inadequate nitrogen supply at some period in growth. Treatment 2, Table III, in which the liquid fertilizer program was supplemented with 1 gram of N from $MgNH_4PO_4 \cdot H_2O$ incorporated in the mix produced excellent results. The results again substantiate the fact that surface applications are more slowly available and longer lasting than comparable applications incorporated in the soil. Figure 6 shows the appearance of plants produced on treatments 1, 2, and 7.

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MEAT TENDERNESS FACTORS

Interrelations of Tenderness, Chronological Age, and Connective-Tissue Fractions of Porcine Musculature

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Tenderness was determined for 206 pork loins from barrows, gilts, and sows 132 to 1173 days old and selected according to five degrees for intramuscular fat. Elastin, acid- and alkali-insoluble collagen, alkali-soluble collagen, and acid-soluble collagen were determined chemically in 20 loins from this group representing all ages but only low levels of intramuscular fat. Advancing chronological age was associated with lower panel tenderness ratings and higher shear-force values; however, the data failed to explain this biochemically. Total quantities of connective tissue did not increase with age, but acid-soluble collagen significantly decreased with advancing age. No significant relations were found between the connective-tissue fractions and various measures of tenderness, suggesting that the physiological age–tenderness relationship may not be a reflection of changes in total quantities of connective tissue in pork muscle.

PALATABILITY characteristics have long been recognized as important yardsticks in determining the acceptance of meat products. Of the various palatability traits, tenderness has received much of the researcher's attention, and the frequency of tough, chewy products undoubtedly accentuates a need for improvement. One approach to the problem has involved the study of connective tissue. The possibility exists that biochemical alterations in the connective tissue may explain at least part of the variation in tenderness of pork products.

Ramsbottom, Strandine, and Koonz (*16*), Mitchell, Hamilton, and Haines (*11*), and Bate-Smith (*2*) have demonstrated large variations in the amount of connective tissue among several anatomical regions of the beef carcass, and have shown the quantity of connective tissue to be inversely related to tenderness. Bate-Smith (*2*) indicated that, as an animal grew older, the proportion of connective tissue in total body protein decreased. An increase in collagen concentration soon after birth was followed by a decrease with advancing age. Moran and Smith (*12*) nevertheless indicated that the degree of meat tenderness was a function of the amount and character of the connective tissue. Research by Wilson (*19*) has partially refuted such claims, because muscle from young veal calves possessed significantly higher amounts of connective tissue than comparable muscle from steers or aged cows.

In cooking experiments, Cover and Hostetler (*5*) found that the collagen content of two muscles of differing anatomical location was not related to tenderness and that the moisture in moist-heat cookery was required primarily to obtain high temperatures and not necessarily to convert collagen into gelatin.

Although the characterization of connective tissue is somewhat incomplete, Briscoe and Loring (*4*), Elden, Noble, and Boucek, (*6*), and Kao, Boucek, and Noble (*9*) have made major contributions in medically oriented research on the role of connective tissue in age-related metabolic diseases. A number of soluble and insoluble connective-tissue fractions have been demonstrated and measured quantitatively by Jackson (*8*). According to Orekhovich and Mazurov (*13*) and Pierce and Hocott (*14*), the more insoluble fractions, such as "elastin" and alkali-insoluble "collagen," increase with advancing age. However, little evidence exists that such changes are associated with differences in meat tenderness.

This investigation was made to study the relation of chronological age to measures of tenderness for the longissimus muscle and, secondly, to determine quantitatively two insoluble and two soluble fractions of connective tissue from swine of various ages and to compare changes in these fractions with variations in tenderness.

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This constitutes one segment of a broader research project initiated to study a number of factors associated with quality attributes of pork.

Experimental Procedures

A total of 206 pork carcasses were selected on the basis of five chronological age classifications (132-, 203-, 282-, 491-, and 1173-day averages) and according to five degrees of intramuscular fat in the longissimus as subjectively scored the cut surface of the muscle at the twelfth costa. Approximately equal numbers of barrows and gilts were selected for the three younger classifications, while packer sows were chosen for the two oldest age categories. About 11 carcasses were obtained for each butcher-type classification and about seven for each sow group.

