MUSCLE PROTEINS HYDROLYSIS BY *LACTOBACILLUS* ISOLATED FROM DRY SAUSAGES. EFFECT OF CURING CONDITIONS.

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Background

Lactic acid bacteria are essential agents of the meat fermentation process that contribute to the hygienic and sensory quality of meat products, which is achieved mainly by the metabolic activities of these bacteria. Their action on carbohydrates and proteins results in sugar depletion, pH reduction and the generation of flavor compounds. The enzymology of dry fermented sausages is quite complex due to the coexistence of enzymes from both, endogenous (cathepsins, calpains) and microbial origin. Therefore, the initial degradation of myosin and actin into peptides is due to cathepsin D, while the later decomposition of peptides into free amino acids is bacterial (6). It is also known that free amino acids are precursor of aroma volatile compounds which have an impact in final texture and flavor (7). However the proteolytic system of dairy lactic acid bacteria has been explored in much detail, the hydrolytic system of lactobacilli involved in meat fermentation is becaming the focus of an increasing number of studies due to the technological roles of these organisms. The effects of curing agents and other technological parameters on the activities of exopeptidases have been reported (8) but studies of the specificities of proteinases and peptidases from lactobacilli for muscle sarcoplasmic and myofibrillar proteins are scarse. The knowledge of the proteolytic events carried out by lactobacilli in meat would be an important contribution to the development of desirable texture and flavor in cured-meat products

Objectives

This work focuses on the proteinase and peptidase activities of different enzyme combinations from two *Lactobacillus* strains on muscle sarcoplasmic and myofibrillar proteins to predict the suitability of these strains and their proteolytic enzymes as starter culture or additives, respectively, in the ripening of dry-fermented sausages. In addition, the rol of curing agents on lactobacilli proteolytic system, as well as the use of the studied strains in a meat system was assayed.

Methods

The strains of *L. casei* CRL 705 and *L. plantarum* CRL 681 isolated from dry-sausages were used for proteolytic assays. The strains were grown in MRS broth at 30°C for 24 h. Cell suspensions (WC) and extracts (CFE) as well as the sarcoplasmic and myofibrillar proteins from pork muscle *(Longuisssimus dorsi)* were obtained as reported by Fadda *et al.* (3). The hydrolysis of muscle proteins were monitored by sodium dodecyl sulfate poliacrylamide gel electrophoresis (SDS-PAGE) analysis (5). Peptide analysis and the change in free amino acids and natural dipeptides were monitored using reverse-phase high-performance liquid chromatography (RP-HPLC), according to Aristoy and Toldrá (1). Curing agents (NaCl 3%, $NaNO_2$ 200 ppm and ascorbic acid 0.1 mg/ml) were individually added to sarcoplasmic and myofibrillar extracts to evaluate their effect at 25°C, using cell suspensions of *L. casei* CRL 705. A mixed culture involving *L. casei* CRL 705 and *L. plantarum* CRL 681 was also assayed in a sausage-like system containing the curing additives obtained according to Deibel *et al.* (4).

Results and discussion

Electrophoretic analysis resulting from the hydrolysis of muscle sarcoplasmic and myofibrillar proteins by the combination of whole cells (WC) and cell free extracts (CFE) of *L. plantarum* CRL 681 and *L. casei* CRL 705 are shown in Fig.1 and 2. WC of *L. casei* CRL 705 drastically hydrolyzed protein bands at 96 h of incubation, while *L. plantarum* CRL 681 also produced a protein degradation but in a lesser extent. Eventhough CFE from both strains also caused reduction in the intensity of protein bands, the additive effect following the incorporation of both WC and CFE resulted in the strongest protein hydrolysis. The protein profiles corresponding to myofibrillar protein extracts (Fig 2) shows that endogenous proteins (control) were responsible for the degradation of protein bands such as myosin and actin and that the hydrolytic effects were intensified when WC+CFE from both lactobacilli strains were added.

Peptide maps (data not shown) of sarcoplasmic extracts were greatly modified by the activities of *L. casei* CRL 705 regardless of the use of whole cells or CFE. The simultaneous addition of both enzymatic sources strengthened the above mentioned effects. The hydrolysis of myofibrillar proteins led to the generation of mainly hydrophilic peptides which are associated with desirable cured-meat flavors (2). When the activity of whole cells of *L. plantarum* CRL 681 was evaluated a generation of hydrophilic peptides from both sarcoplasmic and myofibrillar extracts was observed while no major changes were detected when only CFE were added.

Table 1 shows the free amino acids and natural dipeptides (carnosine and anserine) of technological importance released from sarcoplasmic protein extracts by the activity of *L. casei* CRL 705 and *L. plantarum* CRL 681. The inoculation of WC from *L. casei* caused a decrease in the level of amino acids, specially glutamine and alanine but contributed to the generation of threonine, carnosine and taurine, similar results being obtained when WC of *L. plantarum* were inoculated. The activity of CFE from *L. casei* was involved in the generation of glutamic acid, histidine, alanine and carnosine while a major concentrations of β-alanine, histidine, alanine and anserine were obtained with *L. plantarum.* The content of almost all amino acids analyzed increased by the end of incubation when whole cells and CFE were used together. In myofibrillar extracts there was a general but slight increase in free amino acid and natural dipeptide contents when both cellular fractions were present.

