TABLE II
Characterization of Sulfonates

Sample	Mol wt Osmometry 130C in o-dichloro-benzene	Equivalent wt	Sulfur content, %	
			Calc from eq wt	Observed
A	463 [a]	453 [b]	7.1	7.0
B	546 [b]	500 [d]	6.4	6.5
C	451 [b]	452 [c]	7.1	7.1
D	461 [b]	452 [d]	7.1	7.1
E	479 [b]	463 [d]	6.9	7.2
F	453 [a]	452 [c]	7.1	7.1

[a] Prepared from naturally occurring mineral oil aromatics.
[b] Prepared from synthetic heavy detergent alkylate.
[c] Neutralization of free acid + 14 to compensate for contribution of the CH_3 group to the mol wt.
[d] Saponification equivalent.

change column and titrating the resulting free acid with base. Both methods give reliable and reproducible results. The bomb method was used to determine the sulfur content of the methyl esters.

It has been previously mentioned that the presence of disulfonates would manifest itself as a discrepancy between the observed average mol and equivalent wt, respectively. The presence of sulfones would be indicated by a difference in sulfur content calculated from the equivalent wt and that obtained by direct elemental analysis. In five of the samples examined, the equivalent wt were found to be essentially equal to the mol wt, thus indicating negligible disulfonate content. The equivalent wt observed for Sample B, however, was found to differ significantly from its mol wt, the difference indicating a disulfonate content of ~10%. Likewise, the sulfur content estimated from equivalent wt for five of the samples was found to be in excellent agreement with those obtained from direct analysis, thus indicating a negligible sulfone content. In the sixth sample, sample E, a barely significant discrepancy between the two values, was observed.

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Fatty Acid Components of Fried Foods and Fats used for Frying[1]

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Abstract

Oxidation of fat is accelerated at temp as high as those used for frying. The extent of this oxidation has been studied by frying two kinds of foods (chicken or potatoes) in cottonseed oil and in lard. Fat samples, taken prior to use and following 5 and 10 hr of frying (both for chicken and potatoes) were esterified. Subsequent quantitative assays of methyl esters by gas chromatography showed that the linoleic acid content had decreased from 57–49% after cottonseed oil had been used 10 hr for frying. This decrease was present regardless of whether chicken or potatoes were fried. There was no change in the linoleic acid content of lard after chicken was fried, but a decrease from 11.3–5.8% was noted when potatoes were fried for 10 hr.

The fatty acid content of the fat extracted from the potatoes and the fat used to fry them, was the same when sampled at 5 hr. However, at the end of the 10-hr frying period, fat extracted from the potatoes had a lower linoleic acid content than fat used to fry them. Results were the same for both cottonseed oil and lard. Fatty acid components of the fat extracted from the chicken seemed to be affected as much by the fatty acid composition of the chicken itself as by fat used for frying. Whether the chicken was cooked in fat used 5 or 10 hr made little difference.

Introduction

AUTOXIDATION REACTIONS occurring in fats are accelerated at temp as high as those necessary for deep fat frying of foods (1), but the final degradation products differ with the conditions. Studies under controlled laboratory conditions have shown that chemical changes which occur in a fat during heating are dependent on many factors. Some of these factors are: length of time fat is exposed to heat and the temp (2); presence of accelerators of oxidation such as oxygen or oxidation products (3,4); mixed fatty acid composition and the position of the fatty acid in the triglyceride (5); presence of metallic ions such as iron (1); presence or addition of hematin compounds (6); presence of water vapor (4); amt of fat heated/unit of surface area (7); presence of amino acids and carbohydrates (8,9); and processing conditions used to refine, decolorize and deodorize the fat (10).

The possible toxicity and reduced nutritional value of frying oils is still controversial. Some investigators (2,11–14) believe heated fats lose nutritional value and could be a health hazard, but others (15–18) report no toxic polymers present and only slight changes in nutritional value.

