Color and the Lipid Composition of Pork Muscles[1]

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Abstract

The lipid characteristics of three porcine muscles were investigated. Included in the study were the light and dark portions of the semitendinosus muscle, the semimembranosus (light) and the quadriceps femoris (dark) muscles. The lipids from the semimembranosus and the quadriceps femoris muscles were observed to be statistically different. The light muscles were 20% higher in lipid level and these lipids contained 20% more glycerides and 40% less phospholipids than the dark muscles. The glycerides from both muscle lipids were identical in fatty acid composition but the phospholipid fatty acids differed significantly. The light muscle phospholipid fatty acids were higher in monoenes while the dark muscle phospholipids predominated in polyunsaturates. In the semitendinosus muscle, the light portion was 35% higher in lipid content than the dark portion but the compositions of the lipids from both areas were similar. When these data were compared to the values for the semimembranosus and the quadriceps femoris muscle lipids, the lipid characteristics were found to be intermediate of predominantly light or dark muscle lipids.

Introduction

Muscle color in meat animals is of considerable interest since a desirable color is an important factor in consumer acceptance of the meat product. Pork muscles frequently exhibit pronounced variations in color and consequently the role of certain physical and chemical properties of muscle such as pH, moisture, lipid content and pigments in determining muscle color has long been questioned. The relative importance of these is difficult to evaluate as published data regarding them are incomplete or contradictory. In fact the actual measurement of the color itself is not well defined (9).

The contribution of lipid material to ultimate muscle color has been studied by several investigators with widely different results. The earlier work of Mackintosh and Hall (15) on beef animals, followed by the work of Craig et al. (4) showed a significant correlation between the color of the muscle and its fat content. Craig ascribed greater importance to fat content than to pigment in determining the color of beef muscle. Conversely, Henry and Bratzler (6) and Janicki et al. (9) reported that fat did not influence the color of porcine muscle. Recently, Beecher et al. (2) reported that the majority opinion now supports the findings of Pirko and Ayres (16) that lightness or darkness of the muscle is primarily a function of pigment activity.

While the effect of lipid material on establishing muscle color has been somewhat defined, the correlation of lipid characteristics with color is still open to question. It was the object of this present study to compare the lipid characteristics of three porcine muscles. One of these is the semitendinosus, a unique muscle containing portions of both light and dark muscles. The others are the semimembranosus, a light (or white) muscle, and the quadriceps femoris, a dark (or red) muscle.

[1] Presented at the AOCS Meeting, New York, October 1968.
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Experimental Procedures

Sample Isolation

Four pork carcasses were obtained immediately after slaughter and samples of the semitendinosus, semimembranosus and quadriceps femoris muscles were taken. The muscles were trimmed of external fat and connective tissue and divided into their respective light and dark portions. Muscle samples were frozen with solid carbon dioxide and stored at −40 C for later analysis.

Solvents

All solvents used in any of the operations reported were purified and redistilled in an all glass apparatus.

Lipid Extraction

Portions of the frozen muscle were lyophilized at room temperature for periods of 36 to 48 hr at a vacuum ranging from 50 to 75 μ. The average muscle contained about 70% water. After removal of water, the lipids were extracted with chloroform-methanol (2:1) in the following manner. Five to 50 g portions of the dried fragmented muscles were macerated with mortar and pestle in the presence of 75–100 ml of the extracting solvent. In this technique the material disintegrates into very fine particles allowing excellent contact between the solvent and the tissues. The lipid rich solvent was decanted and the tissue residue was treated again in the same manner with an additional 100 ml of solvent. The chloroform-methanol extracts were combined and centrifuged and the clarified supernatant collected. The residue was reextracted with 50 ml of solvent and this was added to the original extract. The combined extracts were taken to dryness on the rotary evaporator using mild heat (40 C) and vacuum. This residue was extracted several times with 10–15 ml portions of purified hexane which were combined and centrifuged. The clarified hexane solution was made up to a standard volume, usually 50 ml. Extreme care was exercised at all times to minimize the effects of oxidation and where possible operations were carried out under nitrogen. Approximately 0.05% of the antioxidant 4-methyl-2,6-ditert.-butylphenol was added to the solvent solutions of the lipids to protect against autoxidation during chromatography, manipulation and storage (19).

