Titratable Acidity in Water-Saturated n-Butyl Alcohol and Petroleum Ether Extracts of Some Stored Wheat Products [1, 2]

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ABSTRACT

Titratable acidity values of water-saturated *n*-butyl alcohol (WB) and petroleum-ether extracts of Flour Blend A samples in storage were compared. At 90°F., titratable acidity increased at the same rate for both solvents up to 3 months' storage time, and beyond 3 months little change occurred. At 120°F., increases for the two solvents were nearly equal after 3 months, but with longer storage acidities of the WB extracts increased regularly while those of the petroleum-ether extracts changed little. With samples containing 0.5% sodium stearoyl-2-lactylate, the same trends and differences between extractants were observed. WB also gave consistent results with full-fat soy flour/wheat flour blends in storage. The blends gave larger titrations than the corresponding wheat flour samples, and 10%-moisture blends increased less than 13%-moisture blends. In a wheat stored at 14.6% moisture and 100°F. for times beyond complete loss of viability, increases in titratable acidity were small with both extractants. WB extracts showed the increases in titratable acidity due to breakdown of lipid (formation of "free fatty acids") during storage of wheat products at least as satisfactorily as petroleum-ether extracts did, and probably more effectively with extensively deteriorated samples stored at relatively high temperatures. Extraction of lipids with WB was rapid and more nearly complete than with nonpolar solvents, and variations in moisture content of samples were less critical.

During the storage of cereal grains and flours, the titratable acidity of various extracts increases, and the changes often have been followed in attempts to measure deterioration of the stored products. Based on work of Balland (Brooke, 1), aqueous ethyl alcohol extracts were used for many years in various procedures, e.g., the "Greek" acidity method. Later, petroleum ether with Soxhlet extraction, or benzene in a Stein mill, was employed to obtain extracts (2). These less polar solvents came into use after Johnson and Green (3) and Schulerud (4) in the early 1930s concluded that increases in fatty acid content were solely responsible for changes in acidity of flour in storage. Later, Zeleny and Coleman (5) reported that in whole grain the percentage change in titratable acidity of petroleum ether-extracted lipids is much larger than that of aqueous or aqueous alcoholic extracts, and the changes are better correlated with loss of viability of the grain. Extracts in a nonpolar solvent thus were preferred for evaluating the soundness of whole grains too. As Baker et al. (6) observed, however, the moisture content of the sample affects the acidity found in petroleum-ether extracts, and grain should be dried to 11% moisture before extraction or a suitable correction applied to the titration.

Water-saturated *n*-butyl alcohol (WB) dissociates lipid complexes in wheat and wheat flour more effectively than many other lipid extractants (7,8). Some nonlipid material is extracted, but it is mostly carbohydrate and the proportion is

[1]Presented in part at the 57th Annual Meeting, Miami Beach, Oct.-Nov. 1972.

[2]Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

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low (9,10). Normal variations in the moisture content of grain and flour samples should have little effect on the extraction because about 20% water is already present in the solvent. The solvent is easy to use, lipids are extracted rapidly, and extracts are easily filtered and free of turbidity. Therefore it seemed of interest to compare petroleum ether and WB as extractants in a storage study with long-extraction flour. The results and some related observations are reported here.

MATERIALS AND METHODS

Flour Blend A (FBA) was prepared by blending 70 parts unbleached straight-grade bread flour and 30 parts wheat protein concentrate (11). The latter is obtained by a dry remilling of shorts or bran (12). Both the flour and the wheat protein concentrate were commercial products. To maintain 13% moisture in the blend during storage, samples were held in heavy polyethylene bags in metal cans with friction lids. 0.5% Sodium stearoyl-2-lactylate (SSL) was blended into some samples. Soy-wheat flour blends contained 12.7 parts full-fat soy flour and 87.3 parts bread flour, plus 0.5% SSL; moisture content was 12.4%. The SSL was a commercial product (Emplex; C.J. Patterson Co.).