Many problems concerning thermal oxidation of edible oils during use are poorly understood and are the subject of extensive research, yet most of the fatty acid data available are based on raw fats. Chang and co-workers (19) found that lard used for frying doughnuts for 50 min showed very little change in fatty acid composition. They also found the linoleic acid content of corn oil used to fry potato chips for 8 hr was decreased from 55–52.2%. Fleischman and co-workers (20) reported that corn oil used 15 min to fry potatoes showed a decrease from 66.5–54.3% in linoleic acid content. They also found the linoleic acid content of cottonseed oil had decreased 65.46–34.80% after chicken had been fried for 2.5 hr. These decreases seem rather large and some error may have been introduced because the data on the oil before use were not obtained from an original sample but an analysis of the same brand of oil before use.

[1] Journal Paper No. 1130, Mississippi Agricultural Experiment Station.
[2] A portion of the work in this paper is taken from a thesis submitted in partial fulfillment of the requirements for the Master of Science degree at Mississippi State University in January, 1963.

The following experiments were designed to study the changes in fatty acid composition of lard (low linoleic acid content) and cottonseed oil (high linoleic acid content) after repeated frying of two types of foods for ten hr. The two foods chosen were chicken, which has a relatively high fat, protein, and hematin content; and potatoes, which have a high carbohydrate content and low fat, protein, and hematin content. Fat also was extracted from the chicken and potatoes and assayed for fatty acid components.

Experimental Procedure

Preparation of Fat Mixtures. Two separate homogeneous mixtures were prepared from each of two kinds of commercially available fats commonly used for deep-fat frying in the home. They were a lard and a cottonseed oil. Iron frying pans with diam of ca. 10 in. were used. Approx 2.5 lb of each fat mixture was used in each frying pan. One mixture of each fat was used to fry potatoes and the other was used to fry chicken. The experiment was duplicated throughout.

Frying Periods. Each frying period consisted of 1 hr during which the fat was held at a temp of 185 ± 5C, except when the food was being cooked. The fat was heated constantly but the cold food lowered the temp temporarily. The length of time required to raise the temp of the fat to 180C and to cool it to room temp was not recorded. Two frying periods were completed/day, after which the fats were stored overnight in uncovered glass beakers at 9C.

Preparation of Food. Idaho potatoes obtained from a local market were cut into strips (approximately ¼ x ½ x 4–6 in.) and weighed into 0.5-lb lots. Two lots were fried for 12 min apiece during each frying period. Samples of fat and of the fried potatoes were taken from each frying pan after the fifth and the tenth successive frying periods and were stored frozen until analyzed. No replacement were made of fat lost during frying or sampling.

Frying chickens, obtained from the Poultry Dept. of the Mississippi Agr. Expt. Station, were sawed in half while frozen. Halves of the same bird were placed in duplicate frying pans and fried for 30 min of each frying period. Otherwise, frying periods were the same as previously described. Samples of fat and chicken were taken at the same stages as in the potato frying experiment. Fat was extracted from the cooked chicken and potatoes with petroleum ether in a Soxhlet extraction apparatus.

Preparation of Esters. The following procedure which is a composite of known techniques was used to convert the fat to methyl esters: 1) dissolve 5 g sample in 25 ml diethyl ether and add 50 ml methanol and 25 mg KOH pellets; 2) reflux 2.5 hr and transfer the cooled solution to a 250-ml separatory funnel using ca. 25 ml diethyl ether; 3) acidify with concd HCl to a pH of 2–3, add 50 ml H_2O and extract the aqueous phase 3 times using 25 ml ether each time; 4) wash the combined ether extracts with water until the washings are neutral; 5) dry the combined extracts over anhydrous Na_2SO_4 in a Erlenmeyer flask, filter off the solids washing them with ether, and evaporate on a steam bath under nitrogen until the ether is gone, but no longer; 6) reflux the extracted fatty acids for 2.5 hr in 50 ml methanol containing 50 mg *p*-toluenesulfonic acid; 7) transfer the cooled solution to a 250-ml separatory funnel using 50 ml diethyl ether, add 50 ml water, shake thoroughly, and allow the phases to separate; 8) draw off the aqueous phase and extract twice more with ether; 9) repeat steps 4,5,6,7,8, 4 and 5 on the partially esterified fatty acids in that order; and 10) cool the methyl esters under nitrogen and store under nitrogen at −20C until analyzed. . . . This method, probably due to the complete esterification, produced esters that chromatographed well.

