3. Leibetseder, F., and E. H. Ahrens, Jr., Brit. J .Haemat. *5*, 356 (1959).
4. Mulder, E., J. de Gier and L. L. M. van Deenen, Biochim. Biophys. Acta *70*, 94 (1963).
5. Farquhar, J. W., and E. H. Ahrens, Jr., J. Clin. Invest. *42*, 675 (1963).
6. Notham, M. M., and S. Proger, J. Am. Med. Assoc. *179*, 40 (1962).
7. Hurst, R. O., Can. J. Biochem. Physiol. *36*, 1251 (1958).
8. Rouser, G., A. J. Bauman, G. Kritchevsky, D. Heller and J. S. O'Brien, JAOCS *38*, 544 (1961).
9. Rouser, G., J. S. O'Brien and D. Heller, *Ibid. 38*, 1 (1961).
10. Hanahan, D. J., R. M. Watts and P. Pappajohn, J. Lipid Res. *1*, 421 (1960).
11. Nakayama, F., and C. G. Johnson, J. Lab. Clin. Med. *59*, 364 (1962).
12. Rhodes, G. M., and R. M. C. Dawson, "Chromatographic and Electrophoretic Technique," Ivan Smith, ed., Interscience Publishers, New York, 1958.
13. Cornatzer, W. E., W. A. Sandstrom and J. H. Reiter, Biochim. Biophys. Acta *57*, 568 (1962).
14. Skipski, V. P., R. F. Peterson and M. Barclay, J. Lipid Res. *3*, 467 (1962).
15. Block, R. J., E. L. Durrum and G. Zweig, "Paper Chromatography and Paper Electrophoresis," Academic Press, Inc., New York, 1958.
16. Slotta, K. H., and J. K. Powers, Anal. Biochem. *4*, 69 (1962).
17. Bottcher, C. J. F., C. Pries and C. M. van Gent, Rec trav. chim. *80*, 1169 (1961).
18. Williams, J. N., C. E. Anderson and A. D. Jasik, J. Lipid Res. *3*, 378 (1962).
19. Farquhar, J. W., *Ibid. 3*, 31 (1962).
20. Nelson, G. J., *Ibid. 3*, 71 (1962).
21. Reed, C. F., S. N. Swisher, G. V. Marinetti and E. G. Eden, J. Lab. Clin. Med. *56*, 281 (1960).
22. Weed, R. I., C. F. Reed and G. Berg, J. Clin. Invest. *42*, 581 (1963).
23. Farquhar, J. W., Biochim. Biophys. Acta *60*, 80 (1962).
24. Phillips, G. B., and N. S. Roome, Proc. Soc. Exptl. Biol. Med. *109*, 360 (1962)
25. Balint, J. A., H. L. Spitzer and E. C. Kyriakides, J. Clin. Invest. *42*, 1661 (1963).
26. Dawson, R. M. C., N. Hemington and D. B. Lindsay, Biochem. J. *77*, 226 (1960).
27. de Gier, J., and L. L. M. van Deenen, Biochim. Biophys. Acta *49*, 286 (1961).
28. Borkenhagen, L. F., E. P. Kennedy and L. Fielding, J. Biol. Chem. *236 PC*, 28 (1961).
29. Thannhauser, S. J., and N. F. Boncoddo, *Ibid. 172*, 141 (1948).
30. Oliveira, M. M., and M. Vaughan, Fed. Proc. *21*, 296 (1962).
31. Leat, W. M. F. Biochem. J. *84*, 85P (1962).

[Received June 3, 1964—Accepted October 22, 1964]

Fatty Acid Composition Changes in Meat Lipids During Low Temperature Storage[1]

C. K. HUSTON,[2] J. D. SINK, R. C. MILLER and J. W. SHIGLEY, Departments of Animal Industry and Nutrition and Biochemistry, The Pennsylvania State University, University Park, Pennsylvania

Abstract

The effect of low-temp storage on the fatty acid composition of meat lipids was studied. Fat samples were taken from the perirenal and subcutaneous depots of 12 beef carcasses. The samples were divided and one-half from each carcass were stored at −37C for four months. The fat samples (before and after the low-temp storage period) were then extracted, the glyceride fractions separated by low-temp solvent crystallization, and subsequently analyzed by gas-liquid chromatographic techniques. The data obtained in this study and the changes in fatty acid composition noted were not consistent with the pattern of oxidative deterioration but would appear to indicate that non-oxidative mechanisms were responsible for the changes in fatty acid composition observed.

