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EFFECT OF HEXANE IN DOUGHS MADE WITH DIFFERENT FLOURS, WITH AND WITHOUT ADDED FAT[1]

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ABSTRACT

The addition of 0.64% hexane, flour basis, to conventional sponge doughs made with two groups of flours (one group of 11 commercially milled flours and one group of 15 pilot-milled flours) invariably led to marked improvements in grain score and crumb texture. When the hexane was added to doughs not containing added fat, however, the solvent exerted deteriorating effects on the resulting bread instead of functioning as an improver. The extent of deterioration in the doughs was widely different, depending on the flour employed. For both groups of flours, trends in the positive direction were observed between protein and lipid levels in the flours, and susceptibility to deterioration in the dough systems containing hexane but no added fat.

Studies conducted in this laboratory on the unique dough-improving effects of small amounts of hexane, heptane, and related hydrocarbons have indicated that added dough fat is essential for the bread-improving effect to be manifested (1). Without added fat, these solvents in fact were shown to exert a deteriorating action in dough. More recent observations have suggested, however, that the extent of this deterioration might be a function of the particular flour employed in breadmaking. To develop more information in this area, a study was undertaken, encompassing flours both commercially milled and pilot-milled, with results as herein described.

Materials and Methods

Two groups of flours were studied in the present investigation. One group of 11 flours comprised commercially milled, bakers' patent flours; analytical values of these flours are presented in Table I.

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A second group of 15 flours was selected from those evaluated by the Hard Winter Wheat Quality Advisory Council during the 1964 collaborative study of wheat varieties (2). These flours were milled on the pilot mill at Kansas State University from a number of wheat varieties grown in various states. Analytical values, obtained in this laboratory, of the 15 flours are summarized in Table II.

These data show less lipids and less ash in the pilot-milled flours compared to the commercial flours; this is probably attributable to a number of factors, including differences in crop year and variety as well as milling and extraction.

Sponge-dough baking tests were conducted by a laboratory procedure that yielded two 1-lb. loaves per dough, essentially as previously described (3). Optimum mixing times, absorption, and levels of Arkady-type yeast food were determined for each flour before the main baking experiments were undertaken.

Each flour was baked by three different dough systems, varying only in the presence of hexane and lard, and designated as treatments A, B, and C: treatment A, control (3% lard); treatment B, 0.64% hexane, based on flour (3% lard); treatment C, 0.64% hexane (no lard).

The hexane, where employed, was introduced at the dough stage by simply pipetting the solvent into the mixing bowl immediately prior to starting the machine. Each dough made from the 11 commercial flours was produced in triplicate (total of 99 doughs); each dough made from the 15 experimental flours was produced in duplicate (total of 90 doughs). Of the 15 experimental flours, doughs made by treatments B and C were produced several months after the control doughs (treatment A) had been made for participation in the 1964 collaborative study of the Hard Winter Wheat Quality Advisory Council. Insufficient sample precluded a repeat of the control doughs at the later date.

Protein, ash, and total lipids (by acid hydrolysis) were determined by routine methods (4). Lipids were also extracted from the flours by Soxhlet procedure; extractions were carried out overnight (about 18 hr.) using anhydrous diethyl ether, petroleum ether, or ethanol, as noted. Lipid phosphorus was estimated by the method of Harris and Popat (5).

The "defatted" flour used in one of the baking experiments was prepared by extracting a commercial bakers' patent flour with petroleum ether at room temperature. About 4 liters of solvent was added to 10 lb. flour. The slurry was allowed to stand for 2 hr. with occasional stirring, then the solvent was decanted and filtered through Whatman No. 1. The flour was extracted three more times (the last

TABLE I

Analytical Data on Eleven Commercially Milled Flours

Flour	Protein [a]	Ash [a]	Lipids	
			Ether-Extractables [b, c]	Acid Hydrolysis [b]
	%	%	%	%
1	11.2	0.42	1.13	2.26
2	11.7	.44	1.14	2.20
3	11.3	.45	1.22	2.43
4	11.8	.44	1.11	2.58
5	12.0	.44	1.05	2.05
6	11.9	.44	1.15	2.69
7	12.1	.43	1.19	2.30
8	11.7	.43	1.16	2.30
9	11.4	.45	1.20	2.42
10	11.4	.44	1.19	2.46
11	12.0	.42	1.21	2.76
Mean	11.68	0.436	1.159	2.405

[a] 14% Moisture basis.
[b] Flour solids basis.
[c] Diethyl ether.

