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Wheat Hydrocarbons¹

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ABSTRACT

Nonpolar lipids were extracted from HRS wheat flour and bran, and from durum semolina and bran, by shaking with petroleum ether. The lipids were streaked on thick silica-gel plates and developed in carbon tetrachloride, and the hydrocarbon bands were removed. The average total hydrocarbon content of the dry flour and semolina was 0.0036%. All n-hydrocarbons between C-8 and C-33 were separated and identified by gas-liquid chromatography, and the relative amounts were determined in flour, semolina, and bran. C-13 to C-15, C-32, and C-33 were present in very small amounts in all samples analyzed. Flour and semolina contained substantial amounts of C-9 to C-11 and C-17; the bran of both wheat varieties contained more of the long-chain hydrocarbons, particularly C-25, C-27, and C-29. In flour and semolina, 57 and 58%, respectively, of the n-hydrocarbons were odd-numbered; in HRS bran 67%, and in durum bran 64% were odd-numbered. The n-hydrocarbons comprised 65% of the total hydrocarbons analyzed. Other homologous series present were identified as the combined 2- and 3-methyl hydrocarbons, and 1-cyclohexyl derivatives, which comprised 20 and 12%, respectively, of the total hydrocarbons. Squalene, an unsaturated hydrocarbon, accounted for 3% of the total.

The effect of adding organic solvents, particularly hydrocarbons, to dough has been of interest in recent years. Muller et al. (1) found that the addition of alcohols from methanol to nonanol modified both the extensigraph extensibility and resistance. Ponte and co-workers (2,3,4,5,6) studied the effect of 47 organic liquids, including hydrocarbons, on dough and loaf volume. They reported that most of the aliphatic hydrocarbons used (C-5 to C-12) increased farinogram peak time and stability and produced finer grain, smoother texture, and brighter color in bread

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crumb as compared with a control. In most cases, loaf volume decreased. Heptane contained the optimum carbon length for greatest improvement. Fat added to the dough was essential for the improving effect of the solvent; the addition of a solvent in the absence of added fat led to the deterioration of bread quality. Elton and Fisher (7) reported a decrease in loaf volume with the addition of octadecane (C-18) and eicosane (C-20), but generally an increase when docosane (C-22) and tetracosane (C-24) were added.

The naturally occurring hydrocarbons in wheat have received little attention. Drummond et al. (8) found squalene and another similar hydrocarbon in the unsaponifiable material of wheat-germ oil. McKillican and Sims (9) reported no hydrocarbons present in Thatcher, a HRS wheat, or Lemhi, a SW wheat, and only trace amounts in Mindum, a durum wheat. They used column and thin-layer chromatography for their analysis. Meredith (10), however, used column chromatography to fractionate flour lipids extracted with ethyl ether, and reported 0.5% hydrocarbons to be present. Kuksis (11) analyzed the unsaponifiable matter of a commercial wheat-germ oil by gas chromatography. He reported all n-hydrocarbons between C-17 and C-33, and traces of C-34 and C-35. Small amounts of the iso, anteiso, and 1-cyclohexyl chains also were reported. C-29 was most abundant.

The current work reports the findings obtained from hydrocarbon analysis of flour, semolina, and various flour mill streams.

MATERIALS AND METHODS

A majority of the hydrocarbon analyses were performed on Chris, a HRS wheat, and Leeds, a durum wheat, both from the 1968 harvest. Chris was a composite sample of the pure variety grown in several locations in North Dakota. It was milled on a pilot mill (12), and several of the mill streams were saved for hydrocarbon analysis. A sample of Chris, grown at Langdon, North Dakota, and milled on a Buhler mill also was analyzed. Leeds, grown at Langdon, North Dakota, was milled into semolina on an Allis laboratory mill.

The lipids were extracted by shaking the sample in petroleum ether (b.p. 30° to 60°C.) for 0.5 hr. on a Burrell shaker. For determination of total hydrocarbon content, preliminary separation of the nonpolar lipids (hydrocarbons, sterol esters) was performed by silicic acid column chromatography. Petroleum ether was the eluant. This nonpolar fraction was streaked on thick silica-gel plates (0.75 mm.) and developed in carbon tetrachloride. Two bands appeared above the sterol esters. One was identical with squalene in R_f value and the other band, which was near the front, was similar in location to saturated hydrocarbon standards. When exposed to iodine vapors, the squalene band showed greater iodine absorption than the band at the front of the plate. Both bands were scraped from the plate, eluted with petroleum ether:ethyl ether, 1:1, evaporated to dryness, and weighed.