Over 90% of the pigs originated from the University of Wisconsin swine farm. These animals received similar rations and were subjected to similar management conditions.

The loins were aged from 5 to 8 days at 3° C. to simulate normal marketing practices prior to sampling for chemical and taste panel analysis. A 15-member, semitrained taste panel evaluated the center cut loin chops, and ascertained tenderness scores by using a six-point hedonic rating scale. A score of 1 represented unusually tough samples and a score of 6 represented unusually tender samples. The chops were seasoned with salt and baked at 163° C. to an internal temperature of 74° C.

In addition, the Warner-Bratzler shear was used to determine tenderness objectively. Three 0.5-inch cores were removed from a cooked chop representing each loin.

The longissimus muscle was used for the connective tissue analysis because it is usually consumed in a fresh cooked state and therefore is especially susceptible to variations in tenderness, and because it generally contains intermediate amounts of connective tissue, compared to the relatively active biceps femoris and the inactive psoas major. One-inch-thick cross-sectional samples of this muscle were removed opposite the eighth costa from loins selected from 20 carcasses representing both sexes and all age classifications. Samples with low levels of intramuscular fat were selected to facilitate more reliable connective-tissue extractions.

Each sample, free of subcutaneous fat and epimysium and frozen at −65° C., was chipped into minute shavings with a hand food grater. A 2-gram sample was randomly selected and further ground in a mortar with sand. As illustrated in Figure 1, 40 ml. of 0.1*N* acetic acid was mixed with the sample, which was then allowed to stand 20 hours at 25° C. The solution was centrifuged and the supernatant decanted through a cheesecloth filter. The supernatant (fraction A) was stored at −30° C. for subsequent determination of the dilute acid–soluble collagen. A dilute alkali (0.1*N* sodium hydroxide) extraction was completed for the residue to separate the dilute alkali–soluble collagen (fraction B). Then, the residue was separated into a dilute acid– and alkali-insoluble collagen, which was solubilized by heat (fraction C), and elastin (fraction D), by a modified Lowry technique used by Wilson (*19*), also shown in Figure 1. This method was further modified by performing a lipid extraction with 3 to 1 chloroform–methanol and filtering the hot soluble gelatin through glass wool after autoclaving. The dilute acid– and alkali-insoluble collagen and elastin fractions were expressed separately as per cent nitrogen of total nitrogen in the initial 2-gram sample.