The wheat used for germination studies was from a commercial lot of Pacific Northwest soft white, Gaines variety. Its moisture content was brought from 9% to about 15% by adding water, mixing, and holding at refrigerator temperature for 3 days in a closed container. It was then allowed to warm to room temperature and 2-oz. screw-cap jars were filled with the tempered wheat. A double layer of PVC film was placed over each jar before the cap was screwed on. Jars were held either at -10° or at 100°F. in a friction-lid can. At intervals, samples from storage were brought to room temperature. Part of each was ground through a 60-mesh screen (small Wiley mill). Moisture determinations gave values from 14.4 to 14.8% over 0 to 7 weeks' storage at 100°F.

For germination tests on the stored wheats, 400 kernels were dipped in 0.5% NaOCl for 2 min. and rinsed in distilled water twice. In each of four petri plates containing a double layer of S & S No. 596 filter paper, 100 kernels were spread and 4 ml. water added. The covered petri plates were placed in a closed container over a few ml. of water and held at room temperature (70° to 74°F.). After 3 days, all kernels with shoots over about 2 mm. and with a few mm. of roots were removed; and an additional 2 ml. of water was added. A final count of ungerminated kernels was made after an additional 4 days, or less if mold growth became too heavy. This occurred with samples stored a few weeks at 100°F.; wheat stored shorter or longer times gave noticeably less mold growth during the germination test.

Soxhlet extractions of 10-g. samples (mixed with 10 g. Celite filter aid) using petroleum ether (30° to 60°C. boiling point range) were run 16 to 18 hr. Ground wheat samples from the viability storage study were dried for 1 hr. at 100°C. in an air oven before Soxhlet extraction. Solvent was removed on a steam bath to a residual extract volume of about 5 ml., then on a rotary evaporator to dryness. The residue was dissolved in 50 or 75 ml. benzene: 95% ethyl alcohol: phenolphthalein (50:50:0.02, v:v:w) and aliquots of 5 to 25 ml. were titrated with 0.0178N KOH according to the AACC method (2).

Unless otherwise specified, extractions with WB were made by adding 75 ml. to

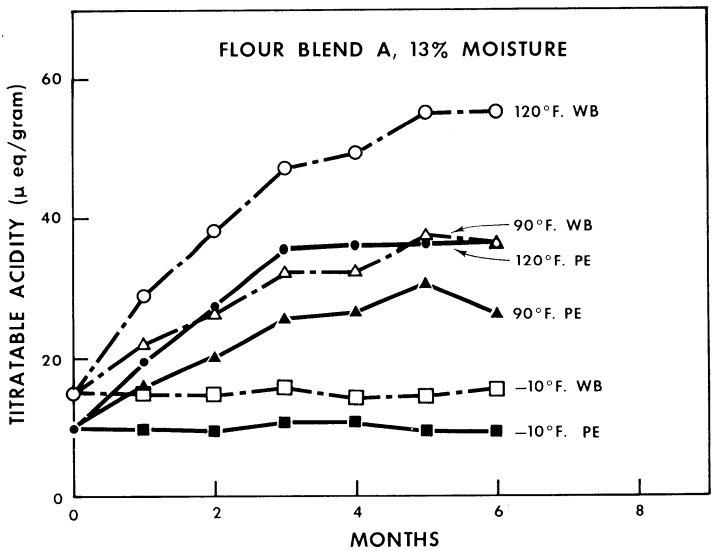

Fig. 1. Development of titratable acidity in FBA stored at different temperatures; comparison of WB and petroleum-ether extracts.

10-g. sample in a 250-ml. glass-stoppered Erlenmeyer flask, shaking for 30 min. (wrist-action shaker), and filtering (E and D No. 509 paper). To 25 ml. filtrate, 25 ml. 95% ethyl alcohol containing 0.04% phenolphthalein was added; and titration then was carried out as with the petroleum-ether extracts.

Results are expressed as μeq. per g. sample. To convert to mg. KOH per g. sample, to compare with work reported on that basis, multiply by 0.05611.

