solvent systems, and as a slow-moving component in nonpolar systems, and was especially prominent in extracts of intact kernels of wheat and rye.

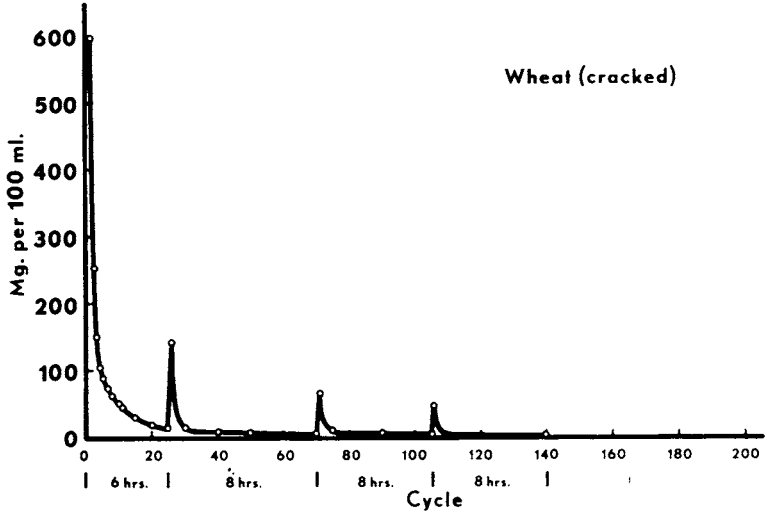

Fig. 4. Hexane Soxhlet extraction of a soft white whole wheat (Genesee, cracked). Sample: 5.6 kg (9.5% moisture basis). Solvent turnover: 2600 ml per cycle.

Lipid Extractions from Flours

Flour lipid yields from various solvents are presented in Table I. With hexane as solvent, soft red flours gave highest yields (1.07–1.15%, dry basis), hard wheat flours gave lowest yields (0.95–0.97%), and soft white flours gave intermediate values (1.01–1.05%). Blends generally reflected these ranges, although one soft-red + soft-white mixture gave the highest yield of any flour (1.21%). Acetone extracted 12–25% more lipid than did hexane, and extractions of a soft wheat blend with chloroform showed a 20% increase over hexane extractions. When acetone and chloroform extracts were taken up on Celite and extracted with hexane on a Goldfisch extractor, yields from the secondary extractions accounted for more than 98% of the residue from the original extract, indicating that less than 2% nonlipid material was extracted by acetone or chloroform.

Lipid Extractions from Whole Grains

Results of extractions of whole grains before and after cracking are shown in Table II. Hexane extraction of intact soft white (SW) wheats gave lipid equivalent to 0.29–0.30% of the dry weight of the kernels, or 21–22% of the lipid

TABLE I
Large-Scale Soxhlet Extractions of Different Varieties and Classes of Flours (Sample Size: 3–6 kg)

Type and Variety[a] of Flour	Solvent	Pretreatment	Number of Samples	Lipid Av % dry wt[b]
SRW				
Thorne	Hexane	None	1	1.14
Arthur + Logan	Hexane	None	3	1.15
Mixture-1	Hexane	None	2	1.07
SWW				
Avon	Hexane	None	1	1.05
Mixture-2	Hexane	None	2	1.01
SRW + SWW				
Mixture-3	Hexane	None	3	1.21
Mixture-4	Hexane	None	4	1.07
Mixture-4	Hexane	Pin-milled	1	1.07
Mixture-4	Hexane	Dried (3% moist.)	2	1.07
Mixture-4	Acetone[c]	None	1	1.33
Mixture-4	Acetone[c]	Dried (2% moist.)	2	1.29
Mixture-5	Hexane	None	2	1.12
Mixture-5	Chloroform[c]	None	2	1.35
HRW				
Comanche	Hexane	None	1	0.95
Mixture-6	Hexane	None	2	0.97
Mixture-7	Hexane	None	7	0.96
Mixture-7	Acetone[c]	Dried (2% moist.)	1	1.08

[a]SRW: Soft red winter; SWW: Soft white winter; HRW: Hard red winter.
[b]Averages of replicates; ranges of replicates within ± 0.01% of averages.
[c]Lipid determined by secondary extraction with hexane.

from cracked kernels. Extraction of intact soft red (SR) wheats gave 0.22–0.24%, or 15% of the yield from cracked kernels, and hard red (HR) wheats gave 0.11–0.13%, or 11–12% of the lipid from cracked kernels. Total lipid from the cracked wheats averaged 1.07% for HR (dry weight basis), and 1.32 for both SW and SR wheats. Lipid yields from intact rye (0.19%) and cracked rye (1.29%) were comparable to yields from wheats, but yields from intact oat groats were very high (3.63%) and represented 56% of the total lipid (6.39%). Yields from single extractions of nonextracted kernels generally agreed with total recoveries from kernels which were extracted intact, cracked, and again extracted. Yields from extraction were slightly higher with acetone than with hexane. Freon-TF extracted lipid equivalent to only 60% of hexane-extracted lipid from cracked wheat and less than 30% of the lipid from intact kernels.