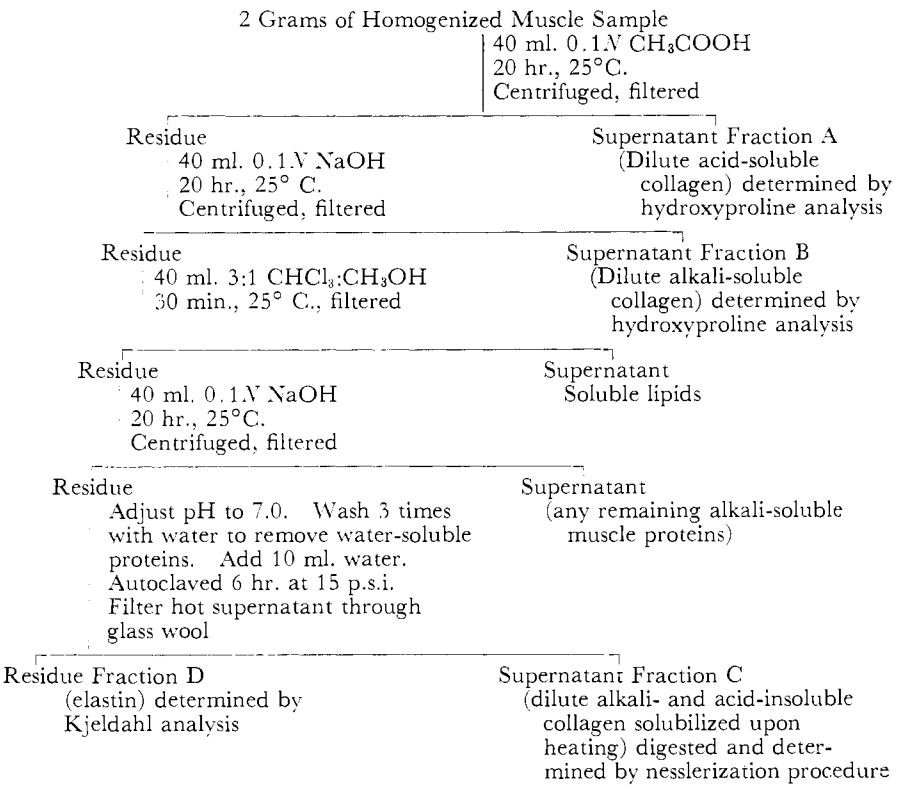

Figure 1. Procedure for separating connective-tissue components

The acid-soluble and alkali-soluble collagen fractions (A and B) were made 6*N* with hydrochloric acid and autoclaved at 15-pound pressure for 9 hours, as prescribed by Wierbicki and Deatherage (*18*). A 1.5-gram mixture of Dowex 10–charcoal was added and filtered to remove the humin. The samples were dried in a 75° C. oven, neutralized with potassium hydroxide, and diluted to 50 ml. Total hydroxyproline was determined on a 10-ml. portion as described by Prockop and Udenfriend (*15*). This procedure was modified by using a 5% solution of *p*-dimethylaminobenzaldehyde (color reagent) instead of the recommended 28% concentration. The hydroxyproline content was multiplied by 7.8 for conversion to collagen, as suggested by Bowes, Elliott, and Moss (*3*). The collagen value was subsequently expressed as micrograms of collagen per milligram of total nitrogen in the sample. After the techniques had been repeated on samples containing known amounts of added hydroxyproline, it was concluded that the hydroxyproline method used in this experiment was reproducible. After isolating dilute acid– and alkali-insoluble collagen (fraction C), the collagen nitrogen was determined by a nesslerization procedure as described by Umbreit, Burris, and Stauffer (*17*). Nitrogen in the final residue, defined as "elastin" (fraction D), was determined by a Kjeldahl procedure (*1*). Quadruplet samples were analyzed for each of the four connective-tissue fractions originating from each muscle sample. These analyses were reproducible within a 10% range of error. Results of the tissue analysis were compared to chronological age and to tenderness measurements as determined by taste-panel scores and Warner-Bratzler shear-force values.

Results and Discussion

Figure 2 illustrates the relationship of animal age to taste-panel tenderness observations and shear-force values for loins originating from 206 pigs. All of the group values were significantly different (as determined by Duncan's multiple range test) from each other, except those between the 282-day-old and 491-day-old groups. Tenderness decreased rapidly with changes in age from 132 to 282 days, and then decreased at a slower rate. Another indication of this relationship is shown in Table I. When variations of tenderness as associated with intramuscular fat were removed, animal age appeared to account for about 18% of the variation in observed tenderness. Because a panel tenderness score of 3 represented an

unacceptably tough product, a score of 4 indicated an acceptably tender product, and a point of acceptance or rejection occurred between these two ratings, it might be concluded that pork from swine more than 203 days old would fail to meet minimum tenderness requirements.

What, then, are the chemical changes responsible for the relation found between animal aging and tenderness? The longissimus contained, on the average, 0.68% total connective tissue on a fresh-weight basis. This composite proportionally contained 51% dilute acid- and alkali-insoluble collagen (fraction C), 43% elastin (fraction D), and 6% acid- and alkali-soluble collagen (A and B). Fractions A and B therefore represented about 10% of the total collagen portion. Tenderness measurements were not significantly related to the solubility of collagen, the quantity of elastin, or the amount of total connective tissue. Furthermore, quantitative changes in only one of the four connective-tissue fractions bore a statistical relation to changes in animal age (Table III). The acid-soluble collagen (fraction A) decreased significantly with advancing age from 142 to 284 days. Table II shows means of the connective-tissue measurements compared to tenderness values, chronological age, and carcass weight. There were no significant quantitative connective-tissue changes within the age range studied. Therefore these findings are somewhat different than those of Hiner, Anderson, and Fellers (*7*), that suggest a quantitative (as determined histologically) increase in connective tissue with advancing age. Perhaps species and method of determining connective tissue may account for such differences.

