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EVALUATION OF COLLAGEN ANALYSIS PROCEDURES IN FRESH AND COOKED BEEF

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BACKGROUND AND OBJECTIVE

The most widely used sample preparation procedure for soluble, insoluble, and total collagen determinations in meat products is described by Hill (1966). This procedure involves separation of soluble and insoluble fractions by heating the sample in 25% Ringer's solution at 77°C for 63 min. It is often used on meat samples that have not received prior thermal processing, such as fresh beef. Twenty five percent Ringer's solution and heating at 77°C for 63 min do not reflect the actual solvent and thermal conditions of the product, respectively. Traditionally, for sensory and instrumental tenderness evaluations, intact steaks are heated to 70°C, at which time they are removed from the oven. This heating regimen is drastically different from heating at 77°C for 63 min. Consequently, this severe heating may mask treatment effects on collagen solubility. Moreover, this procedure may not provide meaningful data when dealing with either fresh or fully cooked beef steaks, because their aqueous phase's ionic environment and pH is significantly different from 25% Ringer's solution. The data obtained using a collagen segregation procedure is of great importance since collagen solubility affects the eating quality of meat and meat products. This study was undertaken to improve the modified Hill (1966) procedure by altering the sample heating regimen and/or changing the solvent solution to accurately mimic conditions used in fresh and cooked beef product manufacture so that sensitivity and accuracy of procedure are maximized. Specifically, our goal was to compare the modified Hill's and the "New" procedures with respect to how well collagen analyses correlate to Warner-Bratzler shear (WBS) forces as a measure of meat tenderness.

METHODS

The design structure for this experiment was a split-split-split-plot with animal as the whole-plot experimental unit, muscle as the sub-plot experimental unit, steak at either raw or cooked state as the sub-subplot experimental unit, and the intact or pulverized steak as the sub-sub-subplot unit. A factorial design of treatments was used with four factors: maturity level (young and old), muscle (*Longissimus thoracis* (LT) and *Semitendinosus* (ST)), meat state (raw and cooked), and protocol (Hill and New). The whole-plot, sub-plot, sub-subplot, and sub-sub-subplot treatments were maturity level, muscle, meat state, and protocol, respectively, which were applied at random to each subplot, using a separate randomization scheme. The order in which protocols were performed was also randomized to reduce experimental error due to the technician skill level. Ten replications were run per maturity level. Data were analyzed using the SAS System (SAS Institute, 1994). Analysis of variance, correlation analysis, and least square means were determined and separated at $P<0.05$.

Samples were obtained from the carcasses of ten, thirteen month, old Hereford-Red Angus crossbred steers and ten, eight year old 5/8 Brahman X 3/8 Hereford or Angus crossbred cows, fabricated at Kansas State University and Prairieland Processors Inc. (Wichita, KS), respectively. Muscles were excised 7d postmortem, trimmed, and frozen (-35°C) for 2 month. Two 2.54-cm thick steaks were cut frozen, using a band saw (Hobart Corp., Troy, OH) from the posterior end of the LT and from the center of each ST. One ST steak was excised distally and the other proximally from the center. Steaks were randomly assigned to two meat states: raw or cooked. Frozen steaks assigned to the raw meat state were sawed into ca. 2.3 cm^3 cubes, deep frozen in liquid nitrogen, pulverized in a Waring Blender (Waring Products Div., New Hartford, CT), and held frozen (-20°C) until testing. Frozen steaks allotted to the cooked meat state were thawed for 24 h at 2°C, and broiled at 168°C in a Blodgett oven (GS Blodgett Co., Inc., Burlington, VT), to 70°C internally, as measured by a thermocouple attached to a Doric temperature recorder (Beckman Instruments, Schaumburg, IL). WBS were determined using a Warner-Bratzler attachment to the Universal Instron Testing Machine (Instron 4201, Canton, MA). Eleven LT and eight ST, 1.27 cm cores from each cooked steak were sheared after cooling at 20°C for two hours. Cores were kept with sample. Cored steaks were frozen and pulverized as previously described. Percent moisture, fat, protein, and ash (AOAC, 1990) as well as pH and cooking yields were determined. Three protocols were compared to a modified version of the Hill (1966) procedure (protocol A) regarding residual (insoluble), soluble (heat-labile) and total collagen. Duplicate frozen, pulverized raw (protocol A and B) or cooked (protocol C and D) samples were weighed (4.0 ± 0.03g) into 50cc polyethylene tubes. Tubes received either 12 ml of 20°C (room temperature) or 50°C (heated), 25% Ringer's solution (ionic strength (IS)= 0.03 M, pH=7.0) for protocol A and C, or the same amount of 20°C or 50°C, 100% Ringer's solution (IS=0.12 M) for protocol B (pH=5.5) and D (pH=5.8), respectively. Ringer's solution (100%) was chosen as the solvent for protocol B and D, since it more closely resembled the 0.15 IS of intracellular muscle fluids (Guyton, 1991). It's pH was adjusted to 5.5 (protocol B) or 5.8 (protocol D), to more closely reflect raw and cooked beef's pH, respectively. Heated 25% (protocol C) or 100% (protocol D) Ringer's solution was used, as 50°C is the temperature at which gelatin remelts.

