Triticale Lipids: Composition and Bread-Making Characteristics of Triticale Flours[1]

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ABSTRACT

Ten lipid-altered flours of southern-grown triticale were evaluated by standard baking tests and other performance tests. The lipid contents of whole-grain and milled flours of triticale were altered by four defatting procedures using three different solvents and by one storage procedure conducted at room temperature in controlled relative humidity. Volume of loaves made from whole-grain and milled flours were affected little when lipids (mostly neutral) were removed with hexane, but volume decreased when the more polar lipids (glycolipids and phospholipids) were also extracted, using 95% ethanol or 80% aqueous butanol. When hexane-defatted, milled flour was fractionated into gluten and starch-water-solubles fractions and the gluten fraction extracted with 80% 1-butanol, the reconstituted flour resulted in a loaf volume of 210 cc compared to a volume of 155 cc for an unfractionated milled flour extracted with 80% 1-butanol. Whole-grain flour stored in air at a relative humidity of 64% for seven weeks gave a better loaf volume than did the control (+15 cc), but milled flour stored under the same conditions resulted in a loaf volume lower than that of the control (−30 cc). Lipid content and composition of the untreated and treated flours are presented to explain the breadmaking characteristics of the defatted triticale flours.

Generally, the quality of breads made with triticale flour is not equal to that of similar breads made with wheat flour (Tsen et al 1973, Unrau and Jenkins 1964). At least two factors, a difference in the structure of the proteins and a considerable amount of α-amylase activity, contribute significantly to the problem (Chen and Bushuk 1970a, 1970b, 1970c; Lorenz 1972). The types and quantities of lipids in triticale also should be factors; wheat lipids, although small in amount, perform an important role in breadmaking (Chung and Pomeranz 1977, Pomeranz and Chung 1978). The present research was undertaken to obtain information on the types and amounts of lipids in a triticale grown in the South and to develop data on the role of lipids in the performance of triticale flour.

MATERIALS AND METHODS

Flour

Portions of a single lot of spring triticale 72S grown in Texas were cleaned and then tempered to 13% moisture. For whole-grain flour, the grain was milled on Lee's Household Stone Grinding mill to give 100% extraction. To produce a milled-flour, grain was milled on a Brabender Quadramat Junior mill. The yield of the flour was 61%. Immediately after milling, the flours were kept at temperatures below −20°C to retard enzymatic actions that would partially degrade the lipid components and change their composition.

Portions of the whole-grain and milled flours were removed from −20°C storage as needed and partially defatted by extraction with solvents. The partially defatted flours were stored at about −20°C until baking tests were made.

A portion of the whole-grain and milled flours was placed in shallow pans and stored in air in a closed glass chamber for seven weeks at room temperature (24°C) and a relative humidity of 64.4%. These referred to as "humidified-stored flour." The humidity, maintained by a saturated solution of sodium nitrite, supplied enough moisture to enhance the enzymatic hydrolysis but not enough to promote mold growth. Baking tests were run with the stored flours.

One whole-grain triticale flour had an unusually high content of free fatty acids when first prepared. This flour, which was stored in air at room temperature (25°C) for one month at an uncontrolled humidity (about 50%), was not used in the baking tests. It is referred to as "aged flour."

Extraction of Lipids

The flour lipids were extracted with 1-butanol/water (80:20, w/w), ethanol/water (95:5, v/v), and *n*-hexane. Hereafter, these aqueous alcohols are termed 80% 1-butanol and 95% ethanol. The alcohols and hexane were reagent grade. The amount of water in the 80% 1-butanol was such that, with the moisture in the sample, the 1-butanol was practically saturated with water.

All extractions with the solvents were performed by repeatedly slurrying the solvent and flour in a glass flask immersed in a water bath at 40°C. An atmosphere of nitrogen was maintained over the slurry. In the first stage of the extraction, 500 g of flour and 3 L of solvent were stirred for 2 hr. The liquid phase and solids were then separated by filtration. The resulting solids were again slurried for 1 hr with 1.5 L of solvent. This double extraction was performed twice for each sample to produce enough lipid-altered flour for analysis and baking tests. The extracted flours were freed of residual solvents by stripping at room temperature with dry nitrogen. All filtrates were combined and freed of solvent by distillation under vacuum and stripping with dry nitrogen. The lipid residues were then extracted with chloroform warmed to 30°C; the chloroform extract was filtered; and the chloroform was removed by heating under vacuum and stripping with dry nitrogen. Chloroform extraction was deemed necessary to remove nonlipids, such as pigments and sugars, extracted by the extensive treatment with hexane and aqueous alcohols. The lipids were stored at −20°C under nitrogen until analyzed.