Column chromatography was not employed as a step in separating the hydrocarbons for gas-chromatographic analysis. Rather, the crude lipids were streaked directly on the thick-layer plates and developed as reported. The hydrocarbon bands were scraped from the plate and eluted from the silica gel, and the volume of the eluant was reduced to about 2 drops. A portion of this solution was injected into the gas chromatograph.

Gas-chromatographic analyses were performed on a Barber-Coleman gas chromatograph, equipped with twin 6-ft. glass columns and flame ionization. Most analyses were programmed from 60° to 265°C. at 7.5° per min. For column packings several stationary phases were tested. These included SE-52, LSX-3-0295, XE-60, SE-30, and NPGS. With the exception of NPGS (neopentyl glycol sebacate), all were silicone gums. LSX-3-0295, a trifluoro propyl, methyl, and vinyl copolymer silicone (13% by weight of the solid phase, Gas Chrom Q), was used for all reported determinations. SE-30 and SE-52 also were satisfactory. XE-60 afforded poorer separation among the short carbon chains. NPGS gave good separation among the short carbon chains, but was satisfactory only to about C-24, probably because the allowable maximum temperature was only 230°C. None of the packings tested would separate the iso and anteiso isomers from each other; they separated as a single peak from the n-hydrocarbons.

The gas-chromatogram peaks were identified by comparing the retention time of standards with the unknowns. The following standards were used: n-hydrocarbons having carbon chain lengths of 8, 9, 10, 11, 12, 13, 14, 16, 18, 20, 22, 24, and 28; 2-methyl heptane, 2-methyl nonane, and 2-methyl undecane; all iso hydrocarbons; 3-methyl heptane, an anteiso hydrocarbon; and squalene, an unsaturated hydrocarbon.

Relative amounts of the hydrocarbons present were determined by measuring peak heights. When exact amounts of C-11, C-18, and C-28 were separated by gas chromatography and their peak heights were measured and compared to the amounts injected, C-11 and C-18 were the same, but C-28 showed a 10% loss in peak height. This correction was included in the calculations. Accurate quantitative determinations were impaired by an irregular base line when the naturally occurring wheat hydrocarbons were measured. This was not true with a mixture of the standards. Consequently, results were calculated only to the nearest percent.

RESULTS AND DISCUSSION

Figure 1 shows the separation of hydrocarbons by thick-layer chromatography. No difference was noted between the hydrocarbon content of the HRS and durum wheat flour. The average value of total hydrocarbons present in two varieties each of HRS flour (Chris and Red River 68) and durum flour (Leeds and Wells) was 0.0036% of the dry weight.

Figure 2 shows a gas chromatogram obtained when the hydrocarbon bands, removed from thick silica-gel plates, were analyzed. All n-hydrocarbons from C-8 to C-33 were identified and are shown in Fig. 2. Squalene, an unsaturated hydrocarbon, occurred between C-28 and C-29.

The peaks just preceding the numbered n-hydrocarbons were identified as combined iso and anteiso hydrocarbons. The peaks just following the n-hydrocarbons were identified tentatively as the 1-cyclohexyl derivatives. No standards were available for these derivatives, but according to Levy et al. (13) and Mold et al. (14) the 1-cyclohexyl derivatives of the hydrocarbons are eluted just after the normal carbon chains, which contain one more carbon than the cyclic derivative. Identification of the cyclic derivatives on this chromatogram is based on this evidence alone.

