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New recommendations for measuring collagen solubility

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ABSTRACT

The heat-solubility of intramuscular collagen is usually conducted in 1/4 Ringer's solution at pH 7.4, despite this ionic strength and pH being inappropriate for post-rigor meat. The current work studied the percentage of soluble collagen and hydrothermal isometric tension characteristics of perimysial strips on bovine semitendinosus muscles in either 1/4 Ringer's solution, distilled water, PBS, or a solution of the same salt concentration as 1/4 Ringer's but at pH 5.6. Values of % soluble collagen were lower at pH 7.4 than 5.6. Increasing ionic strength reduced % soluble collagen. The maximum perimysial isometric tension was independent of the bathing medium, but the percent relaxation was higher at pH 7.4 than at pH 5.6, and increased with ionic strength of the media. It is recommended that future measurements of collagen solubility and tests on connective tissue components of post-rigor meat should be carried out in a solution of concentrations NaCl and KCl equivalent to those in 1/4 Ringer's, but at pH 5.6, a pH relevant to post-rigor meat.

1. Introduction

The measurement of collagen solubility on heating is a very common procedure in studies on meat tenderness. The most popular conditions for this have used quarter-strength Ringer's solution, as originally described by Hill (1966). The only logic provided by Hill (1966) for using Ringer's solution is that it is more effective in weakening intermolecular bonds within collagen than water. Jackson and Bentley (1960) had shown that a low salt concentration is more efficient than water in extracting collagen, and obtained a maximum liberation of collagen at 0.14–0.28 M NaCl, decreasing at higher concentrations. Standard Ringer's solution contains 0.123 M NaCl, as well as smaller concentrations of KCl and $CaCl_2$. However, quarter strength Ringer's solution at pH 7.3–7.4 recommended by Hill contains 0.03 M NaCl. This is neither isotonic to living muscle or to connective tissue. Saline solutions containing NaCl cause substantial swelling in post-rigor muscle tissue (Winger & Pope, 1981). The dramatic decline in pH during the conversion of muscle to meat means that a pH of 7.3 to 7.4 is not relevant to meat. Other authors have chosen media for collagen solubility studies other than Hill's; Christensen et al. (2011) used 0.9% NaCl (0.154 M) to assess collagen solubility in beef meat, whereas Kristensen et al. (2002) used distilled water in their studies on pork. No explanation was given for these choices. It is not clear whether the use of Hill's solution, saline or water would alter the values of collagen solubility measured, or indeed whether any of these reflect the solubilization of collagen within meat as it is cooked under normal conditions.

Lepetit (2008) suggested that heat-induced contraction in intramuscular connective tissue (IMCT) has a considerable contribution to cooking loss and the increase in meat toughness seen above 65 °C. The major mass fraction of IMCT in meat is the perimysium, which on average constitutes about 90% of total IMCT (McCormick, 1994). Cartaginese and Purslow (2014) observed that appreciable loads are generated in isometrically restrained perimysial strips at temperatures above 65 °C. In contrast with less thermally-stable types of connective tissue (Kopp & Bonnet, 1987), perimysium maintains high force overtime when held at high temperatures. The relaxation of isometric force with cooking time has been related to the stabilizing effects of covalent crosslinks between collagen molecules (Allain, Lelous, Bazin, Bailey, & Delaunay, 1978; Kopp & Bonnet, 1987). Variations in pH, salt type and concentration are known to affect the thermal denaturation characteristics of collagen within IMCT (Purslow, 1987; Akta, 2003). If collagen denaturation varies with the ionic species, concentration and pH in the bathing medium, then either the maximum contraction force generated on, or the relaxation of this force overtime at high temperatures may also vary.

This study evaluates the magnitude of differences in the solubility of intramuscular collagen of meat and differences in the thermomechanical stability of perimysial connective tissue in solutions varying in pH and ionic strength. Two basic questions are addressed: (1) Is

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collagen solubility dependent on the conditions used, and if so (2) do the thermo-mechanical properties of perimysium also vary in the same way?

2. Materials and methods

2.1. Composition of bathing solutions

The solutions evaluated were:

(1) H_2O (distilled);
(2) Phosphate Buffered Saline (100%PBS), the most common bathing medium used in connective tissue research. Composition: 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4, pH 7.4 (Cold Spring Harbor Protocols, 2006);
(3) Quarter-strength Ringer's solution (25%R); 30.8 mM NaCl, 1.2 mM KCl, and 1.5 mM $CaCl_2$; pH 7.4 (Hill, 1966); and
(4) A quarter-strength solution (25%S) of the major components of Ringer's solution, but buffered to a pH appropriate for post-rigor meat; 30.8 mM NaCl, 1.2 mM KCl in a 10 mM acetate buffer at pH 5.6.

2.2. Muscle samples

Semitendinosus muscles were excised at 48 h post-mortem from five separate commercially available Aberdeen Angus animals (≥ 18 months old and ≈400 kg live weight). Each muscle was cut transverse to muscle fiber direction into slices (2–3 cm thick), vacuum-packed and stored at 4 °C for 5 days, to bring total time post-mortem to 7 days.