Reconstituted Flours

Portions of whole-grain and milled flours were first extracted with *n*-hexane. The hexane-defatted flour was separated into a gluten fraction and a fraction of starch plus water-solubles by hand-washing with water. The lypholized gluten fraction was extracted with 80% 1-butanol by the lipid extraction procedure described above. The fraction of starch plus water-solubles was also lypholized. Both fractions were then reconstituted into flours for baking tests.

Analytical Procedures

Lipid content was determined from the weight of the dry chloroform extract from each control and treated flour. Protein and ash were determined by AACC methods. Protein content was calculated as N × 5.7.

Moisture content was determined by AOAC method 14.004 (air oven method).

Content of residual lipids was determined by acid hydrolysis, AOAC method 14.019 (fat by acid hydrolysis).

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Thin-layer chromatography was used to separate and quantitate the lipids extracted from each treated flour. Silica Gel 60 (precoated plates No. 5763, EM Laboratories) was the adsorbent. The developing solvent for neutral lipids (including free fatty acids) was chloroform; for glycolipids, chloroform/methanol/water (75:25:4, v/v); and for phospholipids, chloroform/methanol/water/aqueous ammonia, (65:35:4:0.2, v/v). All the separated components were identified with known compounds obtained from Applied Science Laboratories, State College, PA. After the development of the chromatographs, the spots were visualized by spraying the dried plates with a solution of copper acetate-orthophosphoric acid reagent and charring at 175°C for 15 min (Fewster et al 1969). Individual components were quantified with a scanning densitometer. Calibration curves were developed for quantifications. The analyses were repeated three times.

The lipid samples were subjected to a boron trifluoride-catalyzed methanolysis (Metcalfe et al 1966) to obtain the methyl esters of the fatty acids. These esters were analyzed by gas-liquid chromatography on a Barber Coleman model 20 gas chromatograph equipped with a tritium detector and a stainless-steel column (1/8 in. diameter, 15 ft long) containing 12% diethyleneglycol succinate (stabilized) on Gas Chrom P, 10–100 mesh. The column temperature was 184°C.

Baking Procedure

The breads were baked by the straight-dough procedure. The ingredients (on a flour weight basis) were 6% sugar, 4% nonfat milk solids, 3% shortening, 2.5% yeast, 2% salt, 0.5% yeast food, and 0.5% monoglycerides and diglycerides.

The flour and the other ingredients were mixed in a 12-qt bowl by a Hobart A-120 mixer for 3 min at a speed setting of 2. The loaves were scaled to 125 g. Three loaves per batch were mechanically molded, proofed to height at 35°C and 95% rh, and baked at 193.3°C for 20 min. The loaf volumes were measured by rapeseed displacement 2 hr after baking. Farinograms were obtained by the standard AACC farinogram procedure using a 300-g mixer. The terms used to describe farinograms were from the Farinograph Handbook (AACC 1960). The flours were tested on a mixograph at the spring setting of 12. Area under the curve was calculated by cutting out the area under the curve on the chart, weighing it, and using the following formula: area under the curve (cm^2) = weight of the paper × 0.00783. The number 0.00783 was a correction factor obtained by conducting an experiment to determine the accuracy of the method. The correlation coefficient between the area obtained by this method and the actual area measurement was $r^2 = 0.994$ (n = 24).

RESULTS AND DISCUSSION

The whole-grain flour contained 13.7% protein, 1.60% ash, and 12.7% moisture. The milled flour contained 12.2% protein, 0.38% ash, and 12.3% moisture. These values did not change significantly after extraction of the lipids with the different solvents or after storage at 64% rh.

Extractable Lipids

Analytical Samples. Exhaustive extraction of freshly ground samples with 80% 1-butanol removed 2.64 and 1.44% total lipids from the whole-grain flour and the milled flour, respectively. Extraction of residual lipids by acid hydrolysis yielded an additional 0.55% for the whole-grain flour and 0.71% for the milled flour. These analyses are averages of three determinations each. The lipids obtained by acid hydrolysis were, of course, altered by the acid used in the extraction and therefore were not further identified. Lipids not extractable with solvents are apparently so deeply embedded in the endosperm fraction that they have no effect on baking performance (MacRitchie and Gras 1973).