TABLE II

Analytical Data on Fifteen Pilot-Milled Flours

Flour	Protein [a]	Ash [a]	Lipids			Fatty Acids	
			Ether-Extractables [b, c]	Acid Hydrolysis [b]	Phosphorus [d]	Unsaturated	Saturated
	%	%	%	%	%	%	%
Oklahoma							
Triumph	11.8	0.37	0.96	1.72	0.316	21.7	78.3
Comanche	12.3	.39	.95	1.82	.348	21.3	78.7
R.R. Triumph	12.3	.37	.95	1.68	.335	20.8	79.2
CI 13523	12.3	.40	.95	1.65	.342	20.7	79.2
Texas							
Comanche	13.1	.44	.92	1.77	.296	19.6	80.4
391-56-DI-23	14.1	.48	.94	1.79	.364	19.2	80.8
Kansas							
Triumph	12.6	.39	.91	1.46	.381	21.9	78.1
Kaw	11.9	.40	.90	1.72	.346	20.7	79.2
Scout	11.5	.41	.92	1.67	.296	20.7	79.2
Ottawa	12.3	.41	.93	1.73	.348	26.0	74.0
QH CI 13285	12.0	.39	.91	1.65	.392	19.6	80.4
Colorado							
Kaw	13.1	.35	.91	1.60	.375	21.2	78.8
Scout	13.2	.33	.91	1.59	.347	21.7	78.2
Lancer	13.3	.36	.90	1.54	.393	22.1	77.9
Wichita	13.4	.34	.94	1.52	.337	21.1	78.9
Mean	12.61	0.389	0.927	1.661	0.3477	21.22	78.75

[a] 14% Moisture basis.
[b] Flour solids basis.
[c] Petroleum ether.
[d] Based on ether-extractables.

two times with smaller charges of solvent). All the extracts were combined, and the solvent was evaporated off on a steam bath. An average yield of 0.66% lipids was obtained by this procedure.

Fatty acid compositions of extracted lipids were analyzed by gas-liquid chromatography. The lipids were saponified as described by Ast and Vander Wal (6), and esterified by the procedure of Metcalfe and Schmitz (7). Analyses were made with an F & M Model 609 flame ionization gas chromatograph, utilizing a copper column 10 ft. by 1/4 in. o.d., packed with 20% diethylene glycol succinate polyester on 70- to 80-mesh Chromosorb W.

Thin-layer chromatographic analyses were conducted with 20 cm. by 20 cm. glass plates coated with a layer 250 μ thick of Kieselgel (Camag, Switzerland); the adjustable Desaga applicator was used. The plates were activated by drying for 1/2 hr. at 130°C. before use. Lipids to be analyzed were dissolved in petroleum ether to yield 10% solutions. Two microliters of the solutions was applied with a syringe, and the solvent was allowed to run for a distance of 15 cm. from the origin. Spots were visualized by exposure to iodine vapor.

Results

Studies with Commercial Flours. Baking data on the 11 commercial flours are summarized in Table III.

The control doughs (treatment A) produced differences in loaf volume and grain score normally expected from a group of such flours; these differences ranged from 2,737 to 2,890 cc. and from 7.6 to 8.0 respectively.

