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DOUGH-IMPROVING EFFECT OF SOME ALIPHATIC HYDROCARBONS

II. Studies of Dough Lipids[1]

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ABSTRACT

Studies of possible relationships between the lipid system of dough and the improving effect of small amounts of hexane or heptane were conducted. Added fat was essential for the improving effect of the solvents to be manifested in dough; the addition of solvent in the absence of added fat led to deterioration of bread quality. The use of lipid-extracted flour for dough-making in conjunction with added solvent, but no added fat, led to complete inability of the dough to proof to height. Inclusion of hexane in simple unyeasted doughs resulted in increased "binding" of lipid, phosphorus, and protein. Linoleic acid appeared to be preferentially bound when hexane was added to dough.

Reports from this laboratory have shown that (1,2) small amounts of heptane, hexane, and other aliphatic hydrocarbons when added to dough bring about pronounced improvement in bread crumb structure, texture, and color. While the mechanism involved in this effect is unclear, an obvious property of these solvents is that they are non-polar and would probably migrate to the lipid system in dough. Therefore, an investigation of these lipids seemed desirable. Some of the results obtained in such a study form the basis of this paper.

Materials and Methods

The fats and oils employed in these studies were commercially obtained samples. Similarly, the flours utilized were samples of commercially milled bakers' patent bread flours.

For baking studies with solvent-extracted flour, lipids were removed as follows. Batches of 4,000 g. of flour and 3,000 ml. hexane were stirred

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slowly in a mixer employing a bowl from which air was excluded by means of a plastic cover. A stream of nitrogen was fed into the bowl to minimize oxidation of the lipids during extraction. After about 2 hours' extraction, the solvent with dissolved lipids was removed by decantation and suction filtration of the flour. The flour was extracted again with a charge of 2,000 ml. hexane. The hexane was then evaporated from the lipids by vacuum distillation under nitrogen. The amount of lipids extracted was 0.94%, dry basis. The hexane residue was eliminated from the extracted flour by air-drying the flour, thinly spread on sheet-pans.

The studies on lipid binding and on the chemical characteristics of extracted flour or dough lipids were done on material extracted by the Soxhlet procedure. The extractants were anhydrous ethyl ether, followed by ethanol. The extractions were carried out overnight (about 18 hr.). Ether extracts were concentrated and filtered before final evaporation and weighing. The ethanol extracts were evaporated almost to dryness, then taken up in ether before filtration, final evaporation, and weighing. Flour and dough lipids were also estimated by acid hydrolysis (3).

Simple doughs for lipid-extraction studies were prepared by mixing 500 g. flour (solids basis), 446 g. total water, and lard and hexane as noted, for 1 min. in slow speed and 5 min. in second speed of a Hobart A120 mixer, using bowl and hook. The doughs were spread in thin strips on sheets of polyethylene and dried overnight on laboratory benches. A Waring Blendor was then used to pulverize the dried dough strips.

Bread-baking was performed by a conventional sponge dough procedure as previously described (4).

Lipid phosphorus was determined by the method of Bernhart and Wreath (5).

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 16-ft. copper column packed with diethylene glycol succinate polyester as the liquid phase supported on chromosorb.

Soluble protein was determined in doughs made with 700 g. of a bakers' patent flour employing 59% absorption (all on 14% flour moisture basis). The doughs were mixed in a Hobart A200 mixer equipped with a modified McDuffee bowl, for 2 min. in first speed, then the remainder of the time in second speed. Ten-gram portions of dough were sampled at various times (measured from start of mixing in second speed) and immediately placed in jars with 100 ml. of 0.01*N* acetic acid. The jars and contents were shaken for 1 hr. in a mechanical shaker and centrifuged for 10 min. at 2,000 r.p.m. The supernatants were filtered through glass wool; then 20-ml. aliquots were taken for Kjeldahl nitrogen determinations.

Results and Discussion

Studies with Extracted and Reconstituted Flour. The relation of flour lipids, as well as added fat, to the improving action of solvents was explored in an experiment utilizing extracted and reconstituted flour. Hexane was employed for extracting flour lipids; heptane served as the dough-improving solvent. The results of this work are presented in Table I.