RESULTS AND DISCUSSION

Comparison of Extractants on Flour Blend A (FBA)

The titratable acidity of petroleum ether and WB extracts of FBA samples stored at three temperatures are shown in Fig. 1. Values for the −10°F. control samples did not change with time, averaging 15 μeq. per g. sample in the alcohol and 10 in petroleum ether. Comparing the two solvents, the rates of increase are nearly equal through 3 months at 90°F., and also at 120°F. With further storage to 6 months, only WB extracts of 120°F. samples continued to increase consistently in titratable acidity. Actual increases over their respective controls in 6 months at 120°F., in μeq. per g. sample, were 40 for the WB and 26 for the petroleum-ether extracts, i.e., 50% larger for the WB.

Corresponding determinations made on samples of the same FBA to which 0.5% SSL had been added before storage are shown in Fig. 2. The higher base values at −10°F. reflect the presence of material in the SSL that neutralized about 7 μeq. KOH per g. sample. The same patterns of change appeared as were found without SSL. At 90° and 120°F., rates of change were nearly equal for the two extractants for 3 months. With longer storage, only WB extracts of 120°F. samples increased over the entire 6 months. Actual increases were 48 and 29 μeq. per g. sample for

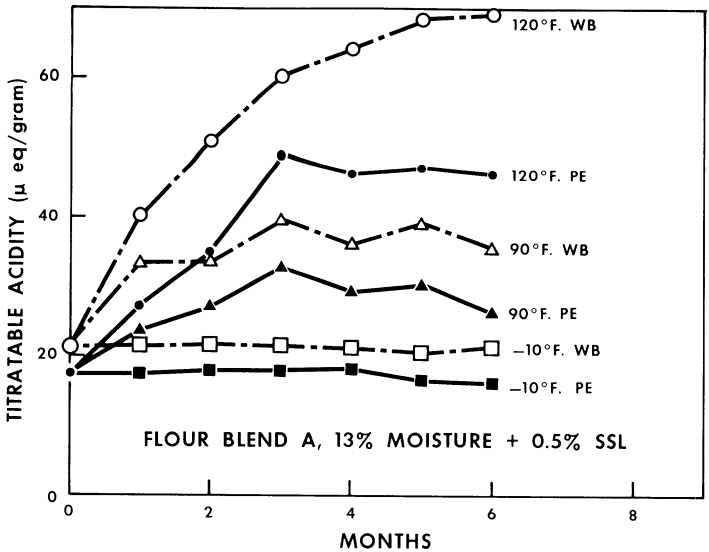

Fig. 2. Development of titratable acidity in FBA containing 0.5% sodium stearoyl-2-lactylate stored at different temperatures; comparison of WB and petroleum-ether extracts.

TABLE I. TITRATABLE ACIDITY WITH INTERCHANGE OF SOLVENTS

Solvent for Extraction	Solvent for Titration, μeq. per g.	
	Benzene[a]	WB[b]
Petroleum-ether	27.5	26.8
WB after petroleum-ether	...	13.5
WB	40.5	41.9

[a] After evaporation of extractant, the residue was dissolved in benzene: 95% ethyl alcohol: phenolphthalein (50:50:0.02; v:v:w). This is the procedure followed with petroleum-ether extracts in the AACC Approved Method for Fat Acidity in grain and flour.
[b] After evaporation of extractant, the residue was dissolved in WB: 95% ethyl alcohol: phenolphthalein (50:50:0.02; v:v:w).

the WB and petroleum ether, respectively, somewhat more than a 50% larger increase for the WB.

Extracts made with the butyl alcohol thus gave at least as large increases in titrations as those made with petroleum ether with relatively short storage times, and at 120°F. after several months the WB extracts gave definitely larger increases–about 1.5 times as large. Zero-time values for the WB extracts were higher than those on the petroleum-ether extracts, but the differences were relatively small compared to the increases during storage at either 90° or 120°F.

Values for the 90°F. samples appear to be less consistent than those for the other temperatures. Samples removed from warm storage were held at 35° to 40°F. until extractions were made. In some cases, the time was as long as 2 weeks although more often only a few days. Possibly slow changes at refrigerator temperature were

a factor that was poorly controlled. As a source of the irregularities in the samples that were stored at 90°F., microbiological activity is suggested. Others (13) have found that microorganism counts decline steadily in flour held at temperatures near 120°F.; thus poor control of conditions before extraction perhaps would be less important for the 120°F. samples, which gave consistent results.