Addition of 0.64% hexane to the dough system (treatment B) brought about the typical improvement in grain score as well as crumb

TABLE III
EFFECT OF TREATMENTS A, B, AND C ON BREAD MADE WITH ELEVEN COMMERCIAL FLOURS[a]

Flour	Loaf Volume				Grain Score			
	A	B	C	B − C	A	B	C	B − C
	cc.	cc.	cc.	cc.				
1	2,742	2,716	2,627	89	7.9	9.2	6.8	2.4
2	2,790	2,799	2,667	132	7.9	8.9	6.9	2.0
3	2,794	2,778	2,617	161	7.9	9.3	7.0	2.3
4	2,794	2,754	2,609	145	8.0	9.2	6.5	2.7
5	2,811	2,770	2,622	148	7.9	9.2	7.1	2.1
6	2,814	2,803	2,614	189	7.9	8.9	6.2	2.7
7	2,757	2,754	2,554	200	7.7	9.1	4.7	4.4
8	2,737	2,745	2,529	216	8.0	8.5	6.6	1.9
9	2,831	2,806	2,622	184	7.9	8.9	7.1	1.8
10	2,890	2,799	2,644	155	7.6	8.7	6.1	2.6
11	2,803	2,786	2,503	283	7.7	9.3	4.0	5.3
Mean	2,797	2,774	2,601	173	7.85	9.02	6.27	2.75

[a] Treatment: A, control (3% lard); B, 0.64% hexane (3% lard); C, 0.64% hexane (no lard).

texture; the mean grain score for all the flours was raised from 7.85 to 9.02. The hexane doughs produced on the average slightly less volume than the controls; as noted in previous reports (1,8), this volume difference is a function of accelerated proofing in the hexane-containing doughs and can be eliminated by proofing to time rather than height.

Addition of hexane, but no lard, to the various doughs (treatment C) consistently lowered both loaf volume and grain score and produced a more variable system. For example, the coefficient of variation for grain score for all doughs made by treatment C was 16.3%, compared to 1.6% and 2.9% respectively for doughs made by treatments A and B.

The extent of variation due to treatment C is illustrated in Figs. 1 and 2. Figure 1 shows representative loaves made with flour 5 by the three treatments; Fig. 2 shows loaves similarly produced with flour 11.

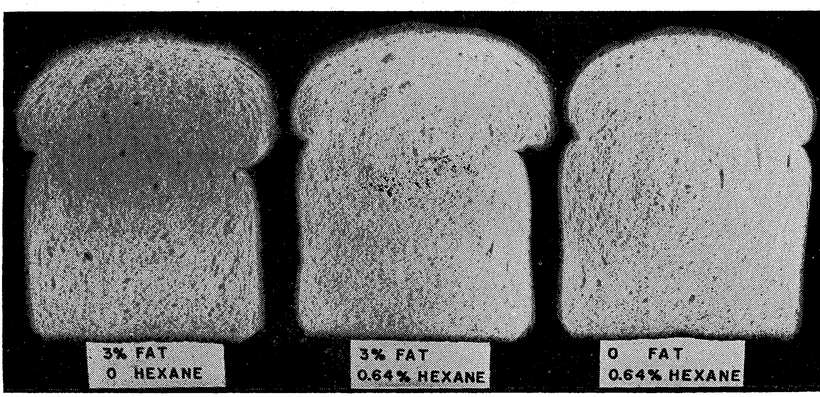

Fig. 1. Loaves made with commercial flour 5 by treatments A, B, and C.

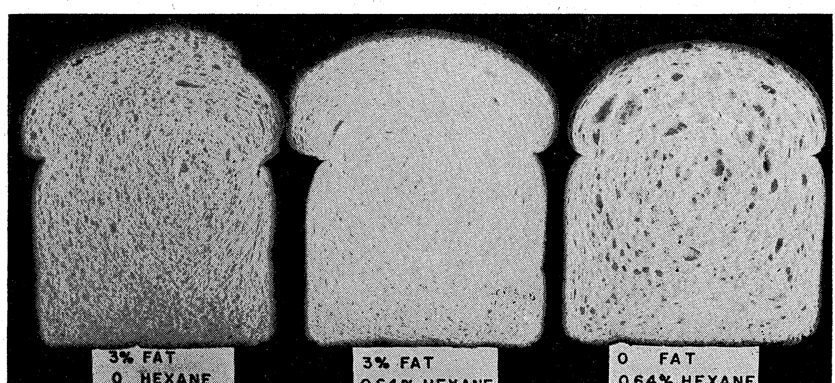

Fig. 2. Loaves made with commercial flour 11 by treatments A, B, and C.