Fractionation of Total Lipids

Silicic acid column chromatography was used for separation of the total lipids into their component lipid classes. The all glass column (21 × 300 mm) was packed with a slurry of 25 g of silicic acid-filter aid (80:20) in 100 ml of nanograde petroleum ether (bp 35–60). The activation and treatment of the silicic acid is described in a previous publication (13). The column was equipped to collect measured volumes of eluate under nitrogen.

About 100–300 mg of weighed lipid in 10 ml of petroleum ether was added to the top of the column

TABLE I

Moisture and Lipid Characteristics of Various Porcine Muscle

Characteristics	No. obs.	Semimembranosus, %	No. obs.	Quadriceps femoris, %	Semitendinosus[a]		
					No. obs.	Light, %	Dark, %
Moisture[b]	10	72.0 ± 4.9	10	70.1 ± 4.1	2	67.0 ± 1.4	66.0 ± 1.8
Total lipid, %[c]	15	19.3 ± 3.0	17	13.1 ± 4.7	2	24.7 ± 0.4	18.7 ± 0.4
Glyceride, %[d]	15	77.1 ± 3.1	20	61.9 ± 8.4	2	81.2 ± 1.0	69.7 ± 2.0
Phospholipid, %[d]	15	19.8 ± 4.3	20	34.3 ± 7.6	2	16.6 ± 1.8	27.4 ± 3.0
Free cholesterol-free acid, %[d]	15	3.2 ± 0.4	20	3.8 ± 0.8	2	2.2 ± 0.4	2.9 ± 1.0

[a] Values from one animal.
[b] Mean value, calculated on fresh weight basis.
[c] Mean value, calculated on dry basis.
[d] Mean values, calculated as per cent of total lipid.

for the charge. After addition of the first eluting solvent the flow rate was adjusted to about 150 ml/hr by regulation of the nitrogen pressure on the system. The order of elution and solvents employed were as follows: (a) hydrocarbons—50 ml of petroleum ether containing 1% of ethyl ether; (b) cholesteryl esters—300 ml of same solvent mixture; (c) glycerides—300 ml of petroleum ether containing 4% ethyl ether; (d) cholesterol-free acids—350 ml of petroleum ether containing 8% ethyl ether; and (e) phospholipids—200 ml of chloroform-methanol (1:1), followed by 100 ml of methanol containing 2% water.

The collected fractions were transferred to suitable flasks and concentrated on a rotary evaporator and weights were obtained on all fractions.

Thin Layer Chromatography

Thin layer plates (20 × 20 cm) of Silica Gel G were coated and activated as previously described (12). Aliquots of the column fractions were chromatographed to follow the column fractionation and final fractions were analyzed for cross contamination of major lipid classes. Approximately 50–100 μg of lipid sample were chromatographed with the developing solvent consisting of petroleum ether-ethyl ether-acetic acid in the proportions 80:20:0.5. The development time was 25 min and the distance of solvent travel was 125 mm. In most cases the developed plates were exposed to iodine vapors for detection of components. Identification of the components was achieved by comparison with authentic lipid standards.

Gas Liquid Chromatography

The analyses were made with an Aerograph 1522 (Varian Aerograph, Walnut Creek, Calif.) chromatograph equipped with dual H_2 flame ionization detectors. The column was an 8 ft × 1/8 in. O.D. (0.093 in. I.D.) stainless steel coiled tube packed with 12% stabilized diethylene glycol succinate (DEGS) on 42–60 mesh Chromosorb W and maintained isothermally at 175 C. The areas under the peaks of the individual components were determined by an electronic integrator (Infotronics, Model CRS-11HSB, Houston, Texas) coupled to a digital printer. Areas and percentages thus obtained were in good agreement with mixtures of methyl esters of similar but known composition.

Cholesterol Analysis

Free and esterified cholesterol were determined by the procedure of Luddy et al. (14).

Statistical Analysis

The statistical analyses performed are described by Steel and Torrie (18).

Results and Discussion

The moisture and lipid characteristics of the various pork muscles are shown in Table I. Data for the semimembranosus (light) and quadriceps femoris (dark) muscles are the mean values obtained from representative samples from the four animals while comparative data for the semitendinosus muscle are from a single animal. The mean values for total lipid of the light and dark muscles differ significantly ($P < .005$) with the lipid yield of the light muscles being considerably higher. Statistical analysis for the lipid yield of the muscles from the individual animal was not possible from the number of samples available but the trend was similar to that shown by the mean values. The light portion of the semitendinosus muscle was higher in lipid content than the semimembranosus (light) muscle. Likewise, the dark portion of the semitendinosus muscle was higher in lipid yield than the quadriceps femoris (dark) muscle. The data for the light and dark muscles agree with those observed by Beecher (3) in his study and are in contradiction with those of Kaucher et al. (8) and Fröberg (5). The findings suggest a difference in the manner or regulation of lipid deposition in these two muscles.