Introduction

THE OXIDATIVE DECOMPOSITION PRODUCTS of meat lipids are prime contributors to the development of undesirable odor and flavor characteristics of meat and meat products (1), and their possible hazard to human health is of current interest (2).

When fats take up oxygen, rancid or off-flavor components are formed. This oxygen uptake and the onset of oxidative deterioration have been shown to be related to the unsaturation of the fat although great variations in natural fats, due to the presence of antioxidants, have been observed (3,4). In an extensive review, Lundberg (5) reported that the oxidative deterioration of food lipids involves autoxidation reactions affecting, primarily, the unsaturated acyl groups. The rate of autoxidation increases markedly with time, and in an exponential manner, with increasing unsaturation.

This problem of flavor changes related to meat lipids has been the subject of extensive research (4,6). Numerous research studies on frozen meats have emphasized the importance of low-temp in retarding rancidity (7–9). Hiner et al. (10) found deterioration in beef and lamb as well as pork in freezer storage to be due primarily to oxidation of the fat. Species differences in susceptibility to oxidation have been observed and have been attributed to the differences in fatty acid composition (11).

It was the purpose of this research to investigate, qualitatively and quantitatively by means of gas-liquid chromatography, the fatty acid composition changes in meat lipids during low temperature storage.

Experimental Procedure

Sampling, Extraction and Storage. Samples of the perirenal and subcutaneous depot fats were taken from the carcasses of 12 steers after the animals had been slaughtered and chilled for 24 hr. Four hundred-g samples were taken from the tip of the kidney knob fat and from the subcutaneous backfat at the 10–13 rib area of each carcass. These samples were stored (less than 24 hr) in a nitrogen atmosphere at −37C in labelled, paraffin-sealed, screw-cap jars until time for analysis. One-half of the samples from each carcass were stored at the same temp for a period of four months.

Eighty-g portions of the fat were cut into small pieces and extracted three times in a Waring blendor with a total of 500 ml of diethyl ether. The ether solution was filtered and dried over anhydrous sodium sulfate. The solvent was removed from the extract by means of a Rinco Evaporator, and the extract was stored in a nitrogen atmosphere. As a check on the completeness of extraction before and after freezer

[1] Journal Series of the Pennsylvania Agricultural Experiment Station, Paper No. 2900.
[2] Present address: U.S. Army Biological Laboratories, Frederick, Md.

TABLE I

Summary of the Analyses of Variance of the Methyl Esters of the Component Fatty Acids

Source of variation	df	Mean squares										
		Chain length of the methyl esters of fatty acid components										
		14	14:1	15	16	16:1	17	18	18:1	18:2	18:3	Sat	Unsat
						Ether extract analysis							
Source of fat	1	0.33	8.00[a]	0.80	17.40	112.24[a]	0.04	2182.95[a]	779.24[a]	1.02	1.88	1704.08[a]	1679.15[a]
Storage time	1	1.54	0.75	0.75	0.32	27.60[a]	4.56[b]	29.30	16.33	45.63[a]	5.67	35.36	41.62
Source × time	1	0.01	0.61	0.19	5.67	0.27	1.02	7.76	28.22	2.52	1.88[b]	0.76	4.38
Error	44	0.40	0.31	0.43	16.39	3.74	0.67	12.40	14.35	3.02	0.32	16.58	17.10
						Glyceride fraction analysis							
Source of fat	1	24.80	16.16	0.39	865.30	190.20[b]	0.06	3374.29[b]	21.06	0.12	0.12	521.43[a]	472.51[a]
Fraction	3	3.15	43.66[b]	0.28	3568.15[b]	469.21[b]	4.12[a]	9755.81[a]	14020.42[a]	193.18	1.59[b]	25589.06[a]	25405.63[a]
Source × fraction	3	6.97[a]	3.12[a]	0.15	289.29[a]	16.17[a]	1.90	184.96[a]	18.45	1.35	0.64	39.17	35.20
Storage time	1	0.00	0.60	1.69[b]	243.91[a]	64.06	9.95[a]	12.76	169.87	103.84	0.47	57.89	25.52
Source × time	1	0.68	0.11	0.00	51.45	1.56	0.00	22.48	0.03	3.47	0.34	11.26	4.61
Fraction × time	3	1.27	0.23	0.11	36.33	16.51[a]	0.94	42.28	239.76[a]	39.21[a]	0.27	70.64[b]	59.40[b]
Source × fraction × time	3	1.50	0.26	0.07	18.61	4.52	0.44	12.55	4.98	0.50	0.97	4.27	5.71
Error	176	0.99	0.38	0.33	13.69	2.29	0.79	17.03	14.00	2.68	0.54	19.49	18.44