The average content of dilute acid- and alkali-insoluble collagen (fraction C) was 1.85%. This was significantly higher than the 1.42% found for the elastin (fraction D). This elastin value for pork is somewhat higher than that reported by Lorincz and Szeredy (*10*); however, it may be partially attributed to the differences in techniques used for the chemical analysis. The alkali-soluble collagen (fraction B) was significantly higher than the acid-soluble collagen (fraction A) when compared to all samples, regardless of age. When either the dilute acid- and alkali-insoluble collagen/elastin ratio (fraction C/fraction D) or the alkali-soluble collagen/acid-soluble collagen ratio (fraction B/fraction A) was compared between age groups, no differences were noted. On the average, these ratios were, respectively, 1.3 to 1 and 1.2 to 1. The biological significance of these ratios may help to explain physiological changes associated with aging and subsequently to explain differences in meat tenderness. However, the results of this investigation did not resolve this relationship.

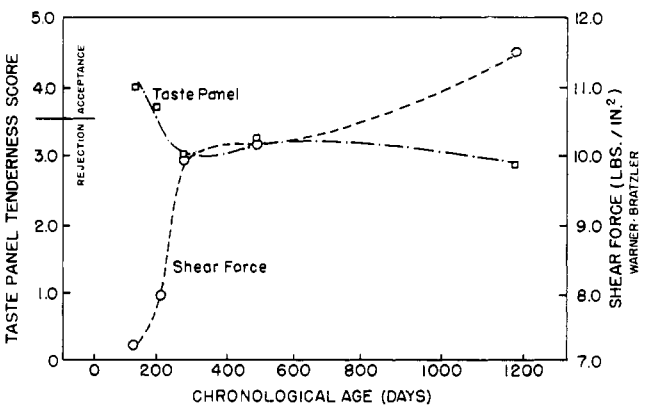

Figure 2. Relationship of chronological age to tenderness of 206 baked loins

Table I. Chronological Age vs. Tenderness of the Longissimus in Pork[a]

Age (Days) vs.	n	Correlations[b] r_{12}	Correlations[b] $r_{12.3}$	Analysis of Variance in Tenderness for Five Age Groups, F
Panel tenderness score (1 = tough, 6 = tender)	206	−0.32	−0.42	8.5
Warner-Bratzler shear force, p.s.i.	206	0.19	0.28	12.9

[a] All values significant at 1% level of probability. [b] Identification of variables. 1 = chronological age, 2 = tenderness measurement, 3 = % intramuscular fat. Partial correlation analysis were calculated to eliminate statistically variations in tenderness that may be attributed to variations in intramuscular fat.

Table II. Comparison of Mean Concentrations of Connective-Tissue Components of 20 Pork Loins with Animal Age and Tenderness

Identification				Connective-Tissue Components: Insoluble[a]		Soluble[b]		Tenderness Measurements	
Age group	n	Age, days	Carcass weight, pounds	Collagen (fraction C)	Elastin (fraction D)	Acid collagen (fraction A)	Alkali collagen (fraction B)	Panel score	Shear force, p.s.i.
Young butchers	7	142	111	2.02	1.53	5.7	6.3	3.7	7.1
Old butchers	7	284	203	1.67	1.17	4.6	5.6	3.3	8.9
Sows	6	828	348	1.86	1.59	4.8	6.1	3.3	9.4
Composite	20	398	214	1.85	1.42	5.1	6.0	3.4	8.4

[a] Expressed as % nitrogen of total protein nitrogen. [b] Expressed as μg. collagen per mg. total protein nitrogen.

