DIFFERENCES IN TOTAL LIPID AND FATTY ACID COMPOSITION OF DOUGHNUTS AS INFLUENCED BY LECITHIN, LEAVENING AGENT, AND USE OF FRYING FAT[1]

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ABSTRACT

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Total lipid and fatty acids in doughnuts were investigated with a 2^3 factorial design using lecithin levels, sulfate-phosphate and tartrate baking powders, and fresh and slightly used frying fat as the variables. The total lipid content of doughnuts increased markedly with increases in lecithin levels; however, the lecithin levels did not affect the fatty acid composition of the extracted lipid. Total lipid content of doughnuts also increased markedly with the use of tartrate baking powder in comparison with sulfate-phosphate baking powder. There was a small but statistically significant tendency for the less polar fatty acids to be present in the sulfate-phosphate leavened doughnuts compared to the tartrate leavened doughnuts. No changes were noted as frying fat use progressed with this relatively short use-time.

Increasing interest in biological systems has been directed to the importance and complexity of the interactions. Phospholipid and lipoprotein complexes have been identified as essential to the integrity of many structures, including gluten (1). Electron micrographs have supported the concept of the probable interaction of gluten with lipid (2), as has the increased molecular weight of gluten mixed with flour lipids (3). Analyses of these flour lipids have revealed 5.8% lecithin (4), indicating perhaps a particular role for phospholipids in the interaction with gluten.

The pH of the gluten may also significantly affect interactions, as marked changes in protein crystalline structure with pH alterations have been demonstrated (5). An isoelectric point for wheat gluten has been suggested as that of pH 7.5 (6,7,8). The pH of a dough is affected by the solubility of the acid salts of the leavening agent (9). Dough differences of pH 6.39 for tartrate baking powder, compared with pH 6.95 for sulfate-phosphate doughs, have been reported (10).

Deterioration of frying fat (11) and substitution of yolk solids (12) for whole eggs are two variables which have been reported to increase total fat absorption in doughnuts. Losses within the frying fat of unsaturated fatty acids, particularly linoleic, occurred during use (13,14). Literature findings show that absorbed frying fat extracted from potatoes after 5 hr of previous use of the cooking fat reflected composition of the cooking fat; after 10 hr the extracted fat contained less linoleic acid than the frying fat (14). Later batches of fried potatoes contained less linoleic acid than those first fried (15). Fatty acids in fritter batter showed a major decrease in linoleic, some decrease in linolenic, and slight increases in palmitic, stearic, and oleic acids compared to the frying fat (16). Removal of egg from the batter coating chicken parts resulted in higher levels of linoleic and

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linolenic acids in the chicken tissue; as use-time of frying fat increased, the oleic and palmitic acids showed greater penetration and linoleic less into the chicken tissue than would be represented by simple fat soakage (17).

Analyses of raw doughs and their baked products have shown no changes in fatty acid composition due to the cooking process (18,19). Since preliminary work in this doughnut project showed no differences in the fatty acid composition of the lipids extracted from the uncooked doughs with change in pH and with the small amount of incorporated commercial egg lecithin, it was feasible to compare the various fatty acid patterns of fried doughnuts as a means of studying tendencies for preferential fat adsorption and absorption. No attempt was made to differentiate in the doughnuts between the adhering fat and absorbed fat since both would be consumed.

EXPERIMENTAL PROCEDURE

The experiments were planned in a 2^3 factorial design with lecithin level, length of time of use of frying fat, and type of baking powder as the chosen variables. The lower lecithin level was that amount present naturally in the two whole eggs in the recipe. The upper lecithin level approximately doubled the amount (20); 3.2 g commercial egg lecithin was added to the mix, simulating the lecithin present in an all egg yolk recipe. Tartrate and sulfate-phosphate baking powders were used in recommended amounts (21). Fresh fat was arbitrarily designated as that which had been used 1–5 frying periods of about 45 min each, and used fat was that used 6–10 frying periods.

Statistical Design

Preliminary work estimated the components of variance for the lipid extraction data to set the experimental design. Analyses of variance gave the best estimates of ϕ^2 batches as 0.947, ϕ^2 doughnuts as 1.711, ϕ^2 samples as 0.466, and ϕ^2 aliquots as 0.025. With three replicates, six doughnuts, one sample, and one aliquot, the 95% confidence interval for the mean of the percentage total lipid would be ±1.29%. Because variation in results due to day-to-day replication was obtained and not all treatment combinations could be used in a single day, the design was arranged for partial confounding of data (22). This was accomplished in the first replication with the effect of the lecithin level confounded, in the second with the effect of the leavening agent confounded, and in the third with the effect of the leavening agent-lecithin-level interaction confounded.

