EFFECT OF LACTIC ACID BACTERIA OR GLUCONODELTALACTONE ON TEXTURAL AND PHYSICOCHEMICAL PROPERTIES OF CALCIUM CHLORIDE MARINATED BOVINE BRACHIOCEPHALICUS MUSCLE

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Background

Natural meat proteolysis seems to be one of the main factors that contribute to tenderisation process during meat aging. Two enzymatic systems are involved in meat tenderness: calpains and cathepsins. However, there are some discrepancies regarding to the cooperative action of both enzymatic systems. It is accepted that these proteolytic systems are similar in all muscles, independently of animal species. Calpain activation could be done by injecting or dipping the meat with/in $CaCl_2$ solutions. Many meat cuts considered as tough due to their high collagen content have a relatively low price during commercialisation. An alternative to obtain more tender meat is the calcium chloride marination, by means of the activation of the calcium dependent or calpain proteolytic system. Acidulating agents produce a high pH reduction. In this way, glucono-δ-lactone in water is hydrolyzed to gluconic acid, and is employed to produce gels in many conditions. In other hand, lactic acid bacteria has been suggested to become protective cultures in meat products due their ability to inhibit the growth of other microorganisms by many ways, like lowering pH by lactic and acetic acid production, competition for the nutrients, or producing hydrogen peroxide, bacteriocins, or antibiotics.

Objectives

We are proposing the calcium chloride marination beside to the application of acidulating agents in order to, first, activate the calpain system, and secondly, decrease pH enough, affecting on a remaining cathepsin activity or a protein degradation by the denaturing effect of low pH, and thus improving meat tenderness.

Materials and methods

A complete factorial design was employed to determine the effect of the acidulating agent, lactic acid bacteria (LAB) or glucono-δ-lactone (GDL), and storage time on texture, water holding capacity, pH and myofibrillar fragmentation index of meat samples. The PROC GLM procedure of the SAS Statistical System v. 8 (SAS Institute, Cary, NC) was employed in order to determine significant differences. Results are the means of three replications. Bovine *brachiocephalicus* muscle was obtained within 48 h *postmortem* in a local slaughterhouse. Muscles were cut in 150 g samples and were marinated in a 150 mM CaCl2 solution at 4°C in a vacuum tumbling machine. After marination, one lot of samples were immersed in a LAB suspension with an optical density of 1.0 (*Pedioccocus pentosaceus* PC-111016, Christian Hansen A/S, Hoersholm, Denmark) at 4°C during 15 minutes. Other lot of marinated samples was immersed in a GDL solution (1% w/v, Lot: 070199-22, Glocona America Inc., Janesville, WI) at 4°C during 30 minutes. Finally, a third lot of samples were keep as control under same storage conditions. All samples where then vacuum packed and stored at 4°C during 1, 5, 10 and 15 days. Warner-Bratzler shear force (WBSF) was determined in a texture analyser model TA-HDi (Texture Technologies, Scarsdale, NY/ Stable Micro Systems, Surrey, UK) equipped with a 50 kg load cell. Samples of approx. 6.0x1.2x1.2 cm were cooked in hot water until an internal temperature of 70°C, as described by Wheeler et al. (1979). Cooked samples were compressed at a constant speed rate of 120 mm/min with the Warner-Bratzler razor, reporting maximum force detected during the test. The pH value was determined using 10 g of sample homogenized with 100 ml of distilled water in a Warning blender during one minute with an Oakton potentiometer EP 2500 (Oakton Corp., Singapore). Water holding capacity (WHC) was determined in agree to the methodology reported by Hamm (1975), with some modifications. Meat samples (300 mg) were placed between two Whatman No. 1 filter papers and compressed during 10 minutes with 1 kg weight between two Plexiglas plates. Water holding

capacity was reported by weigh difference as the percentage of humidity retained in the paper. Sodium dudecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), in denaturing conditions, was performanced in a Mini Protean II Slab cell (Bio Rad, Richmon, CA). Acrylamide concentrations were 4% for stacking and 12% for resolving gels (Bollag & Edelstein, 1991). Myofibrillar fragmentation index was determined using the technique reported by Olson et al. (1976). In order to determine the effect of inoculated LAB on microbial populations, a microbiological analysis were made during storage time. Total coliforms were determined in violet red bile (VRB) agar plates incubated at 35°C during 24 h. LAB were determined in Mann-Rugose-Sharp (MRS) agar plates incubated at 35°C during 24 h. After pertinent dilutions, the colony forming units (CFU) per g of sample were reported in control and LAB treated samples.