The composition of the muscle lipids which includes only the major less polar lipid classes and total phospholipid is also given in Table I. In both the light and the dark muscle lipids, the glycerides

TABLE II

Fatty Acid Composition[a] of Total Lipids Isolated From Porcine Muscles

Fatty acid[c]	No. obs.	Semimembranosus, %	No. obs.	Quadriceps femoris, %	Semitendinosus[b]		
					No. obs.	Light, %	Dark, %
14:0	16	1.6 ± 0.4	14	1.5 ± 0.5	4	1.4 ± 0.1	1.4 ± 0.0
16:0	16	22.7 ± 1.9	14	22.7 ± 2.1	4	23.6 ± 0.1	23.1 ± 0.3
16:1	16	4.5 ± 0.6	14	3.8 ± 0.6	4	4.1 ± 0.1	4.2 ± 0.1
18:0	16	10.0 ± 0.6	14	10.7 ± 0.4	4	10.7 ± 0.1	10.4 ± 0.1
18:1	16	48.2 ± 3.0	14	39.7 ± 3.3	4	48.8 ± 0.5	45.8 ± 0.3
18:2	16	8.4 ± 3.3	14	15.9 ± 3.2	4	8.0 ± 0.3	10.0 ± 0.2
20:4	16	1.2 ± 0.5	14	2.5 ± 0.5	4	0.8 ± 0.1	1.8 ± 0.1

[a] Mean values, expressed as area per cent and include only major components.
[b] Values from one animal.
[c] Number to the left indicates carbon number, to the right double bonds.

TABLE III

Fatty Acid Composition[a] of Glycerides Isolated From Muscle Lipids

Fatty acid[c]	No. obs.	Semimembranosus, %	No. obs.	Quadriceps femoris, %	Semitendinosus[b]		
					No. obs.	Light, %	Dark, %
14:0	14	1.7 ± 0.2	14	1.8 ± 0.3	4	1.7 ± 0.2	1.7 ± 0.1
16:0	14	24.6 ± 1.4	14	24.4 ± 1.3	4	24.7 ± 0.9	24.3 ± 0.1
16:1	14	5.0 ± 0.7	14	5.0 ± 0.6	4	5.3 ± 0.1	5.4 ± 0.2
18:0	14	10.0 ± 0.8	14	9.6 ± 0.8	4	9.9 ± 0.1	9.5 ± 0.1
18:1	14	50.2 ± 0.4	14	48.5 ± 2.0	4	50.2 ± 0.4	49.2 ± 0.7
18:2	14	6.5 ± 1.8	14	7.9 ± 1.8	4	6.4 ± 0.3	7.7 ± 0.3
20:4	14	0.6 ± 0.4	14	0.4 ± 0.1	4	0.2 ± 0.0	0.3 ± 0.1

[a] Mean values, expressed as area per cent and include only major components.
[b] Values from one animal.
[c] Number to left indicates carbon number, to the right double bonds.

were predominant and the concentrations differed significantly ($P < .005$). The semimembranosus (light) muscle lipid was approximately 25% higher in glyceride than the quadriceps femoris (dark) muscle. The phospholipid content of the two muscles was also significantly different ($P < .005$). The quadriceps femoris (dark) muscle had a phospholipid concentration almost 70% greater than that of the light muscle lipid. The mean value for the phospholipid content of the semimembranosus (light) muscle is higher than the values reported by Hornstein et al. (7) and Kuchmak and Dugan (10). The mean value for the quadriceps femoris (dark) muscle lipid is appreciably higher than these values but is in agreement with the findings of Beecher et al. (3) and Allen et al. (1) for the phospholipid content of dark muscle lipids.

The lipid composition of the light and dark semitendinosus muscle was closer to the values of a light muscle lipid than to a dark muscle lipid. The light semitendinosus muscle was 80% glyceride and 17% phospholipid while the dark semitendinosus muscle averaged 70% glyceride and 27% phospholipid.