TABLE I
RELATION OF EXTRACTED FLOUR LIPIDS, ADDED FAT (LARD), AND HEPTANE TO BAKING QUALITY

FLOUR TREATMENT	CRUMB SCORE	LOAF VOLUME	PROOF TIME
		cc.	*min.*
		0 lard, 0 heptane	
1. Control	7.5	2,799	60
2. Extracted	6.5	2,475	56
3. Reconstituted	7.8	2,644	56
		0 lard, 0.43% heptane[a]	
4. Control	7.5	2,578	66
5. Extracted	. .	very small	>90
6. Reconstituted	7.7	2,595	70
		3% lard, 0 heptane	
7. Control	7.8	2,844	59
8. Extracted	7.6	2,672	59
9. Reconstituted	7.8	2,713	58
		3% lard, 0.43% heptane	
10. Control	8.8	2,778	59
11. Extracted	8.8	2,676	57
12. Reconstituted	8.8	2,696	55

[a] Heptane and lard levels based on flour.

The first three doughs were made without added fat (lard) or heptane. Removal of hexane-extractable lipids from the flour led to bread (dough 2) with substantially lowered crumb score and loaf volume, compared to the control. Putting the lipids back in the flour (dough 3) returned the crumb score to normal, but restored only a part of the lost loaf volume. Previous investigators have reported contradictory results on the baking properties of extracted flours; several reviews have treated this subject (8,9,10).

The addition of 0.43% heptane (flour basis) to dough made with the control flour and no fat (dough 4) produced a drop in loaf volume compared to the bread made from dough 1. The crumb score was judged to be about the same as that of dough 1, but the grain was different, being more spherical in nature. Employing the extracted flour in dough (dough 5) in combination with no fat and with 0.43% heptane resulted in complete failure of the loaves to proof to height even after 90 min. of proof. Returning the flour lipids to the dough system (dough 6) enabled the loaves to reach standard proofing height after 70 min.

These data have pointed up the deteriorating effects on dough caused by heptane when no added fat is present; further removal of the hexane-extractable lipids from the system led to complete failure of the dough.

The effects of 3% fat and no heptane on doughs made with the three flours are shown in doughs 7 to 9. In general, the presence of fat increased, somewhat, over-all bread quality as compared to bread made with no fat. Removal of the flour lipids led to loss of loaf volume and a slight loss of crumb quality; only part of the volume was regained by restoration of the flour lipids.

Dough 10 shows the typical improving effect on crumb score caused by 0.43% heptane in the presence of added fat. The removal of flour lipids (dough 11) lowered the loaf volume, but had no effect on the crumb score; again, putting back the flour lipids (dough 12) led to some restoration of the lost loaf volume.

Varying Amount of Fat with Amount of Heptane. A baking series was undertaken to determine the optimum level of fat (lard) to be used with heptane; the results are illustrated in Fig. 1.

The use of 0.43% and especially 0.65% heptane in the absence of fat was injurious to the dough system. At the highest heptane level, the dough failed to proof to height after 106 min., and hence the bread made from this system had very low volume and dense grain.

Increased lard in the absence of heptane generally led to increased loaf volume; the optimum loaf volume was obtained with 3% fat. An over-all trend toward decreased volume with increased heptane was obtained; as pointed out earlier (1), loaf-volume losses in doughs made with 0.43% heptane are eliminated when loaves are proofed to time rather than height as was done in the present work.