Results and discussion

Control pH remained constant during all the storage period since after 48 h *postmortem* and vacuum packed little biochemical changes are expected at 4°C. As expected, LAB and GDL had a significantly effect ($P<0.01$) on pH. WHC was significantly affected ($P<0.01$) by the LAB or GDL treatments during the storage (Figure 1). Control samples (only marinated with $CaCl_2$ solution) presented a little increase in WHC during storage time. LAB treated samples presented higher WHC values than the GDL treatments. The results indicate that the samples treated with acidulating agents had lower WHC values than control sample, since the acidity reduced the pH close to the myofibrillar proteins isoelectric point. Furthermore, low pH values could result in a myofibrillar protein denaturation altering their ability to retain water (Aktas & Kaya, 2001). The texture of the samples was affected by the treatment ($P<0.01$), but not by the storage time ($P>0.05$). GDL treated samples were tougher (high shear force values) than LAB or control (Figure 1). Meat control tenderness increased with storage time, mainly by the $CaCl_2$ effect (Pérez-Chabela et al., 1998). LAB samples force presented a drastic reduction after the 5-day of storage, reaching the same WBSF values than control samples at the 15 day. GDL samples were stronger and more difficult to shear, almost twofold than control samples. In our experiment, GDL samples were tougher than LAB ones. It could be explained from the view that the GDL reduced drastically and quickly the pH, denaturing proteins instead to promote some kind of myofibrillar weakening. When meat pH is below 5.5, meat could be softer due to protein degradation or loss of the structure. MFI increases with storage time and was higher for control samples than for LAB or GDL samples ($P<0.01$)(Figure 2). Control samples presented higher values on the first storage day, with an important increase on day 10. LAB and GDL samples were similar during the first 10 days but LAB had high values at the end of the sampling period. Lin et al. (2000) reported that a high MFI in mule duck marinated in red wine correspond to the acidic environment provided by the red wine, where the cathepsins or lysosomal enzymes could be responsible for protein degradation of myofibrils in acid environment (Saunders, 1994), and MFI is directly related to tenderness (Culler et al., 1978). Microbial population evolution was depicted in Figure 3, where the LAB population increased in LAB treated samples and control, whereas the coliforms population was decreasing in LAB samples. The reduction in coliforms population was important due to inoculated LAB after $CaCl_2$ marination, whereas in control samples in spite of the detection of an relatively high naturally occurred LAB population, coliforms grew with storage time with no apparent relationship with the native LAB. An important natural occurring LAB population was found in control samples. LAB inoculation could contribute in extending shelf life of meat products due to their ability to inhibit pathogens and deteriorative microorganisms by the nutrient competition, displacing them form their ecologic niche and/or the production of antimicrobial substance as lactic or acetic acids, diacetyl, hydrogen peroxide and bacteriocines (Requena & Peláez, 1995; Helander et al. 1997).

Conclusions

The GDL concentrations not affected meat tenderness, probably because of high acidic conditions generated. LAB incorporation had a minor effect on meat tenderness, but reduced the coliforms population. In the present experimental conditions employed, a maximum degradation, and hence tenderness, were reached at the 5th day of storage with LAB as acidulating agent.

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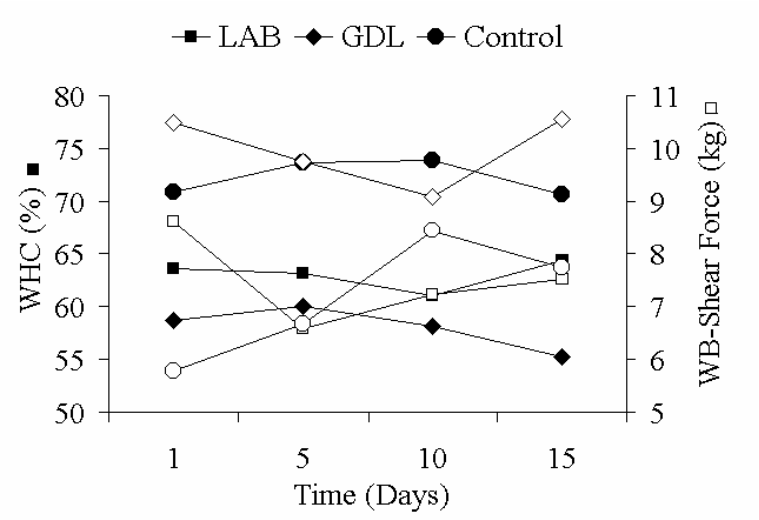

