RELATIONSHIP BETWEEN PYRIDINOLINE CONCENTRATION AND THERMAL STABILITY OF BOVINE INTRAMUSCULAR COLLAGEN$^{1,2}$

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ABSTRACT

Semimembranosus muscle samples were obtained from 49 Holstein beef animals representing different USDA maturities. Intramuscular collagen (IMC) was isolated in the frozen state and evaluated for heat-labile collagen solubility (% Sol), thermal shrinkage temperature ($T_s$), enthalpy ($H_s$) changes, and mature crosslink (pyridinoline) content. These measures were obtained to elucidate a relationship between pyridinoline content of IMC and beef maturity level and to relate IMC thermal stability (% Sol, $T_s$, and $H_s$) to pyridinoline content. With increasing maturity, % Sol decreased ($P < .01$) and $T_s$ increased ($P < .01$), whereas $H_s$ showed no change ($P > .05$). Thus, IMC melted at increasing temperatures, but the amount of energy required to induce this endothermic change remained constant throughout maturation. The pyridinoline content of IMC increased ($P < .01$) linearly with maturity, indicating that this heat-stable, mature crosslink enhances thermal stability of IMC as beef muscle matures. Significant correlations between pyridinoline content and maturity ($r = .56; P < .001$) and $T_s$ ($r = .34; P < .05$) support this contention.

Key Words: Intramuscular Collagen, Thermal Properties, Bovidae

Introduction

The thermal stability of bovine intramuscular collagen (IMC) was shown by Judge and Aberle (1982) to be greater in older animals than in younger ones, as measured by thermal shrinkage temperature ($T_s$). These results support similar findings by Goll et al. (1964) showing that $T_s$ increases with animal age.

Meat tenderness in influenced by the quantity of heat-stable collagen crosslinks; greater amounts of these crosslinks are present in tougher muscles (Light et al., 1985). Judge et al. (1981, 1984) and Judge and Aberle (1982) suggested that the number of total or heat-stable crosslinks influences the heat stability of collagen, as measured by differential scanning calorimetry (DSC). One such heat-stable crosslink of collagen is pyridinoline (Fujimoto and Moriguchi, 1978; Eyre and Oguchi, 1980; Fujimoto, 1980). Nakano et al. (1985) reported an increase in pyridinoline content in porcine epimysial connective tissue with increasing age. Horgan et al. (1988) found a similar concentration-age relationship in caprine (goat) intramuscular collagen. In addition, Horgan et al. (1988) demonstrated a positive ($P < 0.01$) correlation between pyridinoline concentration and thermal transition temperature ($T_m$).

The objectives of this study were 1) to determine the pyridinoline concentration in bovine IMC and relate that concentration to carcass maturity, 2) to determine the associa-
tion between pyridinoline content and $T_s$, solubility, and $H_s$ in bovine IMC, and 3) to investigate the potential of $T_s$ as a means of evaluating IMC crosslinking.

Materials and Methods

Animals and Samples. Forty-nine Holstein cattle were slaughtered at a commercial processing facility. Carcasses were placed into USDA maturity groups (A to E) according to established procedures (USDA, 1976). Semimembranosus muscle samples were obtained from the right side of each animal within 1 h postmortem. Each sample was individually packaged in a sealable plastic bag and placed on dry ice. The muscle samples were immediately placed in a −40°C freezer for later IMC isolation.

Intramuscular Collagen Isolation and Solubility. A portion of each muscle sample was warmed to −5°C before IMC was isolated in the frozen state according to the procedure of McClain (1969). Another portion of each muscle sample was powdered in liquid nitrogen and used in the determination of heat-labile collagen solubility, using the Hill (1966) procedure, wherein the muscle was heated at 77°C for 70 min in 1/4-strength Ringer’s solution.