2.3. Collagen solubility

Muscle slices from each animal were scissor-cut into small pieces and 5 g of fresh muscle added into each of 50 ml-centrifuge tubes containing 12 ml of each solution. Tubes were suspended in a water bath for 60 min at 80 °C with constant stirring. The heat treatment was terminated by transferring the tubes to a cold water bath for 10 min. Supernatant fluids and solid residues were separated by centrifugation and both were dried in an oven at 60 °C. Each fraction was then hydrolyzed in 6 N HCl at 110 °C for 16 h. After hydrolysis, samples were neutralized and the hydroxyproline concentration was determined by HPLC-Fluorescence according to Vázquez-Ortíz, Morón-Fuenmayor, and González-Méndez (2004). The % of soluble collagen was calculated as 100 × the hydroxyproline content of the soluble phase divided by the total hydroxyproline in both the soluble phase and the solid residue.

2.4. Thermo-mechanical measurements on perimysial connective tissue by Hydrothermal Isometric Tension (HIT)

Small strips of perimysial connective tissue were dissected from muscle slices using a surgical scalpel. Each strip was placed in the apparatus described by Purslow, Wess, and Hukins (1998) designed to measure force at a fixed length. Ten perimysial strips from each of the four animals were evaluated (n total = 40). The temperature in the solution was increased at a linear rate 3 °C per minute by an EchoTherm™ Programmable Digital hot plate (Torry Pines Scientific, California, USA) with constant stirring until a target temperature of 85 °C was reached. The temperature was then held constant at 85 °C for a further 30 min. The temperature at which force began to rapidly develop in the strip (T_{onset}) was quantified by back-extrapolating the linear portion of the load-temperature graph to zero load. Peak force at the maximum temperature was recorded. At the end of the 30 min holding period, the residual force in the specimen was measured. The drop in load from peak force to this value was expressed as a percentage (%) relaxation from the peak load.

2.5. Statistical analysis

Data are expressed as mean and standard errors (SE) of five animals per solution. To determine the effects of solution (H_2O; 100%PBS, 25%R; 25%S); ionic force (Ic: 0.000; 0.033; 0.131) and pH (pH: 5.6 and 7.4) data were statistically analyzed by two-way or one-way analysis of variance as appropriate. Post-hoc multiple comparisons were made using Bonferroni's critical range test. Statistically significant differences were considered at $p < 0.05$.

3. Results

Fig. 1 shows means and standard errors across all five animals for the percent soluble collagen (after heating at 80 °C, 60 min) and the percentage relaxation of isometric force in each solution.

In both the solutions at pH 7.4 solutions (100%PBS and 25% Ringer's), the collagen solubility was statistically equal, and lower than in the 25% salt solution at pH 5.6. The collagen solubility in H_2O was similar to that in the salt solution at pH 5.6 (25%S).

In contrast, the presence of salts, independent of their concentration, affected the onset temperature (T_{onset}) in the isothermal tests. This minimal temperature for development of considerable shrinkage force is related to the point where wavy collagen fibers in the perimysium have contracted enough to pull straight and bear load; some denaturation of the collagen has necessarily occurred to reach this point. In all three bathing media containing solutes (25%R, 25%S and 100%PBS) the onset temperature was between 63.5–64.5 °C and lower than the onset temperature in water (67.4 °C).

Values in the peak tension (max. load) generated in the perimysium at 85 °C were similar in all solutions at between 10 and 12 mN.

Table 1 shows the percentage of the total collagen solubilized and parameters from the HIT tests as functions of the variables pH and ionic strength (Ic). For the pH case, values from tests in both solutions at pH 7.4 were pooled (100%PBS and 25%R), and the results in 25%S and H_2O were pooled for the pH 5.6 case. This separation of variables (and analysis of significance by two-way ANOVA) points to a significant effect of increasing pH decreasing collagen solubilization. There is a trend towards lower collagen solubility as ionic strength in the bathing solution increases, and also a trend for the % relaxation of isometric force on heating to increase as ionic strength increases.

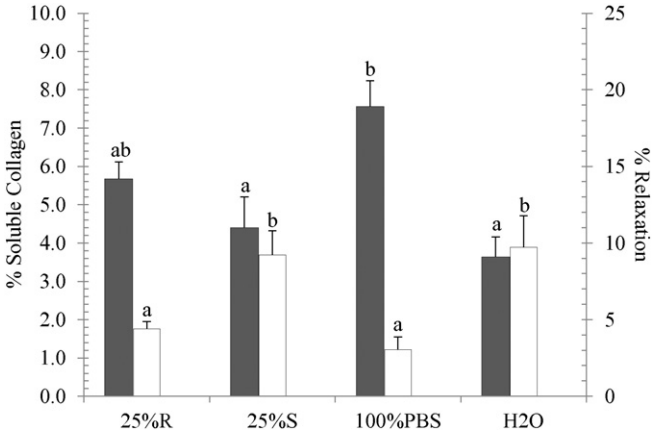

Fig. 1. Soluble collagen percent (left axis) and % relaxation in isometric tension during 30 min heating at 85 °C (right axis) in each bathing solution: 1/4 Ringer's pH 7.4 (25%R); 1/4 salt pH 5.6 (25%S); phosphate buffer solution pH 7.4 (100%PBS) and distilled water (H2O). Bar indicated SE. Vertical bars white: soluble collagen; black: % relaxation.