Average data for the four doughs at each fat level indicated no appreciable loaf-volume difference as a function of fat. There was a

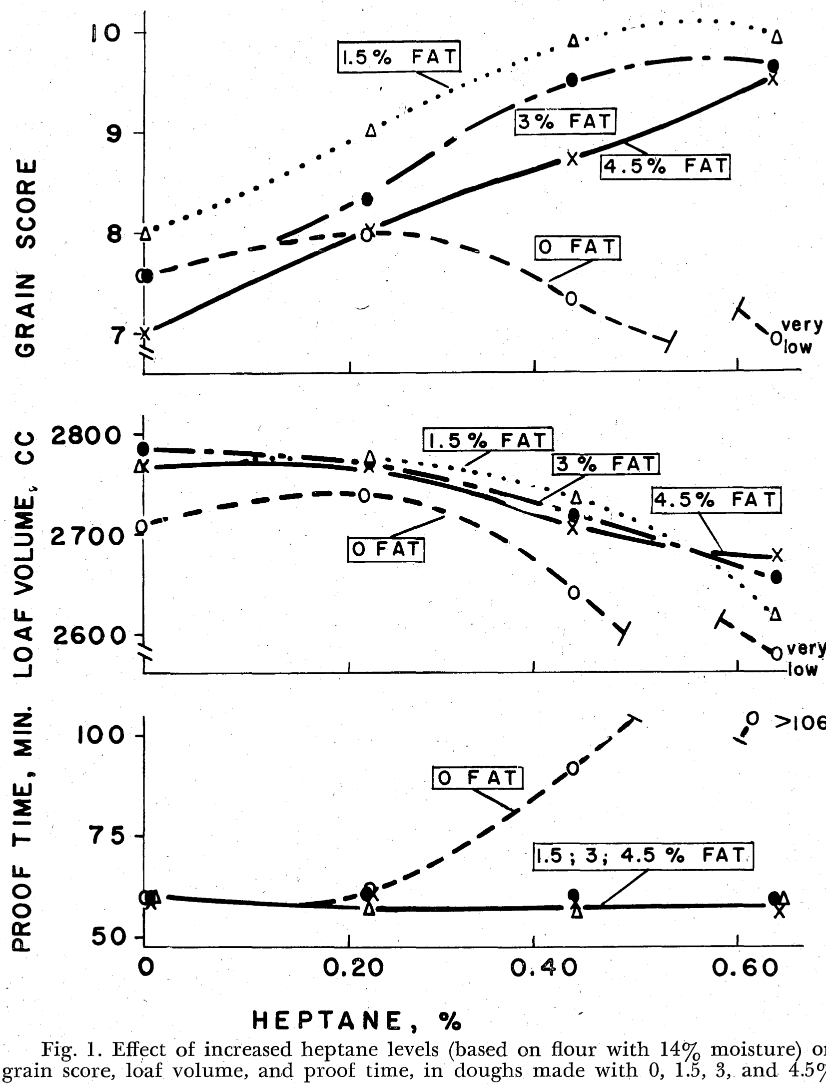

Fig. 1. Effect of increased heptane levels (based on flour with 14% moisture) on grain score, loaf volume, and proof time, in doughs made with 0, 1.5, 3, and 4.5% fat (lard).

trend, however, with respect to crumb score. The average crumb score rose from 7.6 for 0 fat (average of three doughs in this case; dough with 0.65% heptane was not included) to 9.2 for 1.5% fat. Each fat increment thereafter brought about successive small drops in crumb score. It thus appeared that 1.5% fat under these test conditions was the optimum amount to bring about the greatest improving action with heptane.

Relation of Fat System to Hexane Effect. A number of different fats and oils were experimentally bake-tested with and without dough-improving solvent (hexane in this instance), to determine whether a relationship existed between the type of fatty material employed in baking and the improving action of the solvent. The amounts of fatty material and hexane employed were 3 and 0.64% respectively. Table II summarizes the results of this study.