[a] $P < .01$.
[b] $P < .05$.

storage, the tissue residues were treated with chloroform-methanol 2:1 (v/v) for 2 hr at room temp. Also, all the stored samples were examined for peroxide formation.

Low-Temperature Solvent Crystallization. The low-temp solvent crystallization of glycerides has been reviewed by Brown and Kolb (12) and has been used by several investigators to separate the glyceride fractions of various animal fats (13–16). Since the amt of components other than glycerides, i.e., phospholipids, sterols, etc., reported to be found in beef depot fats (17,18) is very small, it was decided for the purposes of this study to assume that the ether extracts of the fats were composed of glycerides only. Considering the number of samples involved and the amt of material available, it was decided to separate the fat into four major glyceride fractions by means of the low-temp solvent crystallization technique. For the purpose of determining at what temps to select the four fractions, several extracts of the fats were selected at random and crystallized from acetone at 10-degree intervals from 20C to −60C. By comparing the iodine values of the crystallized fraction with the crystallization temp it was decided that a separation of the glycerides with respect to unsaturation into four fractions could best be accomplished at the temperatures +20, 0, −20 and −40C, respectively. The crystallization apparatus was constructed similar to that described by Brown and Kolb (12). The fat extracts were dissolved in acetone (10 ml/g of fat) and the acetone solutions were placed in 1000 ml graduated cylinders. Two cylinders plus the Buchner funnels and filter flasks necessary for the filtration could be placed in the crystallization chamber at the same time. The crystallization chamber was brought to the desired temp and held there for a minimum of one hr. At the end of this period the crystallized material was filtered through the Buchner funnel with the aid of a water aspirator, the precipitate was washed twice with a total of 50 ml of acetone chilled 10 degrees below the temp of the crystallization chamber and the solvent was pressed out of the precipitate using atmospheric pressure on a rubber membrane held over the funnel. The precipitate was transferred to crystallizing dishes and the solvent removed.

Gas-Liquid Chromatographic Analysis. Fifty mg of the fat extracts were placed in 12-cm centrifuge tubes. Into each tube was placed a small glass stirring rod and 5 ml of absolute methanol containing 0.5% (by wt) concd H_2SO_4. The methanol-H_2SO_4 solution of the glycerides was heated to 70C and held at this temp with frequent stirring for 2 hr. The tubes were then cooled and the solution was extracted with 2 ml of petroleum ether. The petroleum ether solution was washed with water to remove any traces of acid, dried and placed in a small vial where the excess solvent was removed by a stream of nitrogen gas.

The methyl esters were chromatographed on a Barber-Colman Model 10 gas chromatograph equipped with a 9-ft glass U-tube column. The column was packed with 80–100 mesh Chromosorb W, containing 17% (by wt) ethylene glycol succinate polyester (EGS) as the liquid phase. The chromatography was accomplished under the following conditions: column temp 190C, detector temp 235C, flash heater temp 260C, Argon inlet pressure 40 psi and high voltage of 750 v. Samples of known methyl esters were chromatographed to identify the individual peaks. The peak areas were determined by triangulation.