Differential Scanning Calorimetry Analysis

Triplicate IMC samples were used to determine the $T_s$ (estimated onset). A Model 990 differential scanning calorimeter was employed; the start and limit temperatures were 30 and 120°C, respectively. The program rate was 10°C/min; the time base (T) was set at 1 min/inch and the sensitivity setting (Y) was fixed at 1 Mcal/(s-inch). Enthalpies ($H_s$) of the IMC samples were determined using a high-purity (99.999%), powdered indium metal standard, which has a known enthalpy or heat of fusion of 6.79 cal/g. A unitless calibration coefficient (E) was determined and $H_s$ was determined by the following equation (DuPont Co., 1980): $H_s = (E) (A_s) (T) (Y) (60)/M$, where, $A_s$ is the area under the curve of the thermogram of each IMC sample, $M_s$ is the dry weight (grams) of IMC and 60 is a conversion factor (i.e., 60 s/min). The $A_s$ was determined by the cut-and-weigh method.

Collagen Crosslink Assay

Pyridinoline content was determined using the HPLC method of Eyre et al. (1984). Briefly, IMC samples (100 mg) were hydrolyzed in $6 \text{ N HCl}$ at 121°C and 103 kPa for 24 h. The HCl was removed by a rotary evaporator at 50°C, and samples were washed with deionized water and filtered through a nylon membrane5 (pore size .20 μm) and redried at 50°C. The dried samples were dissolved in 1 ml of 1% n-heptanuorobutyric acid6 (HFBA, an ion-pairing agent) and quantitatively transferred to 1.5-ml vials before a 10-min microcentrifuge run at room temperature. A 10-μl aliquot of duplicate samples was injected onto a 4.6-mm × 250-mm Spherisorb ODS-2 analytical column7, used in conjunction with a 4.6-mm × 30-mm guard column8 packed with identical material. The HPLC system employed a Model 5000 liquid chromatograph9 equipped with a single piston reciprocating pump to which two proportioning valves were mounted. The injector was fitted with a 10-μl sample loop. A Model 650-10S fluorescence spectrophotometer10 monitored the column eluent with excitation and emission maxima at 297 nm and 380 nm, respectively. Peak height was recorded using a Model 3390A integrator11, with a chartspeed of 1 cm/min, peak width of .16 min, attenuation value of 4, and THRSH value of 5. Pyridoxamine12 was used as an internal standard, eluting at approximately 7 min; a pyridinoline standard eluted at approximately 9 min. The following elution buffers were used: A = .01 M HFBA in 5% acetonitrile and B = .01 M HFBA in 100% acetonitrile. The following elution program was used: a gradient of 17 to 21% B during the first 20 min, a column flush of 100% B for 3 min, followed...
TABLE 1. EFFECT OF MATURITY ON BOVINE INTRAMUSCULAR COLLAGEN THERMAL STABILITY AND CROSSLINKING CHARACTERISTICS

<table>
<thead>
<tr>
<th>Variable</th>
<th>USDA maturity group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>No. of Carcasses</td>
<td>9</td>
</tr>
<tr>
<td>Pyridinoline content, mol/mol of collagen</td>
<td>.04b</td>
</tr>
<tr>
<td>Thermal shrinkage temperature, °C</td>
<td>63.20b</td>
</tr>
<tr>
<td>Solubility*, %</td>
<td>6.66b</td>
</tr>
<tr>
<td>Enthalpy, cal/g</td>
<td>5.34</td>
</tr>
</tbody>
</table>

*Least squares means ± SE.

b,c,dMeans not having a common superscript differ (P < .01).

Heat-labile collagen.

Results and Discussion

Pyridinoline concentration (Table 1) in bovine IMC increased (P < .01) linearly with increasing maturity level. These data support the earlier findings of Nakano et al. (1985) and Horgan et al. (1988).