Chromatographic Analysis. The methyl esters of the six dominant fatty acids—myristic, palmitic, palmitoleic, stearic, oleic and linoleic were measured by gas chromatography. The instrument used was a Barber-Coleman Model 20 Gas Chromatograph, equipped with a strontium 90 beta ray detector and a conventional packed column.

Instrument conditions were as follows:

Column	12 ft x ¼ in., 20% diethylene glycol succinate on 60–80 mesh Chromosorb W (acid washed)
Temp	Column 197C, injector 240C, cell 325C
Colunm pressure	65 psi (approx 100 ml argon/1 min)
Scavenger pressure	0
Gain	3
Volts	1250
Sample size	.5 µl

Retention times and peak areas were determined for eight standard (pure methyl esters obtained from The Hormel Foundation, Austin, Minn.) mixtures of the six methyl esters in proportion to cover all esterified fat samples. Two chromatograms were obtained for each standard mixture. When the per cent total peak area of each acid was compared with per cent by wt known to be in the mixture or the mole per cent in the mixture, poor results were obtained. The peak areas were measured by two methods: 1) counting integrator blips underneath peaks and substracting blips for same space with no peaks, and 2) drawing tangents to the peaks and simply counting integrator blips for distance between intersection of tangents and base line.

Standard curves were obtained by plotting per cent of fatty acid by wt vs. per cent of total peak area. The best standard curves were found to be the ones using method 2 for peak area determination, also used in subsequent determinations. The standard curves are shown in Figures 1–6. All esterified fat samples were then chromatographed in duplicate. The chromatogram of the methyl ester in esterified lard used 5 hr for frying potatoes was randomly chosen as the typical chromatogram and is presented as Figure 7.

Peaks representing the esters of the six fatty acids previously named were well defined and completely separated (Fig. 7). Retention times were: myristate, 7 min; palmitate, 12.2 min; palmitoleate, 14.4 min; stearate, 20.8 min; oleate, 24.4 min; and linoleate, 30.4 min. Since no other measurable peaks were observed, it was assumed that no more than trace quantities of other fatty acids were present.

Standard curves were used to convert the per cent of total peak areas for each ester to per cent by wt. When this method of calculation is used, the values for fatty acids are interrelated, i.e., a decrease in the value for one or more acids must produce a corresponding increase in values for other acids. Since the saturated acids are relatively stable at the temp used here, any increase in saturated fatty acids must be compensating or must be produced by dilution of the fat from the material being fried. When potatoes are fried, no measurable dilution can occur and any increase in saturated fatty acids must be compensating. Analyses (Table I) showed the total saturated acids of the cottonseed oil to be 24.0% before use

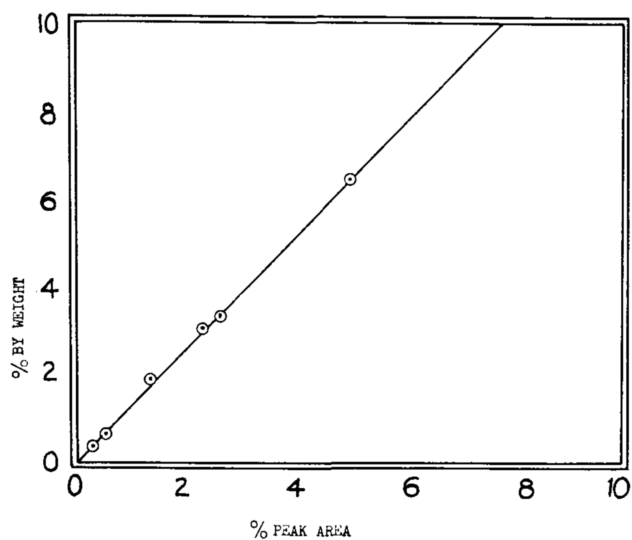

FIG. 1. Standard curve—methyl myristate.