Data Analysis. The results of the gas chromato-

TABLE II

Summary of the Gas Chromatographic Analysis of the Composition of the Ether Extract (Table of Means)

Source of fat and storage time	Weight percentage of the methyl esters of the fatty acid components											
	14	14:1	15	16	16:1	17	18	18:1	18:2	18:3	Sat	Unsat
Perirenal												
Before	3.79	1.18	1.37	24.70	4.24	1.68	25.27	34.14	1.36	1.08	56.81	42.00
After	3.47	1.20	1.24	25.23	5.91	2.01	22.90	34.51	2.85	0.00	54.84	44.47
	−0.32	+0.02	−0.13	+0.53	+1.67[a]	+0.33	−2.37	+0.37	+1.49[a]	−1.08[b]	−1.97	+2.47
Subcutaneous												
Before	3.99	1.77	1.75	26.59	7.45	1.33	10.98	43.73	1.19	0.29	44.64	54.43
After	3.60	2.24	1.38	25.74	8.82	2.24	10.22	41.03	3.60	0.00	43.18	55.69
	−0.39	+0.47	−0.37	−0.85	+1.37	+0.91[a]	−0.76	−2.70	+2.41[b]	−0.29[a]	−1.46	+1.26
All samples												
Before	3.89	1.47	1.56	25.65	5.85	1.51	18.13	38.94	1.28	0.68	50.73	48.22
After	3.53	1.72	1.31	25.48	7.36	2.13	16.59	37.77	3.23	0.00	49.00	50.08
	−0.36	+0.25	−0.25	−0.17	+1.51[b]	+0.62[a]	−1.54	−1.17	+1.95[b]	−0.68	−1.73	+1.86
All samples												
Perirenal	3.63	1.19	1.30	24.96	5.08	1.85	24.08	34.33	2.10	0.54	55.83	43.23
Subcutaneous	3.80	2.00	1.56	26.17	8.13	1.79	10.60	42.38	2.40	0.15	43.91	55.06
	0.17	0.81[b]	0.26	1.21	3.05[b]	0.06	13.48[b]	8.05[b]	0.30	0.39	11.92[b]	11.83[b]

[a] $P < .05$.
[b] $P < .01$.

graphic analysis were punched onto IBM cards which were processed by the IBM 7074 Data Processing System programed for an analysis of variance. The F test was used to test for significant differences among the means of the various groups. Between mean differences from group to group were tested by the method of Tukey as outlined by Snedecor (19). When interactions were significant, lower order interactions and significant main effects were tested by the interaction term.

Results and Discussion

Table I is a summary of the analyses of variance for the ether extract and glyceride fraction data. These statistical analyses were based on the gas chromatographic analyses of the fatty acid composition, before and after four months freezer storage, of an ether extract and four fractions of the perirenal and subcutaneous depot fats from 12 experimental carcasses. Tables II, III and IV summarize the gas chromatographic composition analyses of the ether extract and glyceride fractions with respect to the main effects, source of fat and storage time. In the statistical analyses, the main effects were tested in terms of the appropriate interaction when those interactions were themselves significant. The treatment of the tissue residues with the chloroform-methanol solvent mixture did not extract any additional lipid material. Also, no detectable peroxide formation in the stored samples was observed.

Ether Extract Analysis. From the summary of the analysis of variance (Table I) and the summary of the gas chromatographic analysis (Table II), it can be observed that source of fat was highly significant ($P < .01$) for myristoleic (C14:1), palmitoleic (C16:1), stearic (C18), oleic (C18:1), the saturated and also the unsaturated fatty acids. The perirenal source was shown to contain more saturated fatty acids, particularly stearic, while the subcutaneous source more unsaturated components (myristoleic, palmitoleic and

TABLE III

Summary of the Gas Chromatographic Analysis of the Composition of the Glyceride Fractions of the Perirenal and Subcutaneous Lipid Sources (Table of Means)