Treatment C had relatively little effect on the grain characteristics of bread made with flour 5; it actually produced a somewhat more uniform grain, but the cells were spherical and had thicker walls compared to control. Treatment C had a drastic effect on bread made with flour 11.

The relative effects of omitting lard from the formula have not been separated, in the above experiment, from the combined effects of hexane and no fat (treatment C). Previous work has indicated, however, that the omission of lard causes only relatively small losses of volume and grain score (1) and would not account for the extensive deterioration noted above.

The available analytical data on the 11 flours, presented in Table I, was statistically compared to the baking data to determine whether any patterns might emerge. Following are some of the simple correlation coefficients obtained:

Loaf Volume Difference (Treatment B Minus Treatment C)	
Protein	0.54
Ether-extractables	0.50
Total lipids (by acid hydrolysis)	0.55

As a measure of baking performance, the loaf volume difference between treatments B and C – that is, the dough systems both with hexane but one without added lard – was utilized (Table III). For statistical significance at levels of 5 and 10%, values of 0.60 and 0.52 would have been necessary.

While the above statistics must be interpreted with caution, they do suggest trends. They suggest that flours with higher protein and lipid contents are particularly susceptible to deterioration in dough systems containing hexane but no fat. The statistics are perhaps more impressive when it is considered that the range in analytical values for these commercial flours was quite small: for example, less than 1% for protein.

The lipids of flours 5 and 11, which showed wide differences in behavior toward treatment C, were examined for phosphorus and fatty acid distribution, with results as indicated in the table below.

	Flour 5	*Flour 11*
	%	%
Lipid phosphorus, based on ethanol-extractables	0.40	0.54
Fatty acids, based on ether-extractables		
Saturated	26.4	21.1
Unsaturated	73.6	78.9

The lipids of the flour showing greatest deterioration in the dough system containing hexane but no fat, flour 11, were richer in phosphorus and unsaturated fatty acids than the lipids of flour 5. However, no obvious differences among the flour lipids could be found by thin-layer chromatography with developing solvents varying in polarity; the similarity among the ether-extractables of the 11 commercial flours is illustrated in Fig. 3.

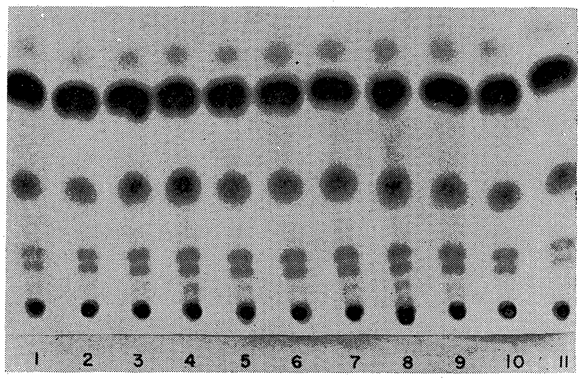

Fig. 3. Thin-layer chromatogram of diethyl ether-extractables obtained from 11 commercial flours. For identification of spots, see Fig. 6. Solvent system was n-hexane: diethyl ether:acetic acid (70:30:2, v:v:v).

Studies with Experimental Flours. A second phase of the baking studies dealt with the 15 flours milled from pure wheat varieties, with results as summarized in Table IV.

These data show a pattern similar to that obtained with the commercial flours, in that the addition of hexane to the doughs, treatment B, caused pronounced grain improvement for all the flours, and that the addition of hexane in the absence of lard, treatment C, caused volume and grain deterioration, to various degrees. A point of difference between the sets of data is that for the pilot-milled flours a small average increase in loaf volume was obtained for treatment B compared to treatment A. As was previously mentioned, hexane ordinarily causes a small volume loss when doughs are proofed to constant height, rather than time, as was done in the present study. Most likely the increase shown for the pilot-milled flours can be attributed to the time difference between production of doughs by treatments A and B (see "Materials and Methods").