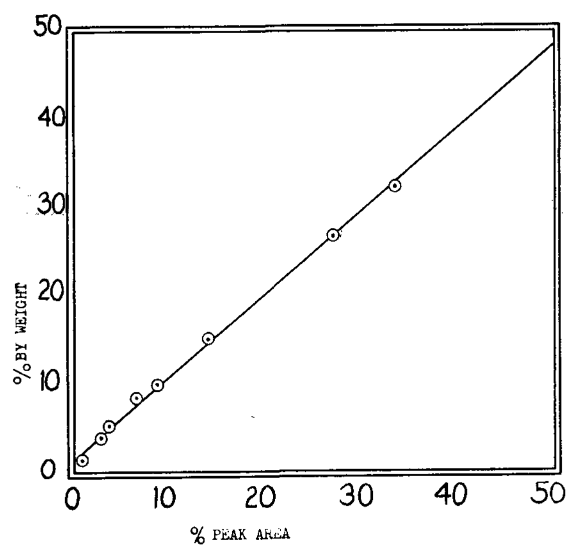

FIG. 4. Standard curve—methyl stearate.

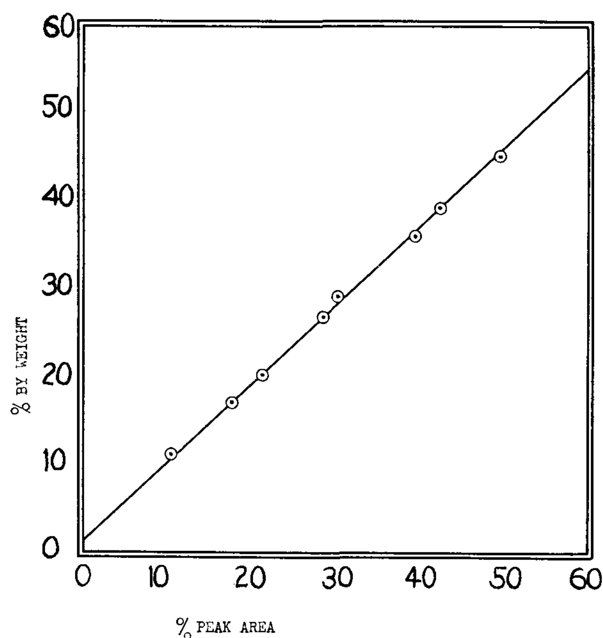

FIG. 2. Standard curve—methyl palmitate.

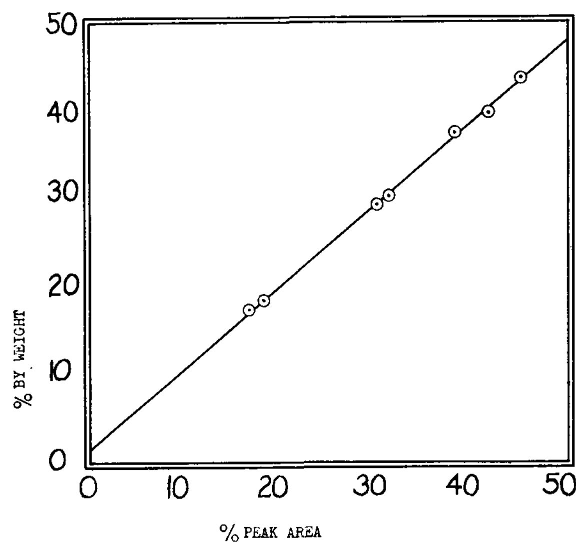

FIG. 5. Standard curve—methyl oleate.

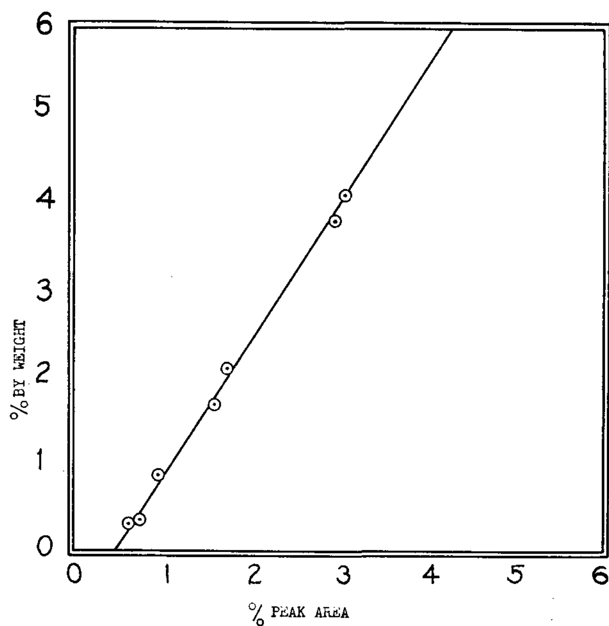

FIG. 3. Standard curve—methyl palmitoleate.