Fraction	Weight percentage of the methyl esters of the fatty acid components											
	14	14:1	15	16	16:1	17	18	18:1	18:2	18:3	Sat	Unsat
Plus twenty												
Perirenal	3.11	1.96	1.13	16.73	6.85	1.38	7.93	54.16	5.03	0.28	30.28	68.28
Subcutaneous	3.58	3.16	1.15	15.00	9.90	0.98	4.45	55.01	4.52	0.54	25.17	73.12
	0.47	1.20[b]	0.02	1.73[a]	3.05[b]	0.40	3.48[b]	0.85	0.51	0.26	5.11[b]	4.84[b]
Zero												
Perirenal	3.18	0.33	1.13	32.07	1.54	1.83	43.70	13.92	0.75	0.24	81.90	16.78
Subcutaneous	4.48	0.48	1.10	39.43	2.33	1.97	32.01	16.24	0.67	0.23	78.99	19.95
	1.30[b]	0.15	0.03	7.36[b]	0.79[b]	0.14	11.69[b]	2.32[a]	0.08	0.01	2.91[a]	3.17[a]
Minus twenty												
Perirenal	3.20	0.87	0.90	26.90	2.54	1.63	31.95	29.38	0.55	0.77	64.58	34.10
Subcutaneous	4.57	1.05	1.10	35.95	3.73	1.48	20.53	28.87	0.67	0.55	63.63	34.89
	1.37[b]	0.18	0.20	9.05[b]	1.19[b]	0.15	11.42[b]	0.51	0.12	0.22	0.95	0.79
Minus forty												
Perirenal	3.64	1.45	1.10	24.15	5.83	1.30	15.55	42.87	2.93	0.43	45.75	53.51
Subcutaneous	3.39	2.25	1.25	26.45	8.77	1.84	8.60	42.85	3.19	0.20	41.53	57.26
	0.25	0.80[b]	0.15	2.30[a]	2.94[b]	0.54	6.95[b]	0.02	0.26	0.23	4.22[b]	3.75[a]
All fractions												
Perirenal	3.29	1.15	1.06	24.96	4.19	1.53	24.78	35.08	2.32	0.43	55.63	43.16
Subcutaneous	4.00	1.73	1.15	29.21	6.18	1.57	16.40	35.74	2.27	0.38	52.33	46.31
	0.71	0.58	0.09	4.25	1.99[a]	0.04	8.38[a]	0.66	0.05	0.05	3.30[b]	3.15[b]
Each fraction												
Plus twenty	3.35	2.56	1.14	15.86	8.36	1.18	6.19	54.59	4.78	0.41	27.72	70.70
Zero	3.83	0.41	1.12	37.75	1.94	1.90	37.85	15.08	0.71	0.24	80.44	18.36
Minus twenty	3.89	0.96	1.00	31.43	3.14	1.55	26.24	29.13	0.62	0.66	64.11	34.50
Minus forty	3.52	1.85	1.18	25.30	7.30	1.57	12.08	42.86	3.06	0.31	43.64	55.39

[a] $P < .05$.
[b] $P < .01$.

TABLE IV

Summary of the Gas Chromatographic Analysis of the Composition of the Glyceride Fractions Before and After Freezer Storage (Table of Means)