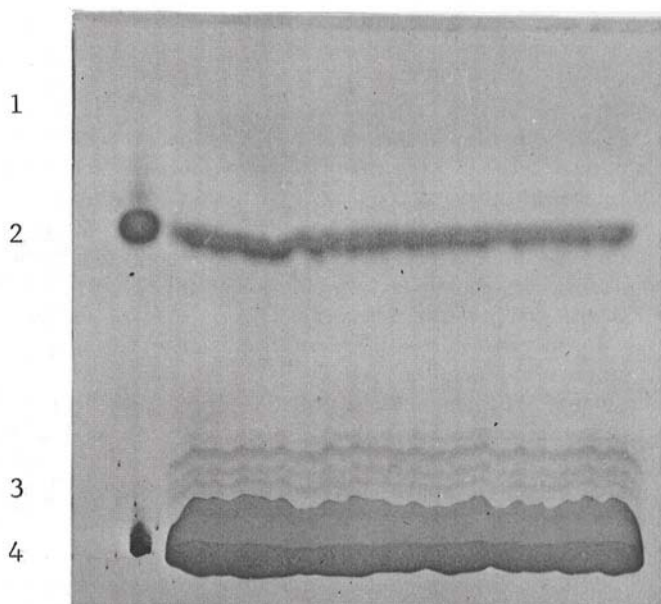


Fig. 1. Separation of hydrocarbons from crude lipids using TLC. Solvent system, carbon tetrachloride; visualization, iodine vapors. The single spot (left) is a squalene standard. The streaked portion is crude durum lipid. Identification: 1, saturated n-hydrocarbons (very little absorption of iodine); 2, squalene; 3, sterol esters; 4, crude lipids, principally di- and triglycerides.

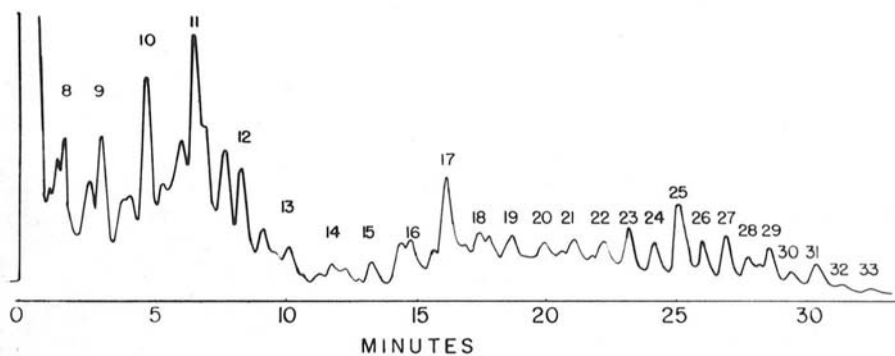


Fig. 2. Gas chromatogram of hydrocarbons extracted from Leeds semolina. The numbered peaks are normal chain hydrocarbons. The peaks just preceding these are the combined iso and anteiso hydrocarbons. The peaks following the numbered peaks are 1-cyclohexyl derivatives having one less carbon than the numbered peaks. Squalene is between C-28 and C-29.

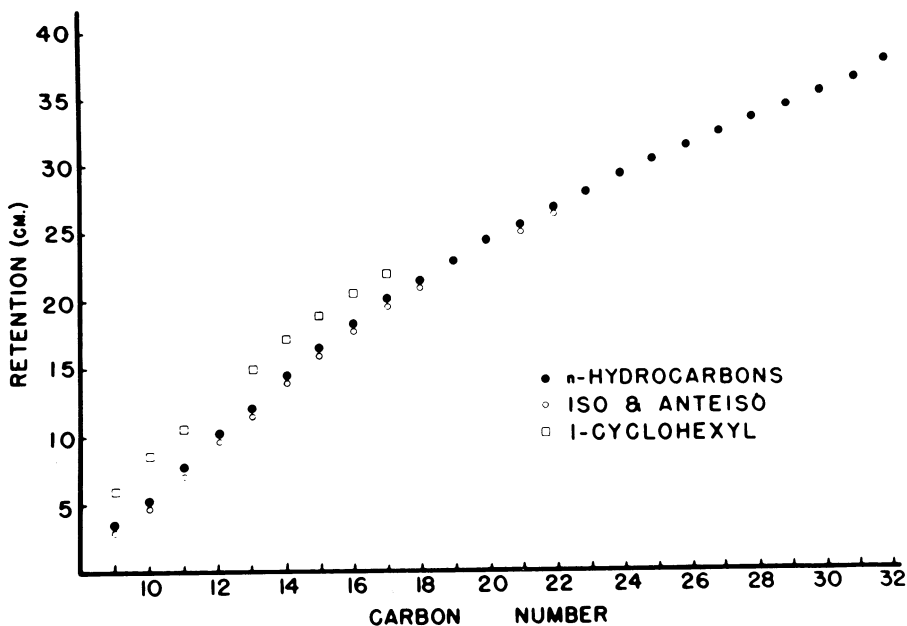


Fig. 3. Plot of retention time vs. carbon number, showing the different homologous series of hydrocarbons.