Doughnut Preparation

Ingredients used in the doughnut preparation were:

	g
Egg lecithin (when used)[2]	3.2
Eggs, fresh shell	96.0
Sugar, granulated	200.0
Shortening, hydrogenated vegetable	23.5
Milk, reconstituted nonfat dry	183.0
Flour, all purpose	460.0
Baking power, sulfate-phosphate	14.4
or tartrate	24.8
Salt	2.4
Cinnamon, nutmeg	1/4 tsp. each

[2]Nutritional Biochemicals Corporation. When lecithin was used, it was mixed with eggs 4 hr before use.

Eggs were beaten with an electric mixer for 1 min at high speed. Sugar was added while beating continued for 1 min. Low speed was used for beating in shortening (20 sec), milk (20 sec), and dry ingredients (45 sec). Doughs were rolled once to 3/8 in. thickness. Sixteen doughnuts were cut and randomly selected for frying, four at a time. Two Sunbeam deep-fat fryers (Model CF-5) were used, one containing fresh, the other used corn oil. The two fryers were used simultaneously to minimize differences in standing time of the raw doughs. Doughnuts were fried at 375° F for 90 sec, turned and fried an additional 60 sec, and drained on absorbent paper toweling. Temperatures were checked with calibrated thermometers as well as with the equipment gauges.

Lipid Extraction

The technique used was a modification of the Folch method (23). Six doughnuts were selected at random from the eight fried in fresh and from the eight fried in used fat, and each was pulverized in a blender. The sample was homogenized with 1:1 chloroform:methanol; after centrifugation, the supernatant was added to 0.01*M* magnesium chloride. The procedure was repeated on the sample with 2:1 chloroform:methanol mixture. The following day, the lower phase (chloroform and fat) was filtered through anhydrous powdered sodium sulfate, and chloroform was evaporated off.

Methylation

A modification of the procedure developed by Stoffel *et al.* (24) was used. The esterification agent was a 2% solution of sulfuric acid and freshly distilled methanol. The method gave 88–97% recovery; unheated corn oil components were in good agreement with those reported in the literature (25,26).

TABLE I
Total Lipid Content and Fatty Acid Composition of Extracted Lipid Values

Fatty Acids or Total Lipid %	Total Lipid %	Palmitic %	Stearic %	Oleic %	Linoleic %
Frying fats					
Corn oil, unheated	...	11.49	2.21	27.77	58.49
Fresh fat, 1–5 frying periods (45 min each)	...	11.79	1.97	27.28	58.95
Used fat, 6–10 frying periods (45 min each)	...	11.51	1.99	27.87	58.63
Doughnuts: Sulfate-phosphate baking powder					
Eggs alone, fresh fat	14.45	12.91	3.32	29.85	53.93
Eggs alone, used fat	14.16	12.89	3.78	29.71	53.96
Added lecithin, fresh fat	19.03	12.18	3.24	28.91	55.68
Added lecithin, used fat	18.95	12.88	3.24	28.68	55.21
Doughnuts: Tartrate baking powder					
Eggs alone, fresh fat	17.25	12.38	2.83	28.82	55.97
Eggs alone, used fat	17.36	12.58	3.03	29.13	55.22
Added lecithin, fresh fat	23.39	12.26	3.04	28.57	56.12
Added lecithin, used fat	23.01	12.28	3.02	29.41	55.40

Chromatographic Analyses

Fatty acid analyses were determined with an Aerograph Hy-Fi Model 600-C gas chromatograph with hydrogen flame ionization detector. Injection port temperature was 220° C; column temperature was 187° C. Nitrogen carrier gas flow rate was 13 cc/min; detector gas rates were 200 cc/min air and 15–20 cc/min hydrogen. Column was coil type, stainless steel, 7 ft × 1/8 in., packed with 20% diethylene glycol succinate on a solid support of 60/80 mesh HMDS-treated Chromosorb W. Peaks were qualitatively identified with standard mixtures from Hormel Foundation; for statistical analyses and discussion, only the same major well-defined peaks were used. Quantitative determinations were made by dividing the area under each peak into the sum of all peak areas and multiplying by 100. These were calculated to be 100% of the total fatty acid content.

pH Measurements

pH measurements were assessed with a slurry of equal weights of dough or doughnut and distilled water.

Index to Size and Shape

All eight doughnuts in each variation were stacked and measured at the highest point, then lined in a row, sides touching, and measured at the widest point (12).

RESULTS

Gas-liquid chromatographic data on the fatty acid composition of the frying fat showed essentially no differences between the fresh fat (1–5 frying periods, 45 min each) and the used fat (6–10 frying periods, 45 min each). Doughnuts fried in the fresh and used fat also demonstrated that no significant changes had occurred during this short use-time; the percentage of total lipid in the doughnuts and the fatty acid composition of the extracted lipid did not differ with use-time (Tables I and II).