The varying deterioration due to treatment C is illustrated in Figs. 4 and 5. Figure 4 shows that treatment C had little effect on

TABLE IV
EFFECT OF TREATMENTS A, B, AND C ON BREAD MADE WITH FIFTEEN PILOT-MILLED FLOURS[a]

Flour	Loaf Volume				Grain Score			
	A	B	C	B − C	A	B	C	B − C
	cc.	cc.	cc.	cc.				
Oklahoma								
Triumph	2,781	2,807	2,520	287	8.3	9.0	7.0	2.0
Comanche	2,775	2,819	2,524	295	8.5	9.0	6.0	3.0
R.R. Triumph	2,786	2,917	2,549	368	8.3	9.0	6.5	2.5
CI 13523	2,876	2,852	2,598	254	8.0	8.8	6.8	2.0
Texas								
Comanche	2,790	2,864	2,578	286	7.8	9.2	5.5	3.7
391-56-DI-23	2,840	3,028	2,496	532	8.1	9.0	4.0	5.0
Kansas								
Triumph	2,775	2,819	2,614	205	7.8	9.0	5.5	3.5
Kaw	2,811	2,872	2,709	163	8.2	8.5	7.3	1.2
Scout	2,832	2,918	2,737	181	8.3	8.5	7.6	0.9
Ottawa	2,770	2,803	2,500	303	7.4	9.0	4.5	4.5
QH CI 13285	2,794	2,897	2,594	303	7.8	8.8	6.5	2.3
Colorado								
Kaw	2,781	2,852	2,574	278	8.2	8.5	7.2	1.3
Scout	2,754	2,705	2,557	148	7.5	9.0	5.0	4.0
Lancer	2,773	2,696	2,492	204	8.3	9.0	4.0	5.0
Wichita	2,778	2,647	2,540	107	7.8	9.5	4.0	5.5
Mean	2,794	2,833	2,572	261	8.02	8.92	5.83	3.09

[a] Treatment: A, control (3% lard); B, 0.64% hexane (3% lard); C, 0.64% hexane (no lard).

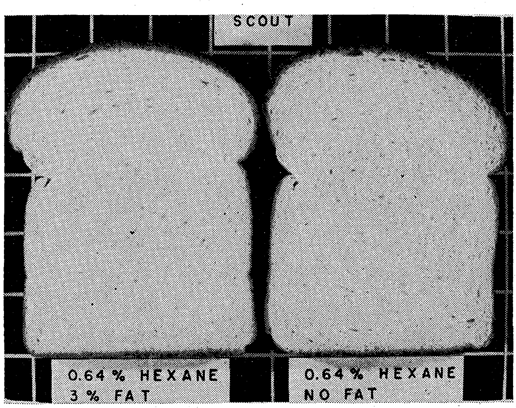

Fig. 4. Loaves made with Scout flour (Kansas) by treatments B and C.

bread made from Scout, grown in Kansas; Fig. 5 demonstrates that treatment C caused serious destruction in variety 391-56-D-23, grown in Texas.

Again, simple correlation coefficients were computed for the relationships between the baking data and the analytical data on the flours (Table IV), with results as shown in the table below. The values in the

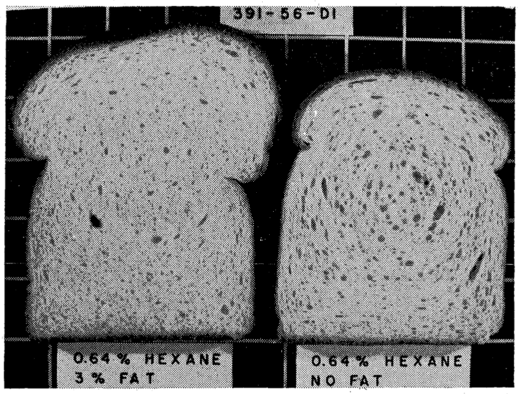

Fig. 5. Loaves made with 391-56-DI flour (Texas) by treatments B and C.