The effect of curing agents on the proteolytic activity of *L. casei* CRL 705 (data not shown) demonstrated that ascorbic acid was the additive that exerted the strongest stimulatory effect mainly on sarcoplasmic proteins breakdown while the presence of the

combined additives (ascorbic acid + NaCl + $NaNO_2$) produced a release of peptides and free amino acids indicating an important peptidolytic activity. In myofibrillar protein extracts, eventhough of NaCl caused the greatest hydrolytic events in the primary proteolysis, there was only a slight increase in free amino acids. On the whole, when all additives were incorporated an important stimulatory effect on the aminopeptidase system (secondary proteolysis) was observed this being of great technological impact.

Fig. 3 shows the sarcoplasmic proteins breakdown of a sausage-like system when *L. casei* CRL 705 + *L. plantarum* CRL 681 as a mixed starter culture was incorporated. After 96 h of incubation a reduction in the intensity of protein bands in the whole molecular mass range was observed indicating the active participation of the satrter culture in the proteolytic events. In Fig. 4 the evolution of amino acid contents, pH and CFU/ml during the incubation at 25°C for 96 hs of both lactobacilli strains (individually or as amixed culture) is observed. Eventhough the presence of *L. casei* CRL 705 showed the higher concentration of total amino acids, the lower pH values was attained when the mixed starter culture was used (Fig. 4d).

Conclusions

The potential contribution of *L. casei* CRL 705 and *L. plantarum* CRL 681 to the breakdown of sarcoplasmic and myofibrillar proteins has been demonstrated. The activities of whole cells together with CFE seem to be indispensable for initiating proteolytic changes. When a starter culture involving these strains was assayed in a sausage-like system the above results were corroborated.

Literature

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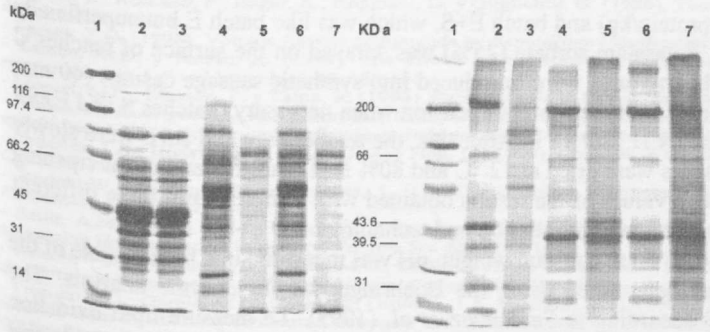

Fig 1: SDS-PAGE of sarcoplasmic protein hydrolysis. Control (lines 2-3), *L. plantarum* CRL 681 (lines 4-5) and *L. casei* CRL 705 (lines 6-7). Samples containing WC+CFE at 0 and 96 h.

Fig 2: SDS-PAGE of myofibrillar protein hydrolysis. Control (lines 2-3), *L. plantarum* CRL 681 (lines 4-5) and *L. casei* CRL 705 (lines 6-7). Samples containing WC+CFE at 0 and 96 h.

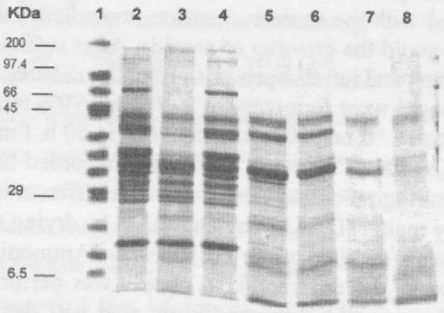

Fig 3: SDS-PAGE of sarcoplasmic proteins hydrolysis of sausage-like system by a mixed culture of *L. plantarum* CRL 681 + *L. casei* CRL 705. Control (lines 2-3), 0, 24, 48, 72 and 96 hs (lines 4-5-6-7-8) respectively.

Amino acids and dipeptides	*L. casei* CRL 705	*L. plantarum* CRL 681
Alanine	CFE / WC+CFE	CFE / WC+CFE
β-alanine	WC+CFE	CFE / WC+CFE
Glutamic acid	CFE / WC+CFE	WC+CFE
Histidine	CFE / WC+CFE	CFE / WC+CFE
Threonine	WC / WC+CFE	WC / WC+CFE
Taurine	WC / WC+CFE	WC / WC+CFE
Anserine	WC / CFE / WC+CFE	CFE / WC+CFE
Carnosine	WC / CFE / WC+CFE	WC / WC+CFE

Table 1: Amino acids and dipeptides released by Whole cells (WC), cell free extracts (CFE) and WC+CFE of *L. casei* CRL 705 and *L. plantarum* CRL 681 after incubation at 37°C from sarcoplasmic proteins.

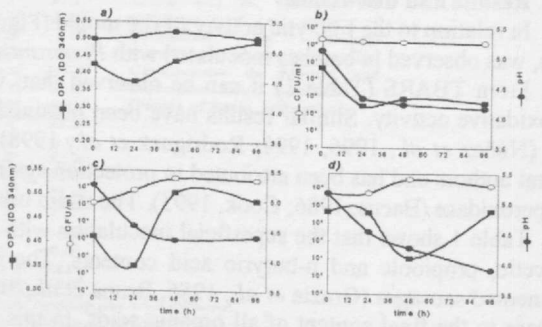

Fig.4: Amino acid contents (OPA), pH and CFU/ml when control (a), *L. plantarum* CRL 681 (b), *L. casei* CRL 705 (c) and a mixed culture (d) were incubated at 25°C during 96 hs.