Fraction	Weight percentage of the methyl esters of the fatty acid components											
	14	14:1	15	16	16:1	17	18	18:1	18:2	18:3	Sat	Unsat
Plus twenty												
Before	3.20	2.42	1.24	15.85	9.40	1.37	6.06	51.68	6.66	0.53	27.73	70.70
After	3.49	2.71	1.04	15.88	7.33	0.99	6.32	57.49	2.89	0.29	27.71	70.70
	+0.29	+0.29	−0.20	+0.03	−2.07[b]	−0.38	+0.26	+5.81[b]	−3.77[b]	−0.24	−0.02	0.00
Zero												
Before	4.00	0.43	1.23	33.89	2.03	2.00	36.99	16.86	0.74	0.32	78.11	20.37
After	3.65	0.39	1.00	37.61	1.85	1.79	38.72	13.29	0.68	0.15	82.78	16.36
	−0.35	−0.04	−0.23[a]	+3.72[a]	−0.18	−0.21	+1.73	−3.57[b]	−0.06	−0.17	+4.67[b]	−4.01[b]
Minus twenty												
Before	3.98	0.89	1.03	29.65	3.19	1.74	27.47	29.11	0.62	0.60	63.87	34.41
After	3.79	1.02	0.98	33.20	3.08	1.37	25.01	29.14	0.62	0.71	64.34	34.58
	−0.19	+0.13	−0.04	+3.55[b]	−0.11	−0.37	−2.46[a]	+0.03	0.00	+0.11	+0.47	+0.17
Minus forty												
Before	3.38	1.82	1.31	24.43	8.43	2.00	1[illegible].87	40.24	4.09	0.36	44.00	54.93
After	3.65	1.88	1.04	26.16	6.17	1.14	11.30	45.49	2.04	0.27	43.28	55.86
	+0.27	+0.06	−0.27	+1.73	−2.26[b]	−0.86[b]	−1.57	+5.25[b]	−2.05[b]	−0.09	−0.72	+0.93
All fractions												
Before	3.64	1.39	1.20	35.96	5.76	1.78	20.84	34.47	3.02	0.45	53.43	45.10
After	3.64	1.50	1.01	28.21	4.61	1.32	20.33	36.35	1.56	0.36	54.53	44.37
	0.00	+0.11	−0.19[a]	−7.75[b]	−1.15	−0.46[b]	−0.51	+1.88	−1.46	−0.09	+1.10	−0.73
Each fraction												
Plus twenty	3.35	2.56	1.14	15.86	8.36	1.18	6.19	54.59	4.78	0.41	27.72	70.70
Zero	3.83	0.41	1.12	35.75	1.94	1.90	37.85	15.08	0.71	0.24	80.44	18.36
Minus twenty	3.89	0.96	1.00	31.43	3.14	1.55	26.24	29.13	0.62	0.66	64.11	34.50
Minus forty	3.52	1.85	1.18	25.30	7.30	1.57	12.08	42.86	3.06	0.31	43.64	55.39

[a] $P < .05$.
[b] $P < .01$.

oleic). A highly significant ($P < .01$) increase in the amt of palmitoleic and linoleic (C18:2) fatty acids can be observed as the result of the low-temp storage treatment. A significant ($P < .05$) increase was also observed for heptadecanoic (C17). A significant interaction affecting linolenic (C18:3) acid was noted and can be explained by the inconsistency of the difference between sources from one storage time to the other time.

Glyceride Fraction Analysis. The data summaries in Tables I and III show highly significant differences in the saturated and unsaturated fatty acid composition of the two sources when totaled over all fractions. The perirenal source contains more total saturates and significantly more stearic and the subcutaneous source more total unsaturates and significantly more palmitoleic.

Highly significant fraction differences were noted for heptadecanoic, stearic, oleic, the saturated and unsaturated fatty acids. Significant mean differences in the content of heptadecanoic were found only between the zero degree and the +20 degree fractions and between the −40 and +20 degree fractions, while significant mean differences between each of the fractions were observed for stearic, oleic, the saturated and unsaturated fatty acids. Significant fraction differences for myristoleic, palmitic (C16), palmitoleic and linolenic acids were also observed. Myristoleic, palmitic and palmitoleic showed significant mean differences between the means of the −20 degree fraction and the other three fractions.

A source × fraction interaction was highly significant with respect to the myristic, myristoleic, palmitic, palmitoleic and stearic components. The gas chromatographic summary in Table III shows that this can be explained by the inconsistent source differences between fractions for the myristic acid content and inconsistent fraction differences between sources for myristoleic, palmitic, palmitoleic and stearic.

The low-temp storage treatment showed highly significant effects on the palmitic and heptadecanoic lipid components and a significant influence on the content of pentadecanoic (C15). The amt of palmitic increases as a result of freezer storage, but that of the odd-carbon components decreases.

The fraction × time interaction was highly significant for the 16 and 18 carbon monounsaturates and for linoleic acid. It was also significant for the total content of saturates and unsaturates. The data summary in Table IV shows that although fraction composition differences varied with storage time, they were not consistent from fraction to fraction.