The ability of the butyl alcohol to dissociate lipids that are complexed with protein and carbohydrate may explain the continuing increase in 120°F. values from 3 to 6 months, in contrast to the relatively stable values with petroleum-ether extraction. This assumes that titratable lipid degradation products continue to form but become associated to some extent with other constituents and are poorly dissociated and solubilized by nonpolar solvents.

Interchange of WB and Petroleum Ether

From the preceding results, it appeared that the WB extracts contained titratable material not extracted by petroleum ether. However, observations of Zeleny and Coleman (5) suggested another possibility. They found that the titration of a 50% ethyl alcohol extract of damaged corn was markedly affected by adjustment of the alcohol concentration up to about 75%. Above 80%, the effect was small. A similar effect with the WB solvent was ruled out by interchanging solvents for extraction and titration.

Extracts obtained from aged FBA with one solvent were evaporated and taken up in the other solvent for titration; duplicate extracts were titrated without change of solvent. As shown in Table I, material recovered from petroleum-ether extracts gave essentially equal titrations whether redissolved in benzene or in WB. The same was true of the material recovered from the WB extracts. Furthermore, the material extracted by WB from samples already extracted with petroleum ether gave a titration accounting for the difference between the two extractants.

Effect of Water Content on Titrations

Bloksma (9) has shown that flour absorbs some water from WB and thus lowers the water content to less than saturated. Obviously, the flour to WB ratio will affect the proportion of water removed, as would the moisture content and water-absorbing properties of particular samples; and the water content could differ slightly among samples both during *extraction* and *titration.* As a further check on water content as a factor in titrations, a sample of an aged FBA was extracted with WB in the usual way. The extract was freed of solvent in a rotary evaporator without heating above 40°C., and the residue taken up in *anhydrous* *n*-butyl alcohol. Aliquots of 20 ml. were taken for titration; to two, 5 ml. water was added, and to a second pair, 5 ml. additional dry *n*-butyl alcohol. All were titrated as usual. The whole experiment was repeated. No significant difference between wet and dry solutions was found; all titrations were between 7.4 and 7.6 ml.

Effect of Water Content on the Extraction

More lipids are extracted from flour by WB than by dry *n*-butyl alcohol (9). The differences are small as saturation with water is approached, but conceivably titratable compounds could be those involved. A sample of an aged FBA containing 12.7% moisture was extracted with WB in the usual way. A second sample of 10 g. was suspended in 75 ml. solvent but then 0.5 ml. water was added to the mixture before shaking for 30 min. Results are shown in Table II in the right-hand column.

TABLE II. EFFECTS OF ADDED WATER AND EXTRACTION TIME ON TITRATABLE ACIDITY OF WB EXTRACTS

Material	Extraction Time min.	No Added Water μeq. per g.	With Added Water[a] μeq. per g.
FBA	20	42.5	...
	30	43.3	43.1
	60	42.8	...
Wheat	30	7.7	7.2
	60	7.5	...
	120	7.8	...
Bran	30	12.1	11.6
	90	11.9	...

[a]0.5 ml. per 10 g. FBA; 1.0 ml. per 10 g. wheat or bran.

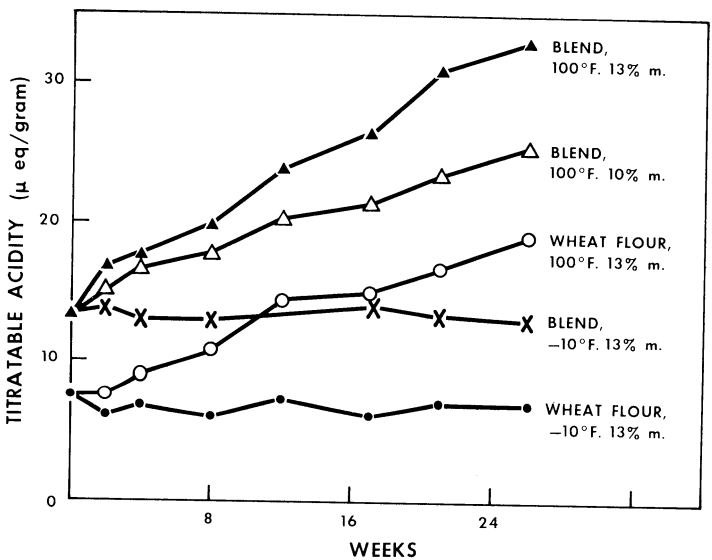

Fig. 3. Changes in titratable acidity of WB of wheat flour and soy/wheat flour blends.