Working Samples. Percentages of lipids extracted from the flour used in the baking tests are given in Table I. As anticipated, the 80% 1-butanol extracted the largest amounts. Hexane extraction removed 73% of the 80% 1-butanol-extractable lipids from the whole-grain flour and 60% from the milled flour. A smaller proportion of lipids could be extracted with 80% 1-butanol from humidified-stored flours. Probably some types of lipids polymerized, oxidized, or both (Lea 1938) or combined from flour components, making them unextractable (Langlois and Wagoner 1967).

Changes occurring in the lipids of aged flour were examined more closely in a separate experiment. This milled flour was an older sample and exhibited a higher original free fatty acid content, 11%, calculated on a total lipid basis. After storage for about one month, the content of free fatty acids in the lipids of the aged flour was 26%. The composition of the total fatty acids, free and combined, also changed on storage (Table II). Linolenic acid ($C_{18:3}$) decreased from 5.7 to 4.5% and linoleic acid ($C_{18:2}$) from 59.6 to 57.7%, whereas palmitic acid (C_{16}) increased from 17.5 to 20.8%. Thus, lipolitic enzymes and oxidation should have a significant role in the effect of lipids on the performance of triticale flours. Small

TABLE I
Lipids Extractable from Triticale Flours Used in Baking Tests

Flour	Lipids[a] (% sample weight) Extracted with		
	Hexane	95% Ethanol	80% 1-Butanol
Whole-grain	1.94	2.41	2.64
Milled	0.87	1.33	1.44
Stored[b]			
Whole-grain	...	...	2.54
Milled	...	...	1.15

[a] All values represent the average of three determinations on a moisture-free basis; overall SD = 0.020.
[b] At 24°C and 64.4% rh for seven weeks.

TABLE II
Fatty Acid Composition[a] (%) of Lipids[b] from Whole-Grain Triticale Before and After Storage in Air at Room Temperature (25°C) for One Month

Fatty Acid	Before	After
C_{14}	0.13	0.17
C_{15}	0.20	0.23
$C_{14:2}$	0.09	0.06
C_{16}	17.50	20.80
$C_{16:1}$	0.60	0.46
C_{17}	0.05	0.06
$C_{16:2}$	0.13	0.16
$C_{17:1}$	0.07	0.06
C_{18}	0.17	0.87
$C_{18:1}$	13.80	14.30
$C_{18:2}$	59.60	57.70
$C_{18:3}$	5.66	4.52
$C_{20:1}$	1.28	0.84
ECL 20:8[c]	0.22	0.84
Free fatty acid (% total lipids)	11.00	26.00

[a] Values represent an average of two determinations; overall SD = 0.08.
[b] Extracted with a solution of 80% 1-butanol.
[c] ECL = equivalent chain length.

TABLE III
Major Fatty Acids in Lipids of Triticale Flour Before and After Storage for Seven Weeks at 64% rh[a]

Fatty Acid	Whole-Grain Flour		Milled Flour	
	Control	Stored	Control	Stored
C_{16}	16.6	18.1	18.4	26.9
$C_{18:1}$	14.7	14.2	11.3	12.3
$C_{18:2}$	60.3	59.0	63.3	53.4
$C_{18:3}$	4.3	4.8	3.2	2.5
Free fatty acid (% total lipids)	5.0	10.3	5.0	6.3

[a] Values represent an average of two determinations; overall SD = 0.08.

amounts of $C_{14:2}$ and $C_{17:1}$ were found in this sample. As far as we are aware, this is the first time that these fatty acids have been reported in whole-grain flour.

Changes in the fatty acid composition of the humidified-stored flour samples are given in Table III. Only the four major fatty acids are listed. Total extractable linoleic acid, both free and combined as an ester, decreased from 60.3 to 59.0% for the whole-grain flour and from 63.3 to 53.4% for the milled flour. Both the whole-grain and milled flours showed an increase in palmitic acid esters and in free fatty acids on storage. The whole-grain flour contained a lower proportion of oxidized fatty acid groups and a higher proportion of free fatty acids, probably because antioxidants and lypolytic enzymes were present at higher levels.

Compositions of the lipids in the control and humidified-stored flours and of the lipids extracted with the three solvents from the flours used in the baking tests are given in Table IV.

After storage at 64% rh, the greatest changes were in triglycerides. An enzymatic hydrolysis of triglycerides yields monoglycerides, diglycerides, and free fatty acids. Yet, in these flours, the percentages of extractable monoglycerides, diglycerides, and free fatty acids changed relatively little.