Doughs and doughnuts containing sulfate-phosphate baking powder had pH values ~ pH 7.0, whereas those containing tartrate baking powder were considerably more acidic, having a range ~ pH 6.0–6.3 (Table III). This agrees with previous literature findings (9,10). In the various treatment combinations, the tartrate-containing doughnuts absorbed significantly more lipid, their total lipid extraction ranging from 17 to 23%, whereas in the sulfate-phosphate doughnuts the total lipid extraction ranged from 14 to 19%. The fatty acid composition of the extracted lipid also differed. A significant difference was noted in the comparison of linoleic acid from the extracted lipids, with the sulfate-phosphate doughnuts containing 53.9–55.7% and the tartrate doughnuts containing 55.2–56.1% linoleic acid. A significant difference was also noted in the comparison of stearic acid from the extracted lipids, with the sulfate-phosphate doughnuts containing 3.2–3.8% and the tartrate doughnuts containing 2.8–3.0% stearic acid (Tables I and II).

In contrast, no significant differences were found in the fatty acid patterns of the extracted lipid in the doughnuts made with added lecithin, even though additional lecithin caused the greatest increment of fat absorption in this study. Doughnuts without added lecithin contained 14–17% lipid, with added lecithin 19–23% (Tables I and II). Doughnuts with added lecithin were quite different in

shape, having slightly lower height but much greater spread (Table IV).

No significant interactions were found. Calculations of the increase in efficiency of the confounded block statistical design over the completely randomized block design (frequently used by experimenters) indicated that in all but two instances, the confounding was a very effective means of reducing the error term. The increase in efficiency for the confounded over the randomized

TABLE II
Analyses of Lipid and Fatty Acids (Mean squares due to treatments are average percentages)

	Treatment[a]							
Fat Analyses	**A**	**B**	**C**	**AB**	**AC**	**BC**	**ABC**	**Error**
Total lipid	75.000**	0.160	41.990**	0.030	0.700	0.004	0.200	0.452
Frying fat fatty acids								
% Palmitic	1.631	0.476	0.701	1.410	0.226	1.075	0.806	0.597
% Stearic	0.309	0.001	0.039	0.098	0.001	0.016	0.012	0.104
% Oleic	0.137	2.117*	0.146	0.002	0.000	0.254	0.203	0.388
% Linoleic	1.226	0.640	0.439	2.342	0.270	0.197	0.086	0.914
Doughnut fatty acids								
% Palmitic	0.114	0.231	0.650	0.064	0.040	0.120	0.379	0.169
% Stearic	0.141	0.173	0.438*	0.187	0.186	0.024	0.014	0.054
% Oleic	1.108	0.224	0.400	0.073	0.497	0.852	0.147	0.364
% Linoleic	2.323	1.382	4.368*	0.082	0.472	0.410	0.104	0.537

[a]Treatment A = lecithin level, treatment B = use of frying fat, treatment C = type of baking powder.

TABLE III
pH of Doughs and Doughnuts

	pH, Average Values	
Formula	**Dough**	**Fully cooked**
Sulfate-phosphate	6.97	7.04
Sulfate-phosphate with added lecithin	6.98	7.02
Tartrate	6.08	6.32
Tartrate with added lecithin	6.08	6.35

TABLE IV
Index to Size and Shape of Cooked Doughnuts

Formula	**Height *vs.* Width (mm, average values)**	
Sulfate-phosphate	235	534
Sulfate-phosphate with added lecithin	227	554
Tartrate	206	548
Tartrate with added lecithin	204	574

block design for total fat absorption was 1488% and for three of the fatty acids was in the 40% range.

DISCUSSION

The chromatographic analysis, coupled with the fat absorption patterns as frying fat use-time increased, demonstrated that, under these conditions, frying time longer than 5 hr would be necessary to change the characteristics of the frying fat. This is in contrast with some reports in the literature which have shown that even with shorter frying periods, the percentage of linoleic acid may be lowered in the frying fat (14,15,16,17) and the total fat absorption in doughnuts may be increased with frying time (11).

In this experiment, the lecithin level was the factor of greatest significance affecting fat absorption, the lower level of lecithin resulting in markedly lower absorption. Electron microscopy has indicated that the fatty acid moieties of the lecithin molecule orient toward the fat phase while the polar groups may provide areas of attraction for protein (27). It is speculated that the phospholipid slip plane areas (1) may have been increased to the extent that they facilitated gluten separation, doughnut tenderness, and therefore more fat penetration. Lecithin decreases the swelling temperature of starch and weakens the gel structure (28,29). Doughs with additional lecithin did give doughnuts with much greater spread (Table IV). It may be hypothesized then that areas were provided for fat absorption in both the starch and the gluten structure.

