CHANGES IN BEEF STRUCTURAL AND PHYSICOCHEMICAL PROPERTIES BY ENDOGENOUS ENZYME ACTIVITY

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Background.

Mechanisms responsible for meat tenderness, although studied by a number of authors, remain elusive. In order to explain this phenomenon, changes in several parameters have been proposed. These parameters are pH, sarcomere length, collagen solubility, glycolysis and post-mortem proteolysis. The changes are also related to extrinsic conditions such as storage temperature and time (*Shackelford et al, 1997*). However, meat tenderness is also affected by animal species and age, it also varies among animals of the same species, among muscles of the same animal and due to post-mortem storage temperature (*Greaser and Fritz, 1995*). Post-mortem proteolysis plays the main role in meat tenderness, the two main changes occurring during this process are disruption of Z-disks and desmin degradation (*Bandman, 1992*). On the other hand, although there are a number of proteases in the skeletal muscle only calpains and lysosomal cathepsins are considered as causing post-mortem proteolysis (*Roncalés et al, 1995*). Other authors consider that a co-operative action of these two enzymatic systems can also occur (*Jaarsveld et al, 1997*). Calpains have been characterised as neutral cystein-endopeptidases activated by calcium (*Saido and Susuki, 1993*). They have two activating conditions: calpains I or m-calpains need 50 to 70 mM calcium for their maximum activity, whereas calpains II or μ-calpains require 1 to 5 μM calcium. The maximum calpain activity occurs at pH 7.5 and 35°C (*Koohmaraie et al, 1988*). Cathepsins are located in the muscle fibre lysosomes, and are liberated when a decrease in pH occurs as a result of a massive respiratory and cardiac arrest. When these functions cease, lysosome membranes disrupt liberating glycolytic, lipolytic and proteolytic enzymes. Cathepsins are the main proteases liberated from the lysosome. They mainly deplete actin and myosin, the most abundant proteins in the muscle fibre. Cathepsin activity reaches it maximum at pH 4 and 37°C (*Chambers et al, 1994*).

Objetive.

To study the changes in some physicochemical and structural parameters of beef subjected to the effect endogenous enzymes, when stored at two temperature conditions.

Methods.

Psoas major muscles were excised from the carcasses 24 hours after slaughtering. Each muscle was divided into two batches and stored at 4 or 15°C. Each batch was divided into 3 portions, vacuum packaged and allocated for sampling at 0, 5 and 15 days. The analysis carried out were:

Physicochemical parameters: pH, water holding capacity (WHC), colour (Hunter Lab), tenderness expressed as shear force (Warner-Bratzler knife) and protein degradation (SDS-PAGE electrophoresis).

Enzymatic activity: total enzymatic activity (Kunitz), acid and neutral enzyme activity.

Microbial populations: Enterobacteria and pseudomonas counts.

Structural integrity: fibre disruption (scanning elctromicroscopy).

Results and discussion.

Physicochemical parameters: Figure 1 shows pH values at the two studied temperatures. pH decreased to lower values when stored at 4°C as compared to samples stored at 15°C. However, there was an increase in pH at day 10 and 4°C due to the production of amino-compounds as a result of protein degradation. The WHC pattern was similar at both storage temperatures (Figure 2) increase at day 15 due to proteolysis that promotes water adsorption within muscle fibres. Shear force results are shown in Figure 3. It decreased as study time increased; the decrease being more marked when the samples were stored at 4°C than at 15°C, as a result of pseudomonads proliferation and consequently action of bacterial proteases upon muscle protein. At the same time, myofibrillar proteins underwent only moderate degradation throughout the study time as observed by SDS-PAGE (Figure 4) with no considerable difference between samples stored at 4 and 15°C. However, the electrophoresis carried out in this study were for low molecular weight proteins in order to detect peptide formation; detection of protein degradation requires electrophoresis for high molecular weight proteins.

Enzymatic activity: Figures 5 and 6 show the enzymatic activity at pH 4 and 7.4, respectively. At pH 4 the maximum activity occurred at day 10. Samples stored at 15°C had higher enzymatic activity than those stored at 4°C, although in both cases the activity decreased at day 10. Activities in samples at pH 7.5 (Figure 6) had the same patter, i.e. a maximum at day 10 and further decrease. Due that at both pH values, 4 and 7.5, the maximum activity was found at 15°C and not 4°C it was concluded that endogenous enzyme activity increased at higher temperatures as they are closed to their optimum temperature. Microbial proteases, mainly from enterobacteria, were also produced and are more active at 15 than at 4°C. However, pseudomas are able to produce very active proteases at chill temperatures.