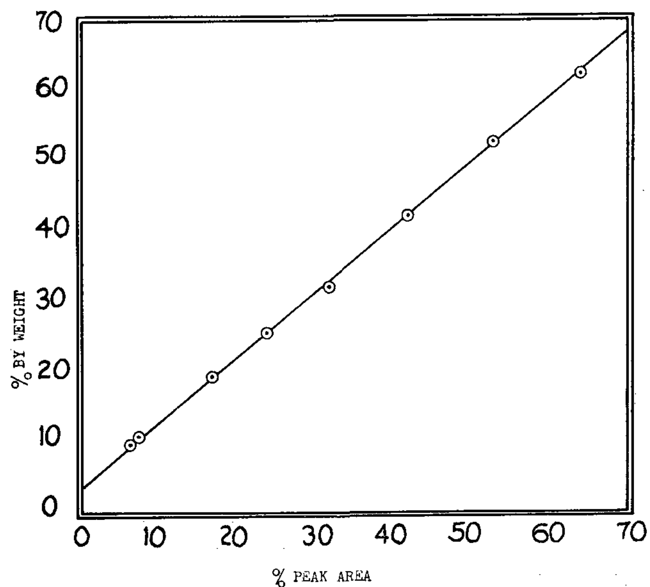

FIG. 6. Standard curve—methyl linoleate.

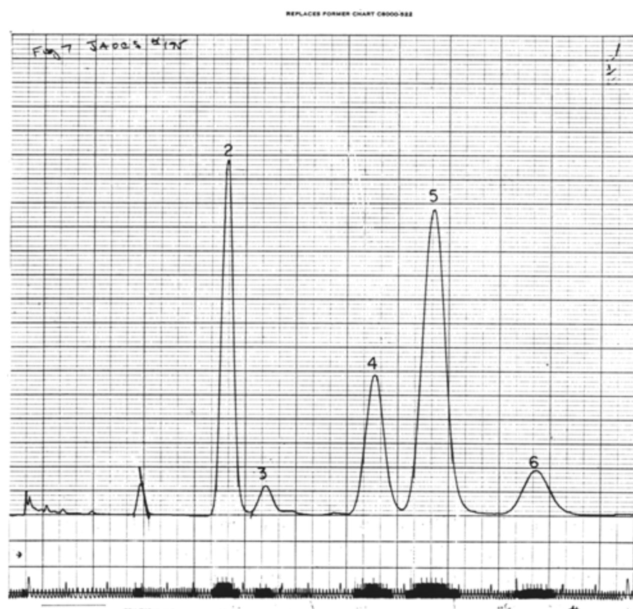

FIG. 7. Methyl esters in esterified lard used 5 hr for frying potatoes.

Peak 1	Myristate	1.27%
Peak 2	Palmitate	25.80%
Peak 3	Palmitoleate	2.38%
Peak 4	Stearate	17.31%
Peak 5	Oleate	44.69%
Peak 6	Linoleate	6.78%

Values are averages from 4 chromatograms.

and 29.0% after frying potatoes. It was assumed that the saturated acids were actually constant, so the values of the unsaturated acids after ten hr of frying potatoes were multiplied by 24.0/29.0 in order to obtain a value for comparing with the unsaturated acids in the unused oil. This same principle was applied to fatty acids in lard used for frying potatoes, and the unsaturated acids in the 10-hr lard sample were multiplied by 39.8/46.3.

Results and Discussion

Fatty acid assays of the cottonseed oil, before and after potatoes or chicken were fried in it, are summarized in Table I. All of the assay data presented here have been reported as per cent of the total fatty acids and are thus interrelated. The original data were analyzed for variance using a factorial design (21), and Duncan's Multiple Range Test (22) was applied to all significantly different means. These statistical analyses show that there was a highly significant loss of linoleic acid after five hr of frying and again after ten hr, regardless of whether chicken or potatoes were cooked.