For protocol A, samples were placed in a 77°C water bath for 63 min, stirring 10 revolutions every 10 min with a separate glass rod, including at the end of 63 min. Tubes were removed from water baths at the indicated times, cooled for 30 min at 20°C and centrifuged at 2°C for 10 min, at 6000 x g (Beckmann, Palo Alto, CA). Supernatant (8 ml) was decanted into 50 ml test tubes with screw caps. Room temperature 25% Ringer's solution (8 ml) was added to the residue and resuspended using separate stirring rods for each tube and centrifuged again under same conditions. Supernatants were decanted into previous test tubes. Concentrated HCl (16 ml 12N) was added to test tubes containing supernatant (soluble collagen) and capped. Distilled, deionized water (8 ml) was used to resuspend pellet. This supension was poured into a separate set of 50 ml test tubes. Concentrated HCl (10 ml 12 N) was added to centrifuge tubes to rinse and pour residue back into pellet containing tubes (insoluble collagen). Tubes were then capped. Supernatants and residuals were autoclaved for 12 hr at 121°C. After autoclaving, hydroxyproline levels were assayed spectrophotometrically (Bergman and Loxley, 1963). Collagen values were expressed as mg collagen/g of sample, using hydroxyproline conversion of 7.25 and 7.52 for insoluble and soluble collagen, respectively (Cross et al., 1973). For protocol B, samples were placed in a 20°C initial temperature water bath and heated to 70°C (medium doneness) at which time they were pulled out (ca. 50 min). For protocol C and D, samples were blended with 50°C solution in a polytron (Brinkmann Instruments, Westbury, NY) for 30 sec and cooled for 30 min at 20°C. Subsequent steps were followed as outlined in Protocol A, except that 100% (pH 5.5, 20°C), 25% (pH 7.0, 50°C) or 100% (pH 5.8, 50°C) was used for protocol B, C and D, respectively, to resuspend pellet. A total of 360 assays were performed. All four protocols were tested using the same experimental unit.

RESULTS AND DISCUSSION

Mean WBS values for LT and ST steaks at 7d postmortem were 3.51 and 5.52 kg, with standard errors (SE) of 0.13 and 0.16, respectively. Muscles from older animals had higher WBS (LT=4.08±0.94, ST=5.96±0.83), than muscles excised from younger animals (LT=2.98±0.49, ST=5.08±0.62). LT steaks had higher cooking yields (78.35%, SE=0.82) than ST counterparts (68.92%, SE=0.82). Animal age did not affect fat level or cooking loss. Fat percentage was greater for LT (6.5%, SE=0.24) than for ST steaks (4.74%, SE=0.24), and lower for raw (4.73%, SE=0.22) than for cooked (6.49%, SE=0.22) steaks. Moisture was higher for cooked LT (63.55%, SE=0.34) than for ST steaks (61.23%, SE=0.34), and lower for cooked steaks from younger (61.49%, SE=0.34) than from older animals (63.30%, SE=0.34). Raw steaks had lower pH's (5.4) than cooked (5.8) counterparts. Correlation coefficients between WBS values and mg/g of soluble, insoluble and total collagen are reported in Table 1. Insoluble and total collagen was correlated ($P<0.05$) to WBS of cooked LT steaks from older animals when protocol C ($r= 0.81$ and $r= 0.79$, respectively) and D ($r= 0.71$ and $r= 0.71$, respectively) were used, but not with any other protocol within that muscle or maturity level. Protocol D resulted in positive correlations ($r= 0.65$) between soluble collagen and WBS in cooked ST steaks excised from older animals. Collagen protocol was poorly correlated ($P>0.05$) to WBS when raw samples were analyzed. Likewise, WBS and insoluble, soluble or total collagen were not significantly correlated ($P>0.05$) for either raw or cooked, LT or ST steaks from younger animals.