The added water, which in effect would raise the moisture content of the sample from 12.7% to about 17%, did not change the titration.

Similar comparisons (Table II) were made with a ground whole wheat, at 8.8% moisture, without and with 1 ml. added water; and with bran, at 8.4% moisture, without and with 1 ml. added water. With these materials,too, the added water had no effect on the results. Thus rather large variations in sample moisture can be tolerated.

Effect of Extraction Time

As shown in Table II, 20 min. was probably adequate extraction time for FBA, and extension to 1 hr. gave no increase over the routine 30-min. extraction. It was

expected that complete extraction of lipids from wheat and especially from bran would be more difficult to achieve. However, extraction times of 2 hr. and 1.5 hr., respectively, did not increase the titrations. The titratable material must be extracted relatively quickly by WB.

Soy Flour/Wheat Flour Blends

WB extracts of soy flours (both full-fat and defatted) were brown, rather than the yellow of FBA extracts, and more intensely colored. Also, the soy flour appeared to retain considerable solvent during filtering. Apparently as a result, values on 100% soy flour samples were somewhat erratic. However, in soy flour/wheat flour blends containing up to 15% soy no difficulties were observed in using WB. Results from a storage experiment in which a full-fat soy flour/wheat flour blend was held at 13% moisture and 100°F. are shown in Fig. 3, together with results on the wheat flour alone. (Note that the scale differs from that of Figs. 1 and 2.)

The changes with time and differences among samples seem consistent with expectations, i.e., with full-fat soy added to the wheat flour to provide additional substrate, lipase in the flour (or its microflora) produced more free fatty acids than in flour alone. Also, at a lower moisture level the production of free fatty acids is slowed. Consequently it appears that WB extraction can be used satisfactorily on wheat-soy blends containing large proportions of soy.

Viability of Wheat vs. Titratable Acidity

Changes in viability of the Gaines wheat sample at 14.6% moisture and 100°F. with time are shown in Fig. 4, along with changes in titratable acidity of WB and petroleum-ether extracts. In contrast to the rapid loss of viability, the acidity of the WB extracts increased slowly. For comparison, in FBA at 13% moisture the titratable acidity increased about 18 and 35 μeq. per g. of sample at 90° and

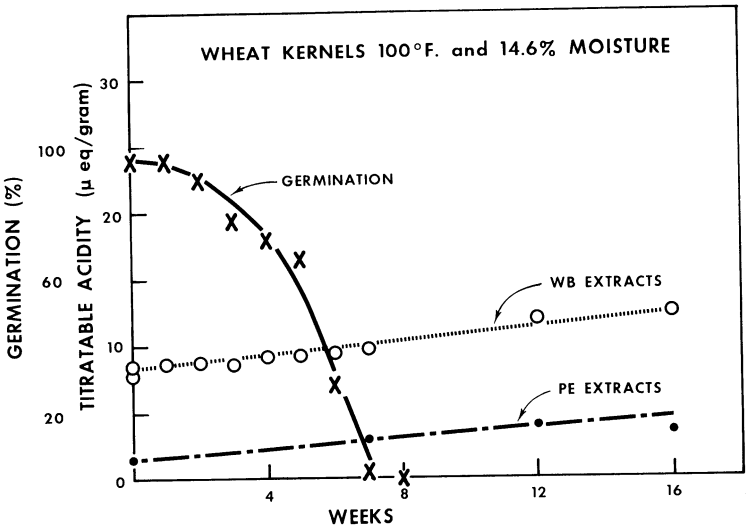

Fig. 4. Loss of viability and increase in titratable acidity of WB and petroleum-ether extracts of wheat.