The assays of the lard are summarized in Table II. Statistical analyses on the data showed a highly significant decrease in linoleic acid after potatoes were fried, but no decrease after chicken was fried. The degradation of linoleic acid probably occurred with the frying process, but the slight dilution with chicken fat, which is relatively high in linoleic acid (approx 20%), could have compensated for the loss. Statistically significant decreases in oleic acid occurred when either chicken or potatoes were fried.

If the saturated acids were considered to be stable as hypothesized previously, the losses in the unsaturated acids would increase. Using the original fats and the same fats after potatoes were fried for ten hr, the saturated fats were calculated to remain constant and the changes in the unsaturated fats are reported in Table III. Now instead of a decrease from 56.8–49.4% in the linoleic acid of cottonseed oil, there is only 40.9% linoleic acid after frying potatoes for ten hr. Also a small decrease in oleic acid is revealed. Oleic and linoleic acids show greater decreases in the lards, also. These percentage values are no longer additive and are only presented to reveal the real destruction of unsaturated fatty acids that occur when fats are used.

Fat Extracted from Potatoes and Chicken. The fatty acid composition of the fat extracted from the potatoes is reported in Table IV. Statistical comparison of these values with corresponding values in Table I and Table II indicate that fat extracted from potatoes fried in fat that had been used for five hr was simply the fat in which it was fried. This was true whether cottonseed oil or lard was used. But after ten hr the fat extracted from the potatoes had about 17% less linoleic acid than the fat in which the potatoes were cooked. It is quite likely that branched chain compounds, hydroxyl compounds, or unstable peroxide linked polymers were formed which were not readily absorbed in the potatoes. This is noteworthy since little research has been done on fat extracted from foods cooked in used oil; even though there is no doubt that fat can be abused to the point of being toxic. When commercial potato chips (7) or fat extracted from meats which were fried, roasted, or broiled under practical home conditions (18) were fed to rats, no damage was noted. Rat feeding studies on fat extracted from fried foods and fat used for frying conducted simultaneously should be informative.

The fatty acid composition of fat extracted from

TABLE I

Fatty Acid Composition of Cottonseed Oil[a]

Treatment of fat	Fatty acids (%)					
	Myristic	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic
Unheated	0.7	21.0	0.5	3.0	17.0	57.0
After frying chicken:						
5 hr	0.7	22.9	0.6	3.3	19.1	53.6
10 hr	0.8	24.5	0.9	3.9	20.6	48.7
Unheated	0.6	20.5	0.7	2.9	17.9	56.8
After frying potatoes:						
5 hr	0.7	22.6	0.9	3.1	18.5	53.4
10 hr	0.9	24.7	1.0	3.4	19.7	49.4

[a] Values represent means of four determinations.

TABLE II

Fatty Acid Composition of Lard[a]

Treatment of fat	Fatty acids (%)					
	Myristic	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic
Unheated	1.1	23.5	1.7	19.6	42.6	9.7
After frying chicken:						
5 hr	1.1	25.0	2.1	20.2	40.5	9.5
10 hr	1.3	26.8	2.6	19.5	39.4	9.3
Unheated	0.9	21.5	1.9	17.4	45.1	11.3
After frying potatoes:						
5 hr	1.3	25.8	2.4	17.3	44.7	6.8
10 hr	1.3	27.4	2.0	17.6	44.1	5.8

[a] Values represent means of four determinations.