Flour extraction curves suggest a peak from an initial rapid extraction superimposed on a curve from a delayed extended extraction. It has been reported that 10–30% of free-flour lipids originate in the germ and are transferred to the endosperm during milling (9,10). The initial peak possibly represents such lipids, but no significant qualitative differences were detected between the early and delayed stages of extraction.

Relatively small amounts of lipid were extracted from intact wheat and rye kernels, and preliminary data indicate much of this was derived from the cuticle. This observation suggests that the intact kernel is impervious to lipid solvents, because gentle cracking released the remainder of the free lipids from the kernel. Germ from nonextracted wheat contained about 9% free lipid, as compared with

TABLE II
Large-Scale Soxhlet Extractions of Intact and Cracked Kernels of Different Varieties of Wheat, of Oat Groats, and of Rye (Sample size: 5–6 kg)

Grain	Lipid (%, dry wt basis)			
	Intact	Cracked (after intact extn.)	Total	Cracked (single extn.)
Wheat				
Soft white winter				
Genesee	0.29	...	...	1.26
Ionia	0.30	1.06	1.36	1.31
Yorkstar	0.30	1.12	1.42	1.39
Soft red winter				
Arthur	0.24	...	...	1.23
Blueboy	0.22	1.22	1.44	1.40
Hard red winter				
Early Blackhull	0.11	0.94	1.05	1.12
Shawnee	0.13	0.95	1.08	1.02
Oats (groats)[a]	3.63	2.76	6.39	...
Rye[a]	0.19	1.10	1.29	...

[a]Commercial samples; varieties unknown.

7% from germ from hexane-extracted intact wheat. Apparently, extracts of intact kernels contain only small amounts of germ lipid. Probably much of the lipid extracted from 'intact' grain is actually derived from the interior of cracked or broken kernels. Presumably, kernels with minor injuries or flaws yield as much lipid as fully cracked kernels upon exhaustive extraction. High lipid yield may thus indicate a high proportion of unsound kernels. The high yield from intact oat groats probably reflected damage resulting from mechanical removal of hulls. On a weight basis, small intact kernels might be expected to yield more lipid than large kernels because of greater surface per unit weight. However, intact small-kernel hard wheats gave less lipid than large-kernel soft white wheats (0.12% *vs.* 0.30%). Kernel hardness may be a factor, but probably does not directly influence the extraction of intact kernels. Perhaps the hard wheat contained fewer damaged kernels than the soft wheat.

Large-scale extractions with flammable solvents entail certain precautions in view of the volumes involved (10–15 l.), but hexane (or petroleum ether consisting primarily of hexanes) remains the solvent of choice. Chloroform has been reported to increase lipid yields (2), and in this study the increase was about 20%. However, chloroform-extracted flours exhibited a strong chloroform taste (but not odor). The residual chloroform was not removed by aeration alone, but hydration (to about 14% moisture basis) followed by aeration eliminated the taste. Freon-TF is used as an industrial degreasing solvent, and offers several advantages (11). However, Freon-TF extraction of cracked wheat yielded only about 60% of the lipid extracted by hexane and was not considered for further study. Acetone gave lipid yields comparable to those of chloroform, but a strong, unpleasant off-odor and flavor were evident in acetone-extracted flour and wheats, and they were not diminished by aeration or hydration followed by aeration.

Acknowledgment

The author acknowledges the technical assistance of G. Kirchhofer.

Literature Cited

1. KISSELL, L. T., POMERANZ, Y., and YAMAZAKI, W. T. Effects of flour lipids on cookie quality. Cereal Chem. 48: 655 (1971).
2. MacRITCHIE, F., and GRAS, P. W. The role of flour lipids in baking. Cereal Chem. 50: 292 (1973).
3. MECHAM, D. K. Lipids. In: Wheat: Chemistry and technology, ed. by Y. Pomeranz. Amer. Ass. Cereal Chem.: St. Paul, Minn. (1972).
4. COOKSON, M. A., and COPPOCK, J. B. M. The role of lipids in baking. III. Some breadmaking and other properties of defatted flours and of flour lipids. J. Sci. Food Agr. 7: 72 (1956).
5. JOHNSON, A. H. Studies of the effect on their breadmaking properties of extracting flours with ether. Cereal Chem. 5: 169 (1928).
6. SKIPSKI, V. P., and BARCLAY, M. Thin-layer chromatography of lipids. In: Methods in enzymology, ed. by J. M. Lowenstein, Vol. XIV. Academic Press: New York (1969).
7. STAHL, E. Thin-layer chromatography: A laboratory handbook. Academic Press: New York (1965).
8. ANONYMOUS. Charring techniques. Tech. Bull. No. 201, Quantum Industries: Fairfield, N.J. (1975).
9. McKILLICAN, M. E., SIMS, R. P. A., JOHNSTON, F. B., and MES, J. C. Low-temperature anaerobic extraction of free and bound lipids from wheat flour. Cereal Chem. 45: 512 (1968).

10. STEVENS, D. J. The contribution of the germ to the oil content of white flour. Cereal Chem. 36: 452 (1959).
11. ANONYMOUS. Freon TF solvent. Bull. FST-1, E. I. DuPont de Nemours & Co., Inc.: Wilmington, Del. (1970).

[Received November 10, 1976. Accepted February 25, 1977]