TABLE II
RELATION BETWEEN TYPE OF FAT EMPLOYED IN BREADMAKING AND IMPROVING EFFECT OF HEXANE

Fat System	Crumb Score (10)			Loaf Volume		
	Hexane			Hexane		
	0%	0.64%	Δ	0%	0.64%	Δ [a]
				cc.	cc.	cc.
Soybean	7.6	9.3	1.7	2,680	2,636	−44
Cottonseed	7.8	9.5	1.7	2,696	2,619	−77
Coconut	7.8	9.5	1.7	2,590	2,606	16
Corn	7.6	9.0	1.4	2,734	2,709	−25
Safflower	7.1	8.3	1.2	2,758	2,676	−82
Lard oil	7.3	8.3	1.0	2,726	2,721	− 5
Hydrogenated vegetable	7.3	8.3	1.0	2,811	2,799	−12
Peanut	7.8	8.7	0.9	2,713	2,696	−17
Lard	7.6	8.5	0.9	2,791	2,742	−49
Hydrogenated animal	8.0	8.3	0.3	2,745	2,762	17
Mean	7.57	8.77	1.18	2,724	2,697	−27

[a] Δ = Difference due to hexane.

These data show that soybean, cottonseed, coconut, and corn oils made bread with the highest crumb scores in the series with hexane and also showed the greatest degree of improvement due to hexane. Relatively less improvement with hexane was shown in loaves made with safflower or lard oils, or hydrogenated vegetable and animal shortenings.

Various typical analytical values for the fats and oils were obtained from the literature and compared to the crumb scores. A rough trend was observed between the linoleic acid content of the fatty materials and the crumb score of the breads made with hexane; with increased linoleic acid, better crumb scores generally resulted. There were two notable exceptions to this trend: safflower oil has a high linoleic acid content, but produced bread of below-average grain; coconut oil has a low linoleic acid content, but produced bread of above-average grain.

Effects on loaf volume were less clear, but seemed to go in the opposite direction to crumb score. Hydrogenated vegetable and animal shortenings, and lard, the three materials probably having the highest level of saturated fatty acids, produced the bread with the highest average loaf volumes when made with hexane.

The mean values indicate an average loaf-volume loss of 27 cc. due to hexane; again, such losses are generally eliminated when loaves are proofed to time rather than height.

Effect of Hexane on Lipid Binding. Lipids extracted from a series of simple doughs, made with various amounts of hexane, were studied to determine whether differences existed in lipid "binding." Unbound or "free" lipids were arbitrarily defined as those lipids extractable from flour or dough by anhydrous ethyl ether. Table III summarizes the data obtained in these studies.

TABLE III
DOUGH LIPIDS BY ETHER EXTRACTION, ETHANOL EXTRACTION, AND ACID HYDROLYSIS

SYSTEM EXTRACTED	ETHER-EXTRACTABLES [a]	ETHANOL-EXTRACTABLES [b]	TOTAL	TOTAL (ACID HYDROLYSIS)
	%	%	%	%
Flour	1.14	0.07	1.21	2.84
Dough				
Flour	0.24	.52	0.76	2.67
Flour + 0.46% hexane	0.11	.62	0.73	2.26
Flour + 1.37% hexane	0.12	.72	0.84	2.01
Flour + 2.3% lard	2.77	.28	3.05	4.83
Flour + 2.3% lard + 0.46% hexane	2.30	.62	2.92	4.71
Flour + 2.3% lard + 1.37% hexane	1.86	0.91	2.77	4.61

[a] All figures in table reported on flour solids basis.
[b] Ethanol extraction conducted on materials previously extracted with ether.

The amount of ether-extractables obtained from the flour employed to make the doughs was 1.14%, a quantity virtually identical to that reported by other workers (8,11). Making a dough from the flour led to the binding of about 80% of the ether-extractable lipids; this effect has been reported by Olcott and Mecham (12) and by Mecham and Weinstein (13). The addition of hexane to the dough resulted in further lipid binding, amounting to a total of about 90%.

The quantity of ether-extractable lipid (flour lipid and/or lard) bound appeared to be decreased slightly as lard was added to the dough system; Mecham and Weinstein (13) found lard to have negligible effects on total lipid binding. The present data further show that addition of hexane to the lard-containing system increased binding appreciably; these figures indicate a nearly linear relationship between hexane level and binding.

More lipids, i.e., "bound" lipids, were extracted from the ether-extracted materials by ethanol. In both dough systems, more bound lipids were found in doughs made with increased hexane. However, the ethanol did not appear to extract all the bound lipids, since the total extractable lipids (sum of ether- and ethanol-extractables) from the doughs made with lard were not constant, but decreased with increased hexane.