TABLE III

Fatty Acid Composition Hypothesizing No Change in Saturated Acids

Fat and treatment	Fatty acids (%)			
	Total saturated	Palmitoleic	Oleic	Linoleic
Cottonseed oil				
Original	24.1	0.7	17.9	56.8
10 hr potato frying	24.1	0.9	16.3	40.9
Lard				
Original	39.8	1.9	45.1	11.3
10 hr potato frying	39.8	1.7	38.0	5.0

TABLE IV

Fatty Acid Composition of Fat Extracted from Potatoes after Frying

Fatty acids (%)	Oil (5 hr)	Oil (10 hr)	Lard (5 hr)	Lard (10 hr)
Myristic	.7	.8	1.4	1.6
Palmitic	23.8	31.2	26.1	30.0
Palmitoleic	.8	1.8	1.8	2.6
Stearic	2.9	3.6	17.2	18.9
Oleic	17.8	21.0	44.7	42.6
Linoleic	54.0	41.2	6.8	4.8

chicken is reported in Table V. Also, in the table is reported the fatty acid composition of a chicken obtained from the same source which had been fed the same ration. The raw chicken was ground and dried at 100C for six hr. Fat extracted from the dry material was used for fatty acid analyses.

These data show that while the fatty acid content of the fat extracted from the chicken reflects the fat it was cooked in, the fatty acid composition also depends quite a bit on the chicken itself. There appears to be little difference in the fatty acid components of chicken cooked in cottonseed oil whether the fat was used for five or ten hr. The same is true of chicken fried in lard. Thus, there is evidence that any catalytic activity of hematin compounds in the chicken was offset by the deterrent effect of fat, amino acids or some unidentified protective agent in the meat.

TABLE V

Fatty Acid Composition of Fat Extracted from Chicken Before and after Frying

Fatty acids (%)	Oil (5 hr)	Oil (10 hr)	Lard (5 hr)	Lard (10 hr)	Raw
Myristic	.9	.7	1.0	.8	.9
Palmitic	26.0	25.4	25.2	25.8	25.4
Palmitoleic	2.7	2.0	2.7	4.9	5.8
Stearic	5.6	5.0	15.1	12.2	8.8
Oleic	28.2	27.4	38.4	39.8	41.9
Linoleic	36.0	38.7	16.6	15.4	17.3

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Hydroxyl Group Determination in High Molecular Weight Alcohols and Complex Organic Mixtures

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Abstract

Acetylation of complex organic mixtures and high mol wt alcohols is inconsistent by existing procedures because of sample insolubility. By choosing a suitable mixed paraffin-ethyl acetate solvent to bring about nearly instantaneous solution, and utilizing the known catalysis property of perchloric acid, acetylation is quantitative in 10 min or less at room temp. The effect of perchloric acid concn on these high mol wt systems was studied, and from 0.05–0.45M consistent results were obtained without difficulty.

Acetylation of polyethylene oxide (P.E.O.) adducts of straight chain monohydric alcohols proceeds normally and without degradation by the prescribed procedure. Extended reaction time results in partial degradation of the polyether linkages and amounts to nearly 8% after 30 min.

Introduction

THE PUBLISHED CLASSICAL base catalyzed acetylation and phthalation procedures, including the AOCS procedure (1), for determining organic hydroxyl groups in alcohols are time consuming, oftentimes inconsistent, and not applicable to high mol wt alcohols, alcohol-P.E.O. adducts and complex mixtures. Increased interest in high mol wt and complex organic systems has necessitated development of a rapid and quantitative procedure applicable to these systems.

Mehlenbacher (7) presents an excellent review of the pertinent literature through 1952. Siggia et al. (8) use pyromellitic dianhydride to esterify alcohols, while Sully (11) employs stearic anhydride and elevated temp. Burton and Praill (2,3) described a reaction mechanism for perchloric acid catalyzed acetylation which was later used by Fritz and Schenk (6), who extended it to include the pyridinium acetylinium ion suggested by Gold and Jefferson (5). Fritz and Schenk (6) also mention p-toluene sulfonic acid as a mild catalyst for acetylation. Stetzler and Smullin (10) use it and elevated temp to acetylate polypropylene oxide adducts of Sorbitol. They, Fritz and Schenk (6) and Critchfield (4), observed that perchloric acid degrades propylene oxide linkages on polyhydric alcohols. Work in this laboratory shows that degradation of alcohol-propylene oxide adducts does indeed occur as described, but for straight chain aliphatic alcohol-P.E.O. products no degradation occurs in the recommended reaction time. In fact, significant degradation occurs only after three times the prescribed reaction time.

Classical base catalyzed acetylation or phthalation of high mol wt and complex organic systems often requires four or more hr. A reaction time longer than