No significant effect on the composition of any of the fatty acids due to the first order (source × time) and second order (source × fraction × time) interactions were observed.

It was also interesting to note the pronounced effect of low-temp storage on the saturated odd-carbon fatty acids.

In this study, some other fatty acids, three of which have been tentatively identified as a 15-carbon branched-chain (C15:Br), and two monounsaturates, pentadecenoic (C15:1) and heptadecenoic (C17:1), were detected in the gas chromatographic analysis but not included in the data analysis.

If oxidative changes were occurring in the fatty acids, one would have expected to see a decrease in the percentage composition of some or all of the unsaturates. The data obtained in this study and the significant changes in fatty acid composition noted were not consistent with the pattern of oxidative change. This would seem to indicate that some relationship exists between the types of fatty acids affected and the mechanism by which these changes in composition are accomplished.

On the basis of the trends evidenced by these few data, the authors propose that perhaps a hydrogenase-dehydrogenase system is in operation during the storage period and that during storage the glycerides may have undergone interesterification reactions. This type of reaction could substantially alter the fatty acid composition of the glyceride fractions during

storage without appreciably changing the over-all fatty acid content of the fat.

REFERENCES

1. American Meat Institute Foundation, "The Science of Meat and Meat Products," W. H. Freeman & Co., San Francisco, 1960, p. 124–131.
2. Lea, C. H., "Lipids and Their Oxidation," The AVI Publishing Co., Inc., Westport, Conn., 1962, p. 3–28.
3. Meyer, L. H., "Food Chemistry," Reinhold Publishing Corp., New York, 1960, p. 32–42.
4. Watts, B. M., Adv. Food Res. *5*, 1–52 (1954).
5. Lundberg, W. O., "Lipids and Their Oxidation," The AVI Publishing Co., Inc., Westport, Conn., 1962, p. 31–50.
6. Watts, B. M., *Ibid.*, 202–214 (1962).
7. Lea, C. H., J. Soc. Chem. Ind. *50*, 215T–220T (1931).
8. Ramsbottom, J. M., Refrig. Eng. *53*(1), 19–23 (1947).
9. Privett, O. S., F. J. Pusch and W. O. Lundberg, Food Tech. *9*, 347–351 (1955).
10. Hiner, R. L., A. M. Gaddis and O. G. Hankins, *Ibid.* *5*, 223–229 (1951).
11. Chang, I., and B. M. Watts, JAOCS *29*, 334–338 (1952).
12. Brown, J. B., and D. K. Kolb, Prog. Chem. Fats Other Lipids *3*, 57–94 (1955).
13. Garton, G. A., T. P. Hilditch and M. L. Meara, Biochem. J. *50*, 517–524 (1952).
14. Hilditch, T. P., and R. K. Shrivastava, JAOCS *26*, 1–4 (1949).
15. Luddy, F. E., G. R. Fertsh and R. W. Reimenschneider, *Ibid.* *31*, 266–268 (1954).
16. Reiser, R., and H. G. R. Reddy, *Ibid.* *36*, 97–99 (1959).
17. Bailey, A. E., "Industrial Oil and Fat Products," 2nd ed., Interscience Publishers, Inc., New York, 1951, p. 142–149.
18. Lange, W., JAOCS *27*, 414–422 (1950).
19. Snedecor, G. W., "Statistical Methods," 2nd ed., Iowa State University Press, Ames, 1956, p. 291–328.

[Received June 1, 1964—Accepted September 29, 1964]

Determination of Free and Bound Gossypol in Swine Tissues[1]

F. H. SMITH, Nutrition Section, Department of Animal Science, North Carolina State of the University of North Carolina at Raleigh, Raleigh, North Carolina

Abstract

Methods have been developed for the determination of free and bound gossypol in the tissues of swine ingesting diets containing free gossypol. Data presented show satisfactory reproducibility and accuracy of the methods.