When the retention time of the hydrocarbons was plotted against carbon number, different homologous series were evident (Fig. 3). These series present further evidence toward identification of the hydrocarbons. It can be seen in Fig. 3 that the derivatives of the n-hydrocarbons contained short carbon chains.

The n-hydrocarbon distribution in HRS wheat flour, several mill streams of the HRS wheat, and durum semolina and bran was measured. All mill streams contained hydrocarbons. Values are shown in Table I for the n-hydrocarbon distribution in flour and bran of a HRS wheat, and in semolina and bran of a durum wheat.

Hydrocarbons having the shortest chain lengths (C-12 and lower) occurred most abundantly in the patent flour and semolina. Bran of both the durum and HRS wheat was quite low in C-9 and C-10. Considerable variation in amounts of the short-chain hydrocarbons was observed, even from different lipid extractions of the same sample. Apparently some loss of short-chain hydrocarbons occurred when the solvent was removed by roto-evaporation, or during excessive exposure of the thin-layer plates to air at room temperature. When these conditions were controlled, i.e., the bands scraped from the thick-layer plates immediately after development (5°C.) and the solvent removed by passing nitrogen over the surface, the amounts of C-9 to C-11 were consistently higher.

C-13 to C-15, C-32, and C-33 were present in very small amounts in most samples analyzed. In Leeds bran (durum), 76% of the hydrocarbons occurred between C-16 and C-31, and in Chris (HRS), 88%. In semolina and flour, 47 and 53% of the n-hydrocarbons were between C-16 and C-31. C-17 was a major

TABLE I. DISTRIBUTION OF n-HYDROCARBONS IN FLOUR, SEMOLINA, AND BRAN

Carbon Chain	Chris Patent	Chris Bran	Leeds Semolina	Leeds Bran	Carbon Chain	Chris Patent	Chris Bran	Leeds Semolina	Leeds Bran
9	11	1	7	6	22	3	5	2	4
10	15	4	15	7	23	3	6	3	4
11	12	1	19	4	24	3	4	2	3
12	4	1	7	1	25	6	14	5	14
13	1	<1	2	1	26	3	3	3	3
14	1	<1	1	<1	27	4	9	3	8
15	1	<1	1	1	28	2	2	3	2
16	3	1	2	2	29	3	14	3	9
17	7	3	8	3	30	1	2	1	2
18	3	4	3	4	31	3	8	1	4
19	3	4	3	4	32	<1	1	1	1
20	3	4	3	5	33	<1	<1	<1	<1
21	3	5	2	5					

hydrocarbon in flour and semolina. The higher-ash mill streams (bran, in particular) contained the greatest amounts of C-25, C-27, and C-29. This would be expected, since these hydrocarbons probably were extracted from the cutin of the wheat kernel. Of the total n-hydrocarbons in bran of Chris, 67% were odd-numbered; in the bran of Leeds, 64% were odd-numbered. In flour (Chris), 57% were odd-numbered, and in semolina (Leeds), 58%.

When all the peaks obtained in a gas chromatogram of the hydrocarbons extracted from Chris patent flour were measured, the following distribution was obtained:

	%
Normal chain hydrocarbons	65
Combined 2- and 3-methyl hydrocarbons	20
1-Cyclohexyl derivatives	12
Squalene	3
Total odd-numbered hydrocarbons	58

CONCLUSION

Hydrocarbons were found throughout the wheat kernel, as determined by analyzing different flour mill streams. The hydrocarbon fraction was made up of an interesting and complex mixture of normal and isomeric hydrocarbons. Although some of the hydrocarbons identified in the flour and semolina may have originated in the bran, some should have been naturally occurring, because a different distribution of carbon chain length was noted when the hydrocarbons in bran were compared with those in flour. The extremely small amounts present in wheat flour make it doubtful but not completely improbable that they could affect baking quality. This small amount of naturally occurring hydrocarbons would have an optimum distribution throughout the flour, and an association that would be hard to duplicate in reconstitution work. None of the reconstitution studies reported in the literature have used amounts of hydrocarbons so small.

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