With only one exception, storage at 64% rh increased the relative proportion of all the polar lipids for which analyses were made. The one exception is the proportion of lysophosphatidyl choline in the milled flour. In this case, storage resulted in a decrease from 14.8 to 5.3%. Considering the decrease in total extractable lipids and in the proportion of triglycerides after storage, the storage apparently increased the absolute amounts of extractable polar lipids. Warwick et al (1979) found a decrease in the phospholipid content in wheat flours that had been stored over a much longer period of time, but their storage conditions were quite different from those reported here.

Table IV also compares the composition of the lipids in the control flours with the lipids removed from the flours before the baking tests. The hexane extractions of both flours removed a greater proportion of nonpolar lipids than did extractions with aqueous alcohols. Extraction with 95% ethanol was relatively effective in removing both nonpolar and polar lipid components but did not remove lysophosphatidyl choline.

TABLE IV
Lipid Composition[a] (weight percent) for Whole-Grain and Milled Triticale Flours[b] Used in Baking Tests

Component[c]	In Sample Flours				Extracted with					
	Control		After Storage for Seven Weeks at 64% rh		*n*-Hexane		95% Ethanol		80% 1-Butanol	
	WF	MF	WF	MF	WF	MF	WF	MF	WF	MF
Monoglycerides	2.40	3.00	2.00	3.50	2.40	3.50	1.50	2.50	2.40	2.40
Diglycerides	5.50	3.00	2.70	3.00	5.00	4.00	4.00	7.00	5.50	3.25
Triglycerides	50.0	26.0	27.0	13.7	65.0	26.0	58.5	31.0	50.0	30.0
Free fatty acids	5.00	6.00	10.3	6.30	7.00	3.50	6.00	4.00	5.50	5.00
Steryl esters	2.50	1.80	2.00	2.00	2.50	2.00	2.60	2.00	1.00	2.40
Monogalactosyl diglycerides	2.50	4.70	5.10	5.30	2.40	3.30	3.50	4.00	4.00	6.00
Digalactosyl diglycerides	8.00	7.30	11.7	10.7	2.00	8.80	7.30	13.50	9.60	10.7
Phosphatidyl inositol	1.70	3.07	9.20	5.07	3.73	2.70	3.47	5.07	1.30	4.00
Phosphatidyl ethanolamine	1.70	3.07	12.1	14.7	3.73	2.70	10.0	14.4	6.20	10.9
Phosphatidyl choline	4.30	1.60	8.03	3.50	2.40	Trace	9.07	3.20	4.10	3.20
Lysophosphatidyl choline	5.60	14.8	6.67	5.33	Trace	None	Trace	Trace	5.00	6.20

[a] Values are an average of three determinations.
[b] WF = whole-grain flour, MF = milled flour.
[c] The lipid fractions also contained other components not identified.

TABLE V
Baking Test Data of Whole-Grain, Milled, and Treated Flours[a]

Property[b]	Control		After Storage for Seven Weeks at 64% rh		Extracted with						Reconstituted After Extraction of Lipids from Gluten Fraction	
					n-Hexane		95% Ethanol		80% 1-Butanol			
	WF	MF	WF	MF	WF	MF	WF	MF	WF	MF	WF	MF
Absorption, %	52.7	44.9	56.90	47.70	56.6	49.7	64.3	53.8	79.0	66.4	54.7	43.2
Volume, cc	265 (± 6.0)[c]	360 (± 6.9)	280 (± 5.9)	330 (± 6.2)	255 (± 4.9)	360 (± 5.2)	185 (± 3.8)	215 (± 4.3)	180 (± 3.3)	155 (± 3.0)	180 (± 3.5)	210 (± 3.6)
Specific volume, cc/g	2.41	3.26	2.59	2.99	2.36	3.20	1.71	1.95	1.70	1.44	1.70	1.92
Mixograph weakening angle, degrees	134 (± 2.8)	137 (± 2.7)	129 (± 2.5)	155 (± 2.8)	111 (± 2.6)	127 (± 2.5)	150 (± 1.9)	163 (± 2.1)	155 (± 2.5)	134 (± 2.2)	161 (± 2.5)	83 (± 1.9)
Farinograph dough development time, min	3.00	3.00	3.50	3.00	3.00	2.75	7.50	6.50	···	4.0	11.00	···
Mechanical tolerance index	100	90	100	140	120	120	30	70	···	30	···	···

[a] WF = whole-grain flour, MF = milled flour.
[b] Each mean for absorption, loaf volume, specific volume and mixograph weakening angle represents three samples. Farinograph data dough development time and mechanical tolerance index are based on the tests carried out once on each sample only.
[c] Standard error of the mean.