Microbial populations: Enterobacteria are generally an index of faecal contamination whereas pseudomonads are an important population in chilled meat, and are able to produce proteases as well as lipases. As expected, enterobacteria populations are larger at 15°C than at 4°C, whereas pseudomonads are more abundant at 4°C than at 15°C. Pseudomonads, a psychrotroph, growth and produce

proteases at chill temperature, whereas enterobacteria may produce proteases at higher temperatures. It was obvious that both types of micro-organisms are present, but their activity was different according to the storage temperature.

Structural integrity: SEM studies reported only a slight fibre disruption. This structural alteration increased throughout the study time. Disruptions were also more noticeable at 15 than at 4°C. Therefore, taking into consideration all enzymatic activities acting upon the fibres, storage at 15°C promoted more active proteolysis than storage at 4°C.

Conclusions.

Tenderness in beef reached higher values when stored at 15°C as compared to storage at 4°C, due to the disruption of muscle fibres. Ageing should be carried out for 10 days only as enzymatic activity during this time is at its optimum.

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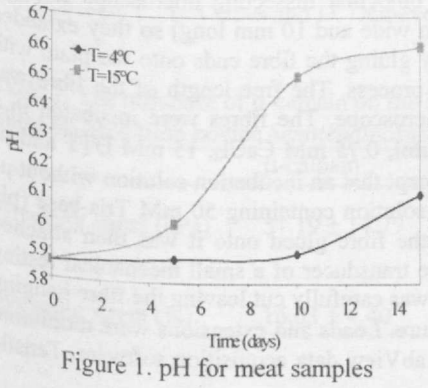

Figure 1. pH for meat samples

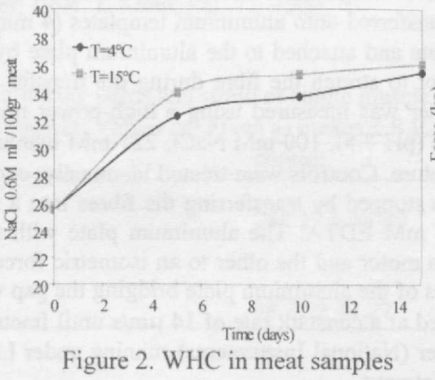

Figure 2. WHC in meat samples

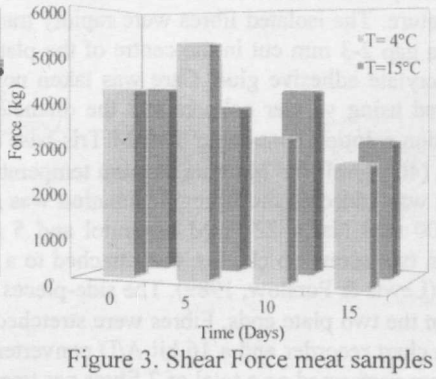

Figure 3. Shear Force meat samples

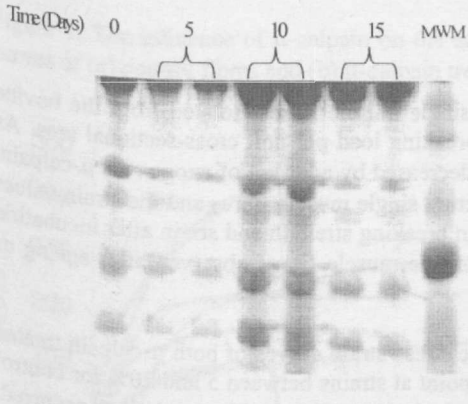

Figure 4. Electrophoregram for meat extracts

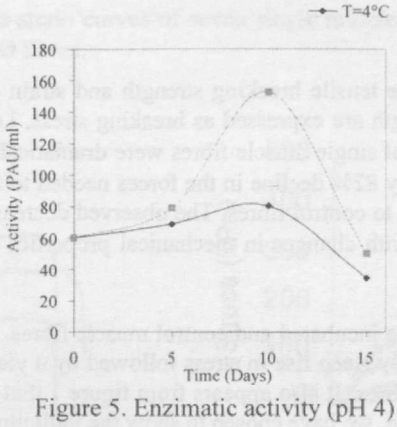

Figure 5. Enzimatic activity (pH 4)

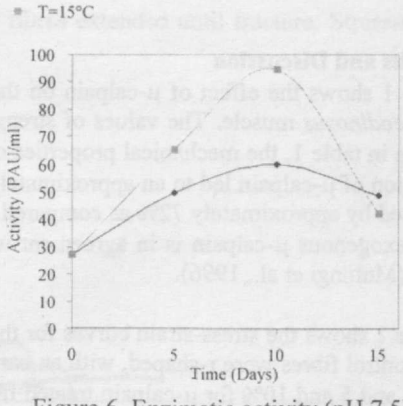

Figure 6. Enzimatic activity (pH 7.5)