No significant difference was found in the concentration of free cholesterol or free acids in the muscle lipids and these minor components were not isolated individually. The cholesterol was determined by a colorimetric procedure (14) and the amount of acids by difference. The mean value for free cholesterol for the semimembranosus (light) muscle was 2.3% and that for the quadriceps femoris (dark) muscle was 2.7%. These cholesterol values are somewhat greater than the values for the semitendinosus light and dark muscle lipids which analyzed 1.0% and 1.2%, respectively. The lipids contained no measurable amount of hydrocarbons or of cholesteryl esters.

The data in Table II summarize the fatty acid composition of the total lipids. They indicate a significant difference in the mean values for all the acids ($P < .05$) in the semimembranosus and the quadriceps femoris muscle lipids except for the C_{14} and C_{16} acids. In accord with the data of Lawrie and Gatherum (11), the dark muscle lipids predominate in the polyunsaturates while the light muscle lipids are characterized by a high content of oleic acid. It is of interest that the mean fatty acid values for the semimembranosus (light) muscle resemble the values for both the normal and PSE (pale, soft, exudative) longissimus dorsi (light) muscles reported by Sink et al. (17). The mean values for the quadriceps femoris (dark) muscle are similar to the data of Allen et al. (1) for the psoas major (dark) muscle.

While the light and dark semitendinosus muscles differed in lipid yield (Table I), the fatty acid data of Table II show these lipids to have almost identical compositions. These fatty acid patterns also resemble those found in the semimembranosus (light) muscle lipids.

The fatty acid data shown in this table and in Tables III and IV reflect only major fatty acids and therefore fatty acid totals are less than 100%.

In Table III, the mean values for the fatty acid compositions of the glycerides of both the semimembranosus (light) muscle lipids and the quadriceps femoris (dark) muscle lipids are summarized. For comparison, the values obtained for the glycerides from the semitendinosus muscle lipids (single animal) are also given. The fatty acid concentrations in the glycerides of all of the muscle lipids were very similar, although a statistical difference ($P < .05$) was observed for the diene acids. The data are similar to those reported by Allen et al. (1) on the neutral lipids from diaphragm, longissimus dorsi (light) and psoas major (dark) porcine muscles.

The fatty acid composition for the phospholipids of the corresponding muscle lipids are shown in Table IV and exhibited a very different pattern, particularly for the unsaturated acids. Statistical differences were observed for the 16:1 ($P < .05$), 18:1 ($P < .005$), and 18:2 ($P < .005$) fatty acids. The semimembranosus (light) muscles had the higher concentration of monoene acids, while the quadriceps femoris (dark) muscle lipids were 25% higher in the diene fatty acids. The fatty acid concentrations

TABLE IV

Fatty Acid Composition[a] of Phospholipids Isolated From Muscle Lipids

Fatty acid[c]	No. obs.	Semimembranosus, %	No. obs.	Quadriceps femoris, %	Semitendinosus[b]		
					No. obs.	Light, %	Dark, %
14:0	12	1.4 ± 0.2	12	2.4 ± 1.4	4	1.1 ± 0.1	1.2 ± 0.2
16:0	12	20.2 ± 2.1	12	19.6 ± 2.3	4	21.8 ± 0.4	21.0 ± 0.0
16:1	12	3.2 ± 0.4	12	1.9 ± 0.9	4	2.6 ± 0.1	1.9 ± 0.1
18:0	12	14.3 ± 2.4	12	15.2 ± 3.7	4	16.1 ± 0.3	21.1 ± 0.3
18:1	12	24.3 ± 2.8	12	16.1 ± 2.6	4	23.2 ± 0.8	16.0 ± 0.3
18:2	12	24.1 ± 2.8	12	30.7 ± 2.6	4	21.8 ± 0.1	22.2 ± 0.3
20:4	12	7.9 ± 0.7	12	8.1 ± 1.1	4	7.3 ± 0.1	8.3 ± 0.0

[a] Mean values, expressed as area per cent and include only major components.
[b] Values from one animal.
[c] Number to left indicates carbon number, to the right double bonds.

in the phospholipids from the semitendinosus light and dark muscle lipids were similar to the values for the semimembranosus and quadriceps femoris phospholipids except for the diene acids. The dark semitendinosus muscle lipid did not exhibit the high concentration of the 18:2 acid that characterized the phospholipids from the quadriceps femoris (dark) muscle lipids.

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[Received April 22, 1969]