Introduction

ISOLATION OF GOSSYPOL from the livers of pigs that had consumed toxic cottonseed meal (2) suggested that studies of deposition and accumulation of gossypol in the organs may be helpful in elucidating the metabolism of this compound in animals. An essential phase in the effectiveness of such a study is analytical methods for measuring the free and the bound gossypol. The procedures used for the estimation of these components in cottonseed meal (3,4) were applied to liver tissue of pigs fed various gossypol-containing cottonseed meals (1). The modifications of these methods for application to animal tissues for the improvement of both the analytical mechanics and accuracy are herein described.

Free Gossypol

Reagents. a) Aniline: reagent, freshly distilled, water clear; b) acetic acid (5): glacial reagent; c) ethyl ether: U.S.P. or purified for fat extraction, must be peroxide free; d) solution A: ethanol + 0.2 ml glacial acetic acid/liter; e) solution B: a 60% ethanol-water solution prepared by diluting 715 ml of 95% ethanol to 1 liter with distilled water and subsequently adding 200 ml of ethyl ether and 0.2 ml glacial acetic acid; f) Hyflo Super-Cel: to remove iron, boil 100 g of Hyflo Super-Cel with 600 ml of distilled water and 50 ml of concd HCl for 15 min, filter through a paper in a Buchner funnel and wash with distilled water. Repeat the acid treatment, wash and dry.

Procedure

The tissues are preserved in a frozen state until analyzed. Just before analysis, the tissues are partially thawed, ground in a food chopper, thoroughly mixed and placed in a glass bottle to prevent moisture changes.

Transfer a 10-g sample of the ground tissue to either the jar of a Serval Omni-mixer or a similar comminuting apparatus, add 50 ml of 95% ethanol (solution A) and 20 ml of ether. Homogenize the mixture for 2 min while the blending jar is surrounded with ice water to prevent heating.

In the meantime, prepare a filter by placing a 5.5-cm filter paper in a size 1 Buchner funnel and after applying vacuum pour a suspension of 2–3 g of Hyflo Super-Cel in ca. 15 ml of 95% ethanol on the paper. After washing the jar cap and blades of the homogenizer with solution B delivered from a wash bottle, suspend 2–3 g of Hyflo Super-Cel in the homogenate and filter through the prepared Buchner funnel. Wash the blender jar with solution B and pour the washings over the tissue residue in the Buchner funnel. Thoroughly wash the tissue residue, which will serve as the sample for bound gossypol, with small portions of solution B to remove all of the free gossypol. Exercise care so that the combined filtrate and washings are not less than 120 ml nor more than 130 ml. Using a flask calibrated to contain 130 ml, dilute the filtrate to 130 ml, mix and refilter a portion of the solution through Whatman No. 1 filter paper if turbid. If the volume should exceed 130 ml, the excess is measured and the calculations are made accordingly.

Transfer 10-ml aliquots in triplicate to 25-ml volumetric flasks, one of which is used as the reference solution after diluting to the mark with solution B. To the other 2 flasks add 0.5 ml freshly distilled aniline and heat them on the surface of the steam bath not directly over the steam for 40 min to convert the gossypol to dianilinogossypol. After the flasks have cooled to room temp, add 2 ml of ether to replace that lost during heating and sufficient solution B to bring the volume to 25 ml. After mixing the contents of the flasks, determine the absorbance at a wavelength of 445 mμ, using the aliquot containing no aniline as the reference solution. Determine the gossypol content of the tissue from a standard absorbance-concn curve prepared from pure gossypol.

Prepare the standard curve by dissolving 0.0250 g of pure gossypol in approx 10 ml of ethyl ether, dilute to 100 ml with solution B and mix. Transfer a 10-ml aliquot to a 100-ml volumetric flask, dilute to volume with solution B and mix. In triplicate and at ml intervals, pipet a series of aliquots ranging from 1–8 ml into 25-ml volumetric flasks. Reserve one flask containing an aliquot at each volume level as a reference. Dilute the remaining aliquots to at least 5 ml with solution B, add 0.5 ml freshly distilled aniline and

[1] Published with the approval of the Director of Research of the N.C. Agricultural Exper. Sta. as Paper No. 1836 of the Journal Series.