Figure 1. Water holding capacity (WHC) and Warner-Bratzler shear force in marinated meat samples during storage.

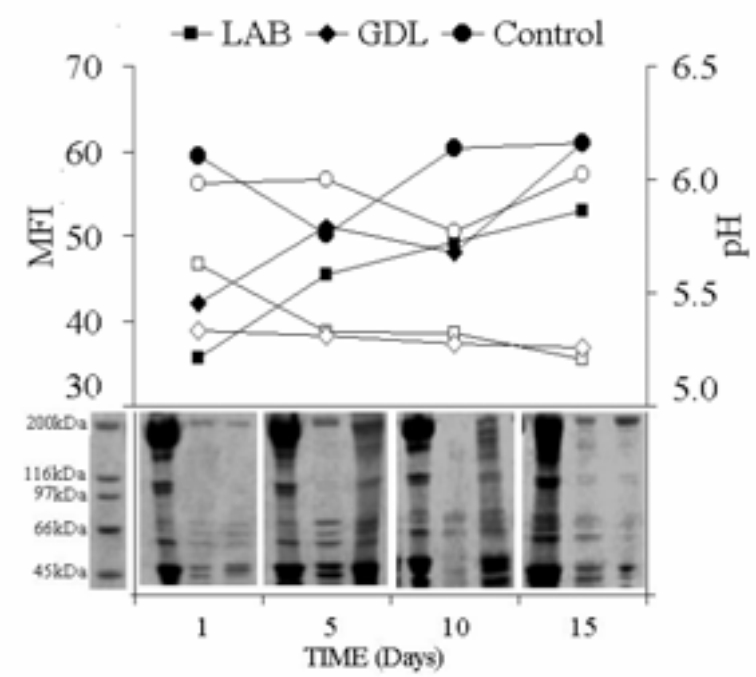

Figure 2. Myofibrillar fragmentation index (MFI), pH and SDS-PAGE for the marinated meat samples during storage (Left: MWM. For each time: Line 1: Control, Line 2: LAB, Line 3: GDL).

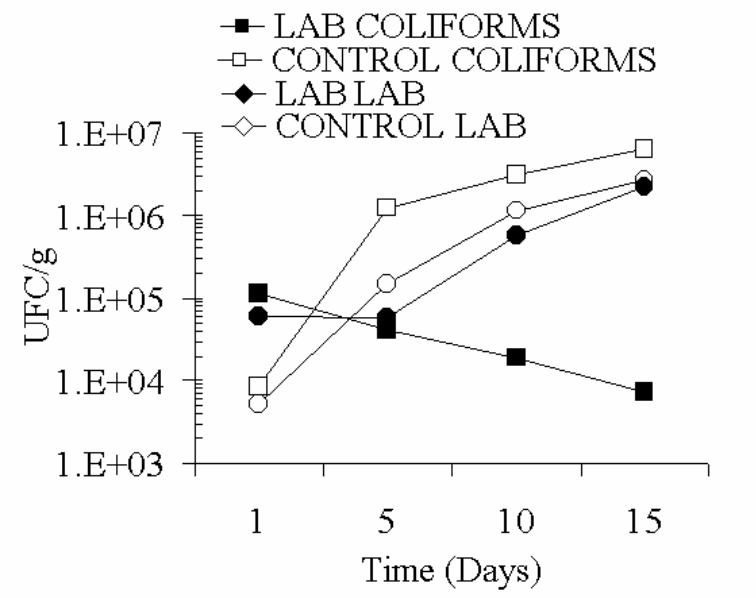

Figure 3. Microbial population in Control and LAB treated samples during storage.