	Loaf Volume Difference (Treatment B Minus Treatment C)	
	All Flours	*Four Flours Excluded*
Protein	0.24	0.79**
Ether-extractables	0.37	0.30
Total lipids by acid hydrolysis	0.60*	0.71*

first column are for the whole set of 15 flours; those in the second column are for 11 of those flours, the four samples from Colorado being excluded. The reason for showing both sets of values is that the Colorado samples appeared to follow a different pattern of behavior compared to the other flours when various data were plotted. A further example of differing properties on the part of the Colorado flours is given below.

In any event, the figures above show the same trends as found for the commercial flours, lending greater significance to the trends. Higher levels of protein, ether-extractables, and total lipids in the flours were associated with greater deterioration in the dough system of hexane without fat.

The trends in phosphorus and fatty acid composition of the experimental flour lipids were similar to those noted for the commercial flours, in that higher levels of phosphorus and fatty acid unsaturation were associated with greater deterioration in doughs made by treatment C; the statistical significance of this relationship, however, was quite low.

The petroleum ether extracts of the 15 flours were examined by thin-layer chromatography, with solvent systems differing in polarity, but no differences were noted that seemed to relate to the baking behavior under discussion. One observation of interest was made, however, as illustrated in Fig. 6. This figure shows that the ether extracts

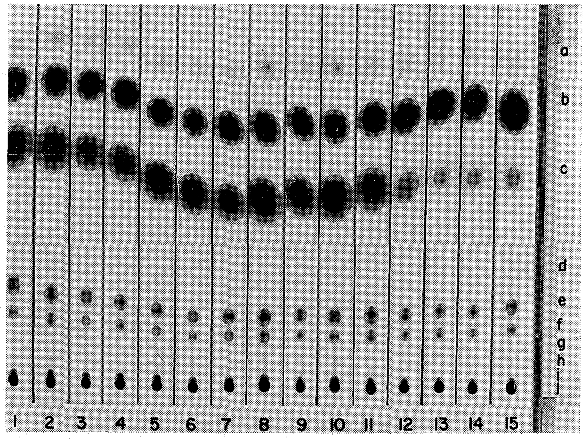

Fig. 6. Thin-layer chromatogram of petroleum ether-extractables obtained from 15 experimental flours. Solvent system was n-hexane:diethyl ether:acetic acid (70:30:2, v:v:v). Principal spots are identified as follows: a, nonpolar components; b, triglycerides; c, fatty acids; e and f, diglycerides; i, monoglycerides; j, phospholipids.

of flours 12 through 15, the Colorado samples, had a smaller ratio of fatty acids to triglycerides compared to the other flour extracts. The reason for the dissimilarity in lipid composition between the Colorado flours on the one hand and the flours from the other states, on the other hand, is not known.

Discussion

The addition of hexane but no lard to dough invariably caused loaf deterioration, but the extent of damage was markedly variable, depending on the flour employed. This is an intriguing phenomenon, inasmuch as flours exhibiting the greatest deterioration still produced superior bread when both hexane and lard were used in breadmaking. In view of the evidence obtained in this and previous studies, it appears that the improving action of hexane involves some interaction between solvent, added fat, and components within flour itself. Recently, publications have appeared demonstrating interactions between nonpolar substances and proteins. Wetlaufer and Lovrien (9) showed that butane and other hydrocarbons interact reversibly with aqueous solutions of bovine serum albumin and beta-lactoglobulin, and Wishnia and Pinder (10) have demonstrated binding of butane and pentane to bovine serum albumin. These investigators as well as a number of others, e.g., Kauzmann (11) and Némethy and Scheraga (12), have on the basis of experimental and theoretical grounds placed emphasis on the role of attractions between nonpolar groups in proteins as a factor in protein structure and stability. In the literature of cereal chemistry, a few speculations have appeared on the possible contribution of nonpolar attractions to protein structure, for example those of Meredith (13) and Bushuk.[2] Because of the foregoing, the question arises as to whether the dough-improving effects of hexane occur through a modification of protein structure by some alteration in the interactions between protein substructures. Further investigation would seem to be warranted.

Acknowledgments

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