The results of the baking tests for the control flours kept at −20°C and the treated flours are summarized in Table V. Storage increased the absorption of both whole-grain and milled flours moderately.

Loaf volume increased moderately after storage of the whole-grain flour and decreased moderately after storage of the milled flour. Storage of the milled flour increased the mechanical tolerance index, indicating a weakening of the flour.

Extraction of the flour lipids with *n*-hexane had little effect on the baking characteristics as measured by absorption, loaf volume, specific volume, and dough development time. Hexane extraction increased the mechanical tolerance index for the milled flour, making the defatted flour weaker. It also decresed the mixograph weakening angle for both flours, a result that is interpreted as a lowering of quality. The effect of extracting lipid with 95% ethanol actually was beneficial according to some of the baking performance data, such as water absorption. However, in the critical index of loaf volume, sizable decreases were found for the 95% ethanol-extracted flour. The latter observation conforms in direction, but not in degree, to that reported by Finney et al (1976) for wheat flour extracted with 95% ethanol. They claimed that extraction of wheat flour with the alcohol impaired the gas-retaining capacity of gluten protein, a 20% decrease in gas-retaining capacity being accompanied by a 70% decrease in loaf volume. Our data on triticale flours extracted with 95% ethanol showed that loaf volume decreased by 30% for whole flour and 40% for milled flour. Extraction of the flours with 80% 1-butanol greatly increased the absorption. Loaf volume decreased greatly, but the percent of decrease was not nearly as marked for the whole-grain flour as it was for the milled flour. In fact, the whole-grain defatted flour gave a better loaf volume than did the corresponding milled flour. Defatting did not significantly affect the development time of the milled flour but did affect the stability of the dough, as measured by the mechanical tolerance index. The flour did not show a tendency to weaken and hence to depart from the top of the curve; therefore the development time and mechanical tolerance index could not be measured for the defatted, whole-grain flour.

Flours from which lipids have been extensively extracted with water-saturated butanol and other alcohols are generally unsuitable for studying the functionality of lipids in baking because these solvents adversely affect other flour components (Finney et al 1976). The usual explanation is that the solvent, such as water-saturated butanol, forms a complex with starch and inhibits gas production during baking (Hoseney et al 1969).

The behavior of the triticale flours on extraction with 80% 1-butanol resembled that reported for wheat flours. This extraction resulted in the formation of a starch-alcohol complex that stopped gas production, as measured by proof height of dough, and apparently denatured the gliaden fraction of the gluten protein.

In an attempt to overcome this deleterious effect, we first separated the gluten from the starch and water-solubles. When the fractionated gluten was extracted with 80% 1-butanol and this completely defatted gluten was reconstituted with the fraction of starch plus water-solubles into flour, the absorption was not significantly different from that of the control (Table V).

For the reconstituted, defatted, whole-grain flour, the loaf volume decreased from the 265 of the control to 180 cc, but this volume was the same as that of unfractionated flour extracted with 80% 1-butanol. In comparison with the control, the mixograph weakening angle increased in the reconstituted defatted whole-grain flour and decreased in the similarly treated milled flour. This behavior is still unexplained. The farinograph development time increased greatly for the whole-grain flour. The mechanical tolerance indices for the milled or whole-grain flour could not be measured; the flours became too stiff and did not depart from the top of the curve.

In summary, hexane extractions of whole-grain and milled flours removed larger proportions of nonpolar lipids than did extractions with aqueous alcohols. Extraction of the flours with 95% ethanol was relatively effective in removing both nonpolar and polar lipids. Loaf volumes were affected little in breads made from the flours extracted with hexane, which extracted mostly neutral lipids. Water absorption was increased when the flours were extracted with 95% ethanol, but in the critical index of loaf volume, sizeable decreases were found for these flours. Extraction of the flours with 80% 1-butanol greatly increased the water absorptions and greatly decreased the loaf volumes. Fractionation of hexane-defatted, milled flour into gluten and starch-water-solubles fractions and extraction of the gluten with 80% 1-butanol resulted in a loaf volume of 210 cc compared to a volume of 155 cc for an unfractionated milled flour extracted with 80% 1-butanol. Whole-grain flours stored in air at a relative humidity of 64% for seven weeks gave a slight increase in loaf volume compared to that of the control.

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