Total material extracted by acid hydrolysis also was not constant; in both the dough systems with or without added lard, less material was extracted by acid as hexane was increased in the dough.

Fatty Acid Composition of Extracted Lipids. Samples of the ether-extractables described in the previous section were subjected to study by gas-liquid chromatography, with results as summarized in Fig. 2.

The ether-extractable lipids from the control flour-water dough (no added fat or hexane) show a decrease in unsaturation compared to the lipids extracted from the flour itself. A further decrease in fatty acid unsaturation resulted with increased hexane; this occurred with both

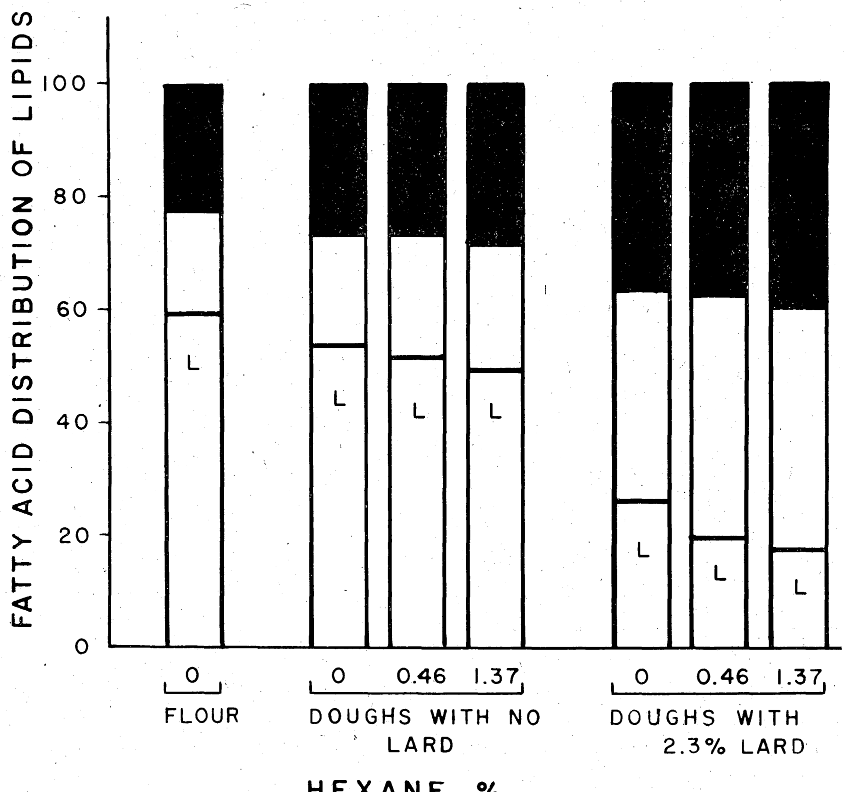

Fig. 2. Effect of hexane (dry flour basis) on the fatty acid distribution of ether-extractable lipids from doughs made with and without lard (lard based on dry flour). Dark areas represent total saturated fatty acids, light areas represent total unsaturated fatty acids, and L indicates linoleic acid.

dough systems. Linoleic acid appeared to be preferentially bound to the doughs as a consequence of hexane addition. Oleic and stearic acids (data not shown) showed reverse trends.

Preferential uptake of unsaturated fatty acid methyl esters by gluten has been reported in the literature.[3] It has also been observed (14,15) that extracted dough lipids contain less linoleic acid than the lipids extracted from the flour used to make the dough.

Phosphorus Binding. The lipids extracted from the various systems were examined for phosphorus, with the assumption made that the phosphorus found was an index of the phospholipids present. Table IV summarizes the phosphorus data obtained, expressed on both an extracted-lipids basis and on a flour-solids basis.

TABLE IV
PHOSPHORUS IN EXTRACTED FLOUR AND DOUGH LIPIDS

SYSTEM EXTRACTED	PHOSPHORUS IN ETHER-EXTRACTABLES BASED ON:		PHOSPHORUS IN ETHANOL-EXTRACTABLES [a] BASED ON:	
	Extracted Lipids	Flour Solids	Extracted Lipids	Flour Solids
	%	$\% \times 10^{-3}$	%	$\% \times 10^{-3}$
Flour	0.203	2.31	0.703	0.49
Dough				
Flour	.054	0.13	.280	1.46
Flour + 0.46% hexane [b]	.029	0.032	.269	1.67
Flour + 1.37% hexane	.038	0.046	.223	1.61
Flour + 2.3% lard [b]	.033	0.94	.346	0.97
Flour + 2.3% lard + 0.46% hexane	.014	0.32	.194	1.20
Flour + 2.3% lard + 1.37% hexane	0.008	0.15	0.174	1.58

[a] Ethanol extractions conducted on materials previously extracted with ether.
[b] Flour solids basis.

These results show that binding occurred, going from flour to dough. A calculation indicates that only about 6% of that phosphorus found in the ether-extractables of flour was recovered from the control dough ether extract (phosphorus based on flour solids); this is in agreement with the results of Mecham and Weinstein (13).

A further binding of phosphorus was caused by addition of hexane to the dough, both when lard was absent or present in the system. In the presence of lard, increased hexane led to greater binding; in the absence of lard, increased hexane had relatively little effect.

On the basis of flour solids in the dough, more phosphorus was extracted from the doughs made with lard than from the doughs containing no lard. As suggested by Mecham and Weinstein (13), it appears that lard may replace some of the flour phospholipid that would otherwise be bound in dough.

[3] Schulerud, A. (cited in ref. 8, p. 61).

Higher phosphorus levels were found in the ethanol extracts (i.e., the more "firmly bound" lipids) compared to the ether extracts. On a flour-solids basis, there was an over-all increase in phosphorus when hexane was added to the dough; again, this demonstrates that hexane caused binding of phosphorus in dough.

The relative binding of lipids and phosporus by hexane was calculated, with results as shown in Fig. 3.

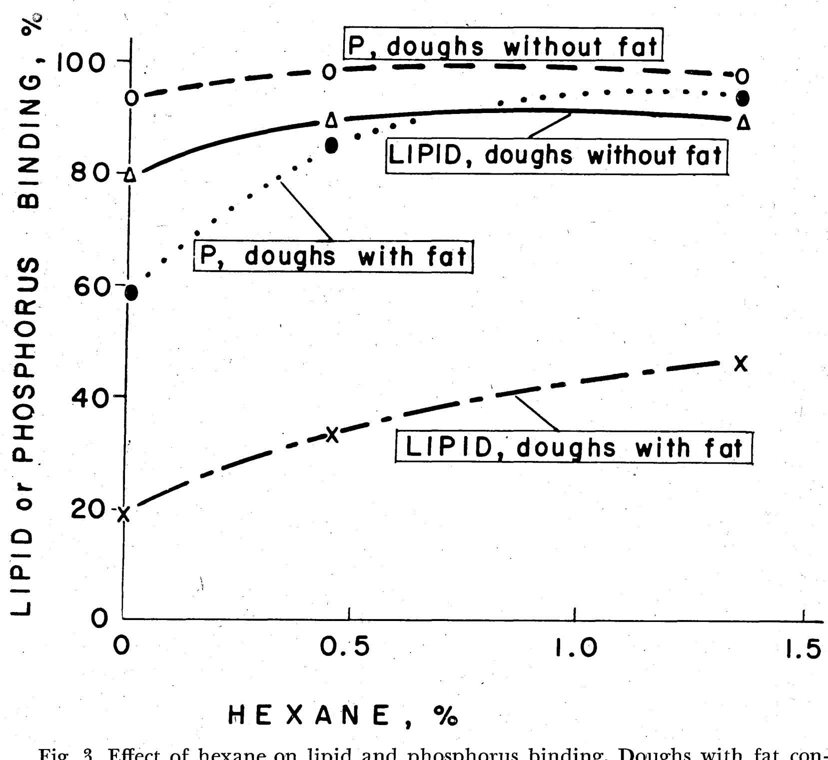

Fig. 3. Effect of hexane on lipid and phosphorus binding. Doughs with fat contained 2.3% lard. All figures on flour solids basis.