CONCLUSION

Existing procedures that assess solubility and quantity of collagen as contributing factors in the tenderness differences between muscles from different maturity levels are not accurate. Modifications to the Hill (1966) procedure, based on sample state of cooking prior to collagen evaluations and on solvent/temperature conditions during collagen determinations, provided the highest correlation coefficients, and were adequate to separate insoluble collagen from cooked *Longissimus thoracis* muscles excised from older animals, but not from the young population or from raw samples. Other researchers have found that collagen characteristics were not significantly related to tenderness, especially within the same muscle from animals that differ in age.

PERTINENT LITERATURE

AOAC. 1990. *Official Methods of Analysis*. 15th ed. Association of Official Analytical Chemists, Washington, D.C.

Bergman, I. and Loxley, R. 1963. Anal. Chem. 35:1961.

Cross, H.R., Carpenter, Z.L., and Smith. G.C. 1973. J. Food Sci. 38:998.

Guyton. A.C. 1991. Texbook of Medical Physiology. 8th ed, W.B. Saunders Company. Harcourt Brace Jovanovich, Inc. Philadelphia, PA.

Hill, F. 1966. J. Food Sci. 31:161.

SAS Institute. 1994. SAS User's Guide, Version 6 ed. SAS Institute Inc., Cary, N.C.

Table 1. Mean, standard deviation (SD), pearson correlation coefficient (r) with Warner-Bratzler shear, and probability values (p) for soluble, insoluble and total collagen as measured by four procedures on raw and cooked *Longissimus thoracis* (LT) and *Semitendinosus* (ST) beef steaks

				Soluble (mg/g)				Insoluble (mg/g)				Total (mg/g)			
Age[1]	Muscle	State	Protocol[2]	Mean	SD	r	p[3]	Mean	SD	r	p	Mean	SD	r	p
Old	LT	Raw	A	0.33	0.09	-0.28	0.43	4.83	1.43	-0.37	0.29	5.16	1.49	-0.37	0.29
			B	0.25	0.13	0.33	0.35	4.36	1.34	0.40	0.25	4.61	1.40	0.41	0.23
			C	0.25	0.09	-0.08	0.84	3.83	0.51	0.81	0.01	4.08	0.52	0.79	0.01
			D	0.26	0.16	0.47	0.17	4.28	1.23	0.71	0.02	4.54	1.33	0.71	0.02
Old	ST	Cook	A	0.26	0.08	0.37	0.29	5.71	1.06	0.24	0.50	5.97	1.10	0.26	0.47
			B	0.21	0.10	0.05	0.90	4.59	1.98	0.05	0.90	4.80	2.05	0.05	0.90
			C	0.27	0.18	0.31	0.39	6.18	2.37	0.47	0.17	6.45	2.51	0.47	0.17
			D	0.22	0.10	0.65	0.04	6.53	2.86	0.62	0.06	6.75	2.96	0.62	0.05
Young	LT	Raw	A	1.55	0.85	0.15	0.69	2.66	0.92	-0.14	0.70	4.21	1.60	-0.00	0.99
			B	1.50	0.57	0.23	0.52	3.27	0.87	0.29	0.42	4.77	1.30	0.29	0.41
			C	1.21	0.43	0.11	0.77	3.40	0.57	0.15	0.69	4.61	0.80	0.16	0.66
			D	1.67	0.91	0.48	0.16	4.14	1.35	-0.02	0.95	5.80	1.60	0.25	0.48
Young	ST	Cook	A	0.62	0.11	0.30	0.40	3.57	0.86	0.17	0.63	4.19	0.90	0.20	0.57
			B	0.63	0.11	-0.40	0.25	5.05	1.17	-0.43	0.21	5.68	1.24	-0.45	0.20
			C	0.87	0.17	-0.00	0.99	6.36	0.65	-0.48	0.16	7.23	0.75	-0.42	0.23
			D	0.95	0.22	0.18	0.63	7.14	1.32	0.62	0.06	8.08	1.50	0.57	0.09

[1]Old=8 year old cows, Young=13 month old steers.

[2]Protocol A: Modified Hill (1966), solvent=25% Ringer's solution, IS=0.03M, pH=7.0, 20°C, exp.conditions= 77°C/63 min (water bath);
Protocol B: solvent=100% Ringer's solution, IS=0.12M, pH=5.4, 20°C, exp.conditions= heated to 70°C (water bath)(ca. 50 min);
Protocol C: solvent=25% Ringer's solution, IS=0.03M, pH=7.0, exp.conditions= blended with 50°C solution;
Protocol D: solvent=100% Ringer's solution, IS=0.12M, pH=5.8, exp.conditions= blended with 50°C solution.

[3]Correlation coefficients / Probability > |R| under Ho:Rho=0, N=10. $\alpha = 0.05$