The increase (P < .01) in T_s of the bovine IMC paralleled that of pyridinoline content (Table 1). The T_s of IMC from the older maturity groups (D and E) was greater (P <

TABLE 2. CORRELATION COEFFICIENTS AMONG BOVINE CARCASS MATURITY AND CHARACTERISTICS OF INTRAMUSCULAR COLLAGEN

<table>
<thead>
<tr>
<th>Item</th>
<th>Maturity^b</th>
<th>Pyridinoline</th>
<th>T_s^c</th>
<th>Solubility^d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyridinoline content</td>
<td>.56**</td>
<td>.34*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T_s</td>
<td>.64**</td>
<td>.34*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solubility</td>
<td>−.47**</td>
<td>−.26†</td>
<td>−.60**</td>
<td></td>
</tr>
<tr>
<td>Enthalpy</td>
<td>−.09</td>
<td>.14</td>
<td>.01</td>
<td>−.22</td>
</tr>
</tbody>
</table>

^N = 49.

^bUSDA maturity group (A to E).

^cThermal shrinkage temperature.

^dHeat-labile collagen.

†P < .10.

*P < .05.

**P < .001.
.01) than that from the younger maturity groups (A, B, and C). There was a greater (P < .01) concentration of pyridinoline in maturity Group E than in all other maturity groups. Table 2 illustrates the highly positive (P < .001) relationship between physiological maturity and both T and pyridinoline content of bovine IMC. Horgan et al. (1988) showed a similar relationship between pyridinoline content and age. However, the pyridinoline concentration in caprine IMC was found to be 3 to 5 times greater than that found in bovine IMC of the present study. This might be due to the IMC isolation buffer containing sodium citrate used by Horgan et al. (1988) and may have solubilized some of the immature collagen, thus diluting the total collagen content of the muscle analyzed. This would increase the amount of pyridinoline found when expressed on per-mole-collagen basis, thereby giving a false impression of relatively high pyridinoline concentrations. This sodium citrate effect on immature collagen was not studied further. However, in unpublished results from our laboratory, using IMC isolated by the Fuji and Murota (1982) chemical method, slightly elevated pyridinoline concentrations, on a per-mole-collagen basis, were shown to exist in IMC isolated by the chemical method (KCI-KI) compared with the McClain (1969) procedure from the frozen state.

A positive relationship (P < .05) between pyridinoline concentration and T (Table 2) was observed. This lends support to the findings of Horgan et al. (1988) of a significant correlation between the two parameters in caprine IMC. From these data, use of T determined by differential scanning calorimetry is an effective means of assessing IMC crosslinking. The amount of soluble, heat-labile collagen decreased (P < .01) with increasing maturity (Table 1); it was reduced by more than one-half from the youngest maturity level (A) to the oldest (E). Solubility was inversely (P < .001) related (Table 2) to T and maturity. Thus, the increasing stability of IMC to thermal changes as maturity increased resulted in reduced amounts of available collagen that could be solubilized. This enhanced structural stability of IMC with age, however, did not result in a significant relationship between solubility and pyridinoline content (Table 2), although an inverse trend (P < .10) was noted. The H of bovine IMC did not (P > .05) change with increasing maturity (Table 1). As illustrated in Table 2, the IMC H value was not (P > .05) related to pyridinoline concentration. This indicates that a change in the heat-stable mature crosslink quantity alone is not sufficient to alter the amount of heat required to denature collagen, although the denaturation temperature (T) is modified (Table 1).

**Implications**

Strong relationships between pyridinoline concentration vs USDA maturity level and thermal shrinkage temperature indicate that pyridinoline is involved in the maturation-induced stabilization of intramuscular collagen thermal characteristics and thermal shrinkage temperature is a useful means of evaluating intramuscular collagen crosslinking.

**Literature Cited**


Judge, M. D. and E. D. Aberle. 1982. Effects of chronological age and postmortem aging on thermal shrinkage temperature of bovine intramuscular colla-
PYRIDINOLINE AND COLLAGEN THERMAL STABILITY