Table III depicts a number of simple correlations among the four connective-tissue components, and between connective-tissue measurements and animal age. Total quantities of soluble (fractions A and B) or insoluble (fractions C and D) connective tissue did not increase with advancing age. However, a significantly negative relation of −0.48 did exist between age and the amount of acid-soluble collagen (fraction A). The correlation coefficient of 0.59 between age and ratio of alkali- to acid-soluble collagen (fraction B/fraction A) was also significant. In addition, a negative and highly significant association was shown between alkali-soluble collagen (fraction B) and either dilute acid- and alkali-insoluble collagen (fraction C) or elastin (fraction D) or the sum of the insoluble components (fractions C and D). Also the amounts of dilute acid- and alkali-insoluble collagen (fraction C) and elastin (fraction D) were positively related. These findings suggest that perhaps the dilute acid- and alkali-insoluble collagen (fraction C) and elastin (fraction D) change together, and tend to decrease when alkali-soluble collagen (fraction B) increases. This does not hold true for the acid-soluble collagen (fraction A), which appears to change independently of the other fractions. The negative association between the total insoluble connective tissue (fractions C and D) and the alkali-soluble collagen (fraction B) may imply that the insoluble fractions (C and D) are either the precursors or the products of alkali-soluble collagen. An understanding of the significance of these changes in various connective tissue fractions with animal age must await their further characterization. Perhaps a chemical change in nonconnective-tissue components of muscle may partially account for the decrease in tenderness as age increases.

Table III. Simple Correlations between Connective-Tissue Components and Chronological Age of 20 Pork Loins

	Fraction C, Dilute Acid- and Alkali-Insoluble Collagen	Fraction D, Elastin	Fraction A, Acid-Soluble Collagen	Fraction B, Alkali-Soluble Collagen	Fractions C and D	Fractions A and B	Fractions B/A
Fraction D	0.92[a]						
Fraction A	−0.03	−0.22					
Fraction B	−0.65[a]	−0.65[a]	0.23				
Fractions C and D			−0.15	−0.66[a]			
Fractions A and B					−0.60[a]		
Age[b]	−0.31	0.07	−0.48[c,d]	0.37	−0.08	0.19	0.59[c]

[a] $P = 0.01$. [b] $n = 11$. [c] $P = 0.05$. [d] Partial correlation coefficient; % intramuscular fat statistically held constant.

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ESSENTIAL OILS

Sources of Monoterpene Hydrocarbons

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Oils of savin, cubeb, sweet marjoram, galbanum, thuja, bay, lemon, and orange were investigated as sources for authentic samples of α-thujene, 3-carene, sabinene, α-terpinene, γ-terpinene, myrcene, β-phellandrene, and terpinolene. Also analyzed were secondary components of commercial samples of terpenes. α-Terpinene, γ-terpinene, and terpinolene were obtained by silica gel isomerization of limonene. Infrared Spectra of α-thujene, 3-carene, sabinene, γ-terpinene, myrcene, and terpinolene are presented.

OBTAINING authentic samples of specific monoterpene hydrocarbons is frequently difficult. Some of the more common terpenes, such as α-pinene, β-pinene, limonene, α-phellandrene, camphene, and *p*-cymene, can be obtained from chemical supply houses, but their purity, and occasionally their authenticity, are questionable. A variety of essential oils have been reported to contain certain of the monoterpenes, but identification has sometimes been limited to the evidence of gas chromatographic retentions. Because of a need for a more complete stock of these compounds for use as chromatographic standards, the authors were interested in establishing sources for these materials. Among the compounds of particular interest were sabinene, α-thujene, 3-carene, α-terpinene, γ-terpinene, myrcene, β-phellandrene, and terpinolene.

Although gas chromatography is a powerful tool for separating complex mixtures, relying on it as a sole source of identification is unwise. Once the composition of a mixture is established and evidence of specific compounds is conclusive, gas chromatography becomes somewhat more reliable for determining the incidence and estimating the quantity of a particular compound in a natural mixture.

These investigations involve gas chromatographic separation and infrared characterization of fractions isolated from oils of savin, cubeb, sweet marjoram, galbanum, thuja, bay, lemon, and orange. Also examined were fractions contained in commercial samples of terpenes (which are, unfortunately, rarely pure).

Apparatus

Preparative-scale gas chromatography utilized an Aerograph Autoprep with a 20-foot by 1/4-inch aluminum column