120°F., respectively, in 4 months, while the increase in the wheat was only 4 μeq. at 100°F. The latter value of course is about a 50% increase over the original value but still reflects a difference of somewhat less than 1.0 ml. in titration. Changes in the titrations of petroleum-ether extracts were not significantly different, i.e., an increase of about 3 μeq.

Zeleny and Coleman (5), with 2 lots of HRW wheat, and Glass et al. (14), with 2 lots of HRS wheat, found that free fatty acids increased consistently during storage, with the changes becoming larger with increases in moisture and temperature. The increases found with the wheat used in our work were relatively small. Our 100°F. storage temperature was higher than the 67° and 86°F. temperatures used by Glass et al. (14), and our sample lost viability much more rapidly. They found nearly 90% viability after 7 weeks at 86°F. and up to 16% moisture, and about 80% viability after 12 weeks at 15% moisture. Although they found fat acidity to increase, approximately 1.5 to 2 times, in 6 weeks at 13% moisture, the increases at 67°F. appeared to equal those at 86°F. Thus free fatty acids may not form increasingly rapidly as storage temperature is raised, and despite rapid loss of viability the increase in free fatty acids may be quite small with some wheats and storage conditions.

GENERAL DISCUSSION

Despite the repeated use of titratable acidity of various extracts of flour to detect or indicate deterioration, reports differ as to the degree of correlation with baking properties. For example, Fifield and Bailey (15) showed that acidity values of 85% ethyl alcohol extracts of flours increased with greater rapidity as the extraction rate of the flour increased, but the acidity value was not considered important to baking performance. Cuendet et al. (16) stored patent, first clear, second clear, and whole wheat flours at 100°F. and moisture levels from 3% to 14%. For each flour, loaf volumes after various storage times were negatively correlated with fat acidity. The losses in loaf volume per unit increase in fat acidity were much larger for patent flour than for second clear or whole wheat flour, however. Deterioration in breadmaking properties involved other (nonlipid) constituents, too, but changes in lipids of deteriorated flours were considered to have a marked influence. Nelson (17) also found increases in fat acidity in patent and clear flours that were negatively correlated with baking performance.

The method described here is capable of indicating a similar negative correlation as shown by the results of Fellers and Bean (18). In their work with FBA, losses in loaf volume were closely related to increases in WB-extractable titratable acidity. Nelson (17) did not consider the increase in fat acidity to be a direct cause of the loss of loaf volume but only to occur simultaneously with the deterioration, because he found that loaf volume decreases occurred as rapidly during storage of ether-extracted flours in which fat acidity changed much less. The degree of correlation between flour deterioration, as shown by baking performance, and development of acidity thus appears to depend upon the nature of the samples examined and the stored conditions imposed.

The situation with whole grain subjected to poor storage conditions is not clear. Our observations showed only small increases in the free fatty acids of one wheat sample, but other adverse storage conditions must favor the development of fungal

or wheat enzymes that would form more free fatty acids. (If adverse storage conditions sometimes approach those favorable for germination, then changes might be relatively rapid. For example, Tavener and Laidman (19) found that triglyceride levels in the endosperm of wheat kernels were halved in 2 days under germinating conditions.)

If it is assumed that formation of free fatty acids reflects enzymatic activity of either microbiological or wheat origin on easily accessible lipids, then petroleum-ether extraction probably is suitable as an extractant for showing a relationship to loss of viability in seeds or baking performance in flours. However, it seems more logical to use a measurement that would also monitor the breakdown of more complex and difficulty extractable lipids, particularly as they seem essential to normal baking behavior (20). Extraction with WB would be more likely to fulfill such a requirement because WB is a more effective extractant than are nonpolar solvents. In the present work, petroleum ether and WB gave very similar results in early stages of storage. After longer storage at high temperatures, however, WB indicated better the deterioration.

WB extraction was shown to be unaffected by wide variations in sample moisture. In addition, WB extracts can be obtained rapidly and filtered readily and changes observed are larger than those in petroleum-ether extracts. Compared to more volatile and nonpolar solvents, WB has some advantages and appears to have no significant disadvantages for the determination of changes in titratable acidity arising from lipid degradation in wheat products.

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