Extent of binding was based on the amounts of lipid or phosphorus extractable from the flour by ethyl ether; in the case of the dough systems made with lard, lipid binding was based on the sum of flour ether-extractables and lard added to the dough.

More phosphorus than lipid was bound throughout both dough systems. The spread between lipid binding and phosphorus binding was greater in the system containing lard than in the system without lard. The effect of hexane on binding was especially pronounced in the former system.

Protein Binding. In view of the evidence that lipids and phosphorus were bound by aliphatic hydrocarbon present in dough, studies were conducted on possible binding of protein. Changes in solubilization of nitrogen during mixing of flour-water doughs are shown in Fig. 4.

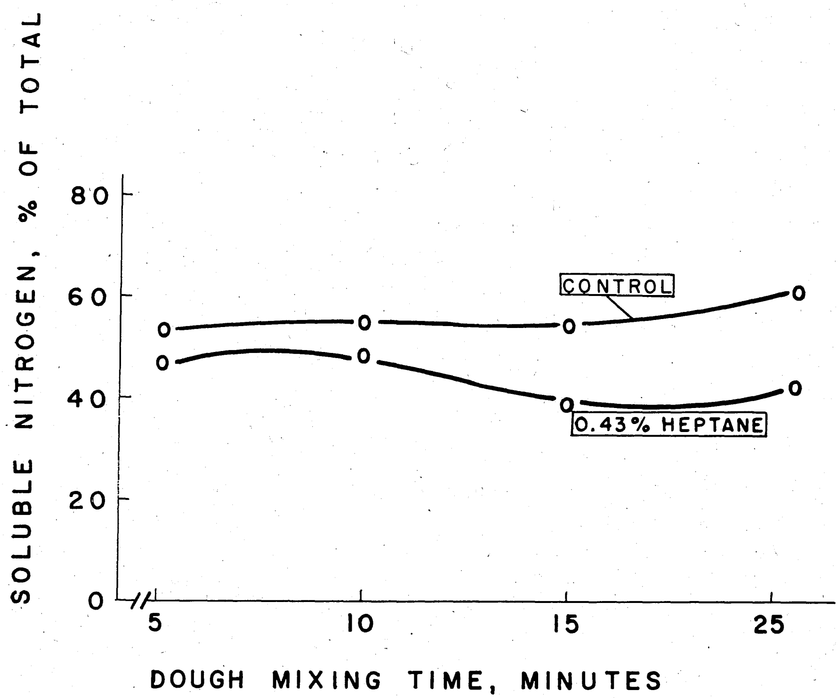

Fig. 4. Change in soluble nitrogen (dry basis) during mixing of flour-water doughs. Heptane level based on flour of 14% moisture.

Soluble nitrogen in the control dough increased with mixing, which confirms the findings of Mecham, Sokol, and Pence (16). The extent and increase (about 8%) of solubilization was not as great as shown by these investigators, most likely because of the more exhaustive extraction techniques they employed.

Addition of 0.43% heptane to the dough altered the pattern of solubilization. Less nitrogen was solubilized soon after the beginning of mixing compared to control, and an over-all decrease in solubilization with increased mixing was obtained.

In this context, it may be mentioned that interactions between aqueous solutions of certain proteins and nonpolar hydrocarbons have been demonstrated (17).

Thus, the data given in the last several sections have shown that relatively small amounts of aliphatic hydrocarbons bind lipid, phosphorus, and protein in dough. It follows that these solvents have induced some modification of the lipoprotein system, a system generally held to be of importance with respect to flour and dough quality (e.g., 10,18,19). Whether or not the observed modifications are actually related to the bread-improving mechanism of hexane or heptane has yet to be conclusively demonstrated.

Acknowledgments

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