An additional rationale for the high fat soakage may be that one of the characteristics of natural lecithin is its ability to lower surface tension (30). It was consistently observed after frying that a slight and fairly stable foam of fat bubbles remained on the crust of only the lecithin-containing doughnuts, indicating that the surface tension of those doughnuts may have been lowered, permitting greater fat absorption.

Lack of significant differences in the nature of the fatty acids of the doughnuts with added lecithin demonstrated general attraction for fat rather than for fats containing specific fatty acids. While part of the reason for the increased fat absorption reported in previous work with egg yolk doughnut mixes may have been due to the delayed coagulation temperature of the yolk proteins (12), the distinctive functional role of the phospholipid of egg yolk must also be considered a major factor.

The summation of some of the complex interactions of phospholipid within a dough product may be theoretically supported by 1) interaction of lecithin with both fat and protein (27), 2) gluten phospholipid slip planes (1), 3) lowered swelling temperature of starch, 4) weakened starch gel structure (28,29), and 5) lowered surface tension of the product (30).

The type of baking powder used affected the pH and caused significant differences in fat patterns both quantitatively and qualitatively. Wu and Dimler (8) suggest a pI of 7.5 for gluten proteins; Tschoegl and Alexander (6,7) demonstrated changes in gluten at pH 7.5. Casein and egg albumin have isoelectric points in the range of pH 4.6–4.8 (31,32). The average isoelectric point of the structural proteins in this mix, therefore, might be expected to be somewhat lower than pH 7.5, and it is assumed that the sulfate-phosphate dough formulation was closer to the pI of the structural proteins than was that of the

tartrate-containing dough. A possible rationale is that the sulfate-phosphate dough proteins had minimal protein solubility (33,34), more ordered α-helical conformation (5), and greater resiliency and ability to withstand extension and stress (35,36); hence, it was less easy for the frying fat medium to penetrate the structure. In contrast, the proteins of the tartrate-containing doughs were more randomly organized (5), less attracted to one another and more attracted to the starch (37), with a lessened ability to withstand extension stress (35,36), and were more easily penetrated.

A comparison of fatty acid patterns of the doughnuts evidenced that the sulfate-phosphate doughnut attracted the less polar fatty acids to a greater extent than did the tartrate doughnuts (Table V). Linoleic acid has been shown to be involved in flour components and with frying fat changes to a greater extent than any other of the fatty acids examined (13,14,15,16,17). If the lessened attraction of the sulfate-phosphate doughnut for linoleic acid were the major change, the increase in percentage of the remaining fatty acids would be expected to rise in proportionate amounts. The percentages of palmitic, stearic, and oleic acids did increase; but there was evidence that the more polar the fatty acids, the less likely they were to be pulled into the sulfate-phosphate system.

These results prompt speculative proposals on the particular interactions that may be occurring.

Assuming that linoleic acid alone decreased in the sulfate-phosphate variation, if the remaining fatty acids simply soaked into the doughnuts, they would have increased in proportion to their previous percentage composition. The other three fatty acids represent a total of 44.3% of the fat that was contained in the tartrate-leavened doughnuts. Of this remaining fat, the percentage of palmitic acid is 27.92; of stearic acid 6.72; and of oleic acid 65.36. Multiplying these percentage areas by 0.98% should give an indication of the magnitude of increase in fatty acid percentage that might be expected.

	Calculated value increase	*Determined value increase*
Palmitic acid	0.27	0.34
Stearic acid	0.06	0.42
Oleic acid	0.64	0.31

The conformation of the structural proteins may explain these differences between the calculated and determined value increases. Close to the isoelectric

TABLE V
Comparison of Type of Baking Powder on Fatty Acids in Doughnuts

	% Fatty Acids			
	Palmitic	**Stearic**	**Oleic**	**Linoleic**
Average value for all sulfate-phosphate doughnuts	12.72	3.40	29.29	54.70
Average value for all tartrate doughnuts	12.38	2.98	28.98	55.68
Average difference in values	0.34	0.42	0.31	0.98
F test differences	3.85	8.11*	1.10	8.13*

point of the proteins, the reactive groups would have lessened hydrophilic potential. With decreased attraction of the polar groups on the protein molecule available for the polar portions of the fatty acids, it can be speculated that the less polar fatty acids would more easily penetrate the rigidly organized, less polar structure. This is not in harmony with a study by Greene and Kasarda (38), which indicated that as the pH was moved closer to the pI of α-gliadin, the accessibility of the protein's apolar regions apparently decreased. However, it should be noted that in their study, pH 3.1–pH 5.0 were used.

The results reported in these experiments indicate that not only total fat absorption but individual fatty acid patterns can be changed with alterations in a dough system. This study makes manifest the need for more sophisticated and more extensive investigations of the lipid bonding mechanisms. Furthermore, the potential envisaged lies in the engineering of structured products which would contain specific fatty acids